ISSN: 2643-671X

Research Article

Journal of Agriculture and Horticulture Research

Regeneration of Transgenic Taro (*Colocasia esculenta* var. *esculenta*) Via Microprojectile Bombardment of Embryogenic Cell Suspensions

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Submitted: 2026, Oct 05; **Accepted:** 2026, Nov 09; **Published:** 2026, Nov 19

Citation: Deo, P. C., Tyagi, A. P., Taylor, M., Harding, R. M., Becker, D. K. (2026). Regeneration of Transgenic Taro (*Colocasia esculenta var. esculenta*) Via Microprojectile Bombardment of Embryogenic Cell Suspensions. *J Agri Horti Res, 9*(4). 01-11.

Abstract

This study outlines the development of a gene delivery system for taro (Colocasia esculenta var. esculenta) using biolistic transformation. The binary vector pART-TEST7, carrying the green fluorescent protein (GFP) reporter and the neomycin phosphotransferase II (nptII) selectable marker genes, was introduced into embryogenic suspension cultures of genotypes CPUK and THA-07. Cells were bombarded with gold particles at 550 kPa helium pressure under -85 kPa vacuum, positioned 7.5 cm from the microprojectile launch point. Green fluorescence was visible within 72 hours, indicating transient GFP expression. Notably, placing the cells in an osmotic medium (0.2 M mannitol and 0.2 M sorbitol) for two hours before and after bombardment markedly enhanced transformation frequency. A total of six genetically modified plantlets were regenerated, and transgene integration was validated via PCR. To evaluate promoter activity, a fluorometric assay measuring uidA (GUS) gene expression was conducted. Results demonstrated that the maize Ubi-1 promoter outperformed the CaMV 35S promoter by 1.4-fold and surpassed the TaBV-600 promoter by 23-fold. These findings offer a platform for advancing taro improvement through genetic transformation and future gene-editing strategies, contributing to the development of resilient cultivars of this underutilized crop.

Keywords: *Colocasia esculenta var. esculenta*, Taro, Genetic Transformation, Biolistic Delivery, Somatic Embryo Development, Embryogenic Suspension Cultures, Gene Expression Promoter

1. Introduction

Taro (*Colocasia esculenta var. eculenta*) is vulnerable to a wide range of pests and diseases, which severely impact its production, threaten regional food security, and result in economic losses in trade. Although genetic improvement of taro through traditional breeding holds promise, progress has been limited due to the lengthy breeding cycles and the scarcity of disease-resistant germplasm [1]. Additionally, cultivar preference often varies across cultural and ethnic groups, posing challenges to the broad adoption of newly developed lines.

In contrast, genetic engineering offers a precise and efficient alternative by enabling the direct insertion of one or a few specific genes into elite, farmer-preferred cultivars. This approach also allows the use of gene sequences from diverse biological sources, beyond the gene pool of taro itself. The establishment of a reliable transformation platform for taro would therefore be a valuable tool for accelerating crop improvement.

Genetic transformation has been successfully applied in various monocotyledonous crops including sugarcane, banana, maize, wheat, and rice, using both *Agrobacterium tumefaciens* and particle bombardment (biolistics) [2-7]. However, limited progress has been made in the genetic transformation of *Colocasia esculenta var. esculenta*. This may be attributed to technical hurdles in developing efficient regeneration systems, as well as the crop's relatively low priority in research agendas of industrialized countries. To date, only two published studies report successful taro transformation—one using microprojectile bombardment and the other using *Agrobacterium*-mediated delivery [8,9]. The biolistic method yielded a single confirmed transgenic plant, while the *Agrobacterium*-based approach produced six stably transformed plants, indicating modest gains.

Successful genetic transformation requires several key components: a reliable gene delivery technique, a suitable promoter to drive transgene expression, an effective selection strategy to isolate transformed cells, and a high-frequency regeneration system to recover whole plants from single transformed cells [10]. Somatic embryogenesis protocols for taro have been previously established, including the development of regenerable embryogenic suspension cultures [11,12]. These cultures are particularly well-suited for transformation studies due to their capacity for rapid proliferation, their structure as fine cell aggregates that facilitate contact with the transforming agent, and their ability to regenerate plants from single cells, thereby minimizing the risk of producing chimeric tissues [13-15].

Another crucial consideration in transgenic plant development is the selection of an appropriate promoter to ensure effective and tissue-specific expression of the introduced gene. In taro, promoter activity has only been assessed in leaf tissues subjected to particle bombardment, highlighting a gap in the evaluation of promoter strength in embryogenic cells [16].

In this study, we report a biolistic transformation protocol for taro using embryogenic suspension cultures derived from selected genotypes. The GFP gene was used as a reporter for its ability to be directly visualized in live cells, while NPTII enabled antibiotic-based selection. Transformation conditions were systematically optimized to enhance efficiency, and stable gene integration was confirmed. In addition, the relative activity of three promoters—Cauliflower Mosaic Virus 35S (CaMV-35S), maize polyubiquitin-1 (Ubi-1), and Taro Bacilliform Virus (TaBV-600) was assessed in embryogenic cells using a fluorometric assay [16].

2. Materials and Methods

2.1. Plant Material for Transformation

Embryogenic suspension cultures derived from two taro (*Colocasia esculenta* var. *esculenta*) accessions, THA-07 and CPUK, were selected as the target explants for transformation experiments. These cultures were established and routinely maintained through weekly subculturing in liquid callus maintenance medium (CMM_L), following the protocol outlined by [12].

2.2. Plasmids

Figure 1 illustrates the schematic representations of the plasmid constructs used in this research. For the promoter analysis, the plasmids pGem4z-TaBV-GUS, pGem4z-Ubi-GUS, and pUC-CaMV-35S-GUS were generously provided by Dr. Jason Geijskes from Centre for Tropical Crops and Bio commodities, Queensland University of Technology. The transformation vector, pART-TEST7, containing the GFP reporter gene and NPTII selection marker, was kindly provided by Dr. Ben Dugdale (Centre for Tropical Crops and Bio commodities, Queensland University of Technology).

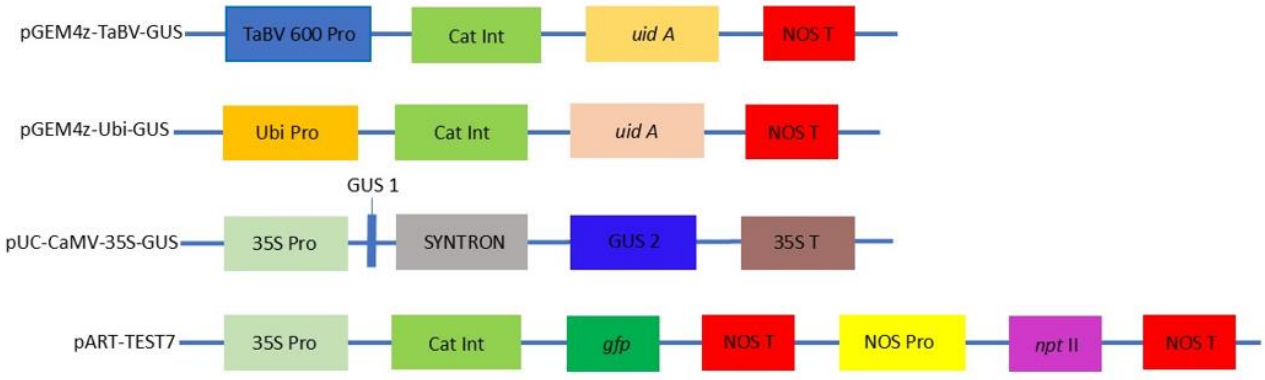


Figure 1: Diagrammatic representation of plasmid constructs used for gene delivery into taro embryogenic suspension cells. Abbreviations: 35S Pro – Cauliflower Mosaic Virus 35S promoter; nptII – neomycin phosphotransferase II gene (selectable marker); 35S T – CaMV 35S terminator; TaBV 600 Pro – promoter derived from Taro Bacilliform Virus; Cat Int – catalase gene intron; uidA – β-glucuronidase reporter gene (GUS); GUS 1/GUS 2 – first and second exons of GUS, respectively; SYNTRON – synthetic intron; NOS T – terminator from the nopaline synthase gene; Ubi Pro – promoter and first intron from the maize polyubiquitin-1 gene; gfp – green fluorescent protein gene (reporter); NOS Pro – promoter element originating from the nos gene of *Agrobacterium tumefaciens*, used for constitutive gene expression

2.3. Kill Curve Analysis

To establish an effective selection regime for eliminating non-transformed cells, taro suspension cultures were exposed to varying concentrations of two antibiotics: kanamycin (0, 50, 100, 150, 200, and 250 mg/L) while hygromycin concentrations between 0–50 mg/L at 10 mg/L intervals. Each treatment was tested using five biological replicates.

Suspension cells were prepared by allowing them to settle in a graduated Falcon tube, and the volume ratio of settled cells to fresh medium was adjusted to 1:5 (v/v). After thorough resuspension, 250 µL aliquots were dispensed onto Whatman filter paper (70 mm diameter) placed on agar-solidified cell maintenance medium (CMM_S) supplemented with the respective antibiotic concentrations in standard Petri dishes (90 mm × 15 mm) [12]. Cultures were incubated in the dark at 25 °C, and cellular responses were monitored weekly over a two-month period, with subculturing performed at monthly intervals.

2.4. Preparation of Target Materials

Four days post-subculture, suspension cultures were harvested and filtered through a 500 µm stainless steel mesh to remove larger clumps. The filtrate was allowed to settle for 10 minutes, after which the supernatant was carefully removed to achieve a settled cell volume to liquid medium ratio of 1:5 (v/v). The cell pellet was then gently resuspended, and 250 µL portions were pipetted onto 70 mm Whatman filter paper discs placed atop cell maintenance medium (CMM_S) in 90 mm × 15 mm Petri dishes. These were incubated in the dark at 25 °C for six days prior to particle bombardment.

2.5. Bombardment Conditions

Genetic transformation was performed using a particle inflow gun (PIG), following the protocol of Finer [17]. Gold particles (1.0 µm diameter; Bio-Rad) were selected as the carrier for DNA delivery. A total of 120 mg of gold was pretreated by washing three times with ethanol and then three times with sterile distilled water. A 1.0 mL volume of sterile glycerol (50% v/v) was used to resuspend the purified particles.

For each bombardment preparation, 25 µL of the gold suspension was sonicated for 30 seconds and then combined with 2.0 µL of plasmid DNA (concentration: 1.0 µg/µL), 25 µL of 2.5 M calcium chloride ($CaCl_2$), and 5.0 µL of 0.1 M spermidine (free base). All components were maintained on ice during preparation. The mixture was gently vortexed every 20–30 seconds over a five-minute period to keep the particles in suspension, followed by a 10-minute incubation on ice to allow DNA precipitation. Subsequently, 22 µL of the supernatant was discarded, and the remaining pellet was vortexed just prior to loading. A 5.0 µL aliquot of this final suspension was used per bombardment.

The gold-DNA mixture was applied to the centre of a Swinney-type syringe filter unit, and the target embryogenic tissues were positioned 7.5 cm beneath the delivery point. A stainless-steel mesh (270 µm pore size) was placed between the filter and target to diffuse the particle stream. Particle bombardment was performed using helium pressure set at 550 kPa and a vacuum level of negative 85 kPa. Seventy-two hours after transformation, cells were examined for green fluorescent protein (GFP) expression, and a colorimetric β-glucuronidase (GUS) assay was carried out.

2.6. Effect of Osmoticum on Transformation Efficiency

To evaluate the influence of osmotic pretreatment on transformation success, suspension cultures plated on cell maintenance medium (CMM_S) and incubated in the dark for six days were transferred to an osmotic adjustment medium. The medium comprised CMM_S enriched with 0.2 M concentrations of both mannitol and sorbitol. The cultures were exposed to this medium in darkness for two hours prior to bombardment.

Following gene delivery (as outlined previously), the cells remained on the osmotic medium for an additional two hours before being returned to standard CMM_S lacking osmoticum. Transfers were carried out by relocating the filter paper discs containing the cells from one medium to another, without disturbing the cells.

2.7. Promoter Activity Assay

Transient expression in taro suspension cultures was evaluated using three promoters: Cauliflower Mosaic Virus 35S (CAMV 35S), maize polyubiquitin-1 (UBI-1), and Taro Bacilliform Virus (TaBV-600) [16]. The corresponding plasmid constructs, pGem4z-TaBV-GUS, pGem4z-Ubi-GUS, and pUC-CaMV35S-GUS (as illustrated in Figure 1), were used to coat gold particles and delivered into the cells via particle bombardment, following the procedure previously described.

A total of three independent transformation trials were performed, each comprising six replicates per promoter construct. Five of these replicates were designated for quantitative fluorometric analysis, whereas one replicate was reserved for histochemical staining to detect GUS expression.

2.8. Reporter Gene Detection and Transient Expression Analysis

Transient expression of the GFP reporter gene was evaluated three days after transformation using a Leica MZ12 stereomicroscope equipped with a GFP Plus fluorescence module (Leica Microsystems).

Expression of the *uid* A gene encoding β-glucuronidase (GUS) was assessed using both histochemical staining and fluorometric quantification, following the protocol established by Jefferson [18]. For the fluorometric assay using 4-methylumbelliferyl-β-D-glucuronide (MUG), total soluble protein (TSP) was extracted from embryogenic cell suspensions (ECS) using GUS extraction buffer. Cell lysis was achieved by probe sonication for 30 seconds at 50 Hz, followed by solubilization on a rotary shaker for 10 minutes. After centrifugation at 14,000 rpm, the lysate was clarified and the supernatant carefully harvested for analysis.

Fluorescence intensity was measured using a Perkin Elmer LS50B luminescence spectrophotometer. GUS enzymatic activity was quantified as picomoles of 4-methylumbelliferone (MU) produced per minute per milligram of protein (pmol MU/min/mg protein), with background values from non-transformed controls subtracted. Total protein content was determined using the Bradford assay, with bovine serum albumin (BSA) as the standard. A standard curve based on 4-MU was used to quantify enzyme activity.

2.9. Selection and Regeneration of Transformed Cells

Following transformation, selection of putative transgenic cells was performed on cell maintenance medium (CMM_S) supplemented with 150 mg/L kanamycin. Three days after bombardment, both treated and control embryogenic cultures were transferred to this selection medium and incubated in the dark at 25 °C. Cultures were maintained under these conditions for two months, with monthly subcultures carried out by relocating the filter paper discs onto fresh selective medium.

After the initial selection phase, the surviving cell masses were transferred to embryogenesis medium (EM) also containing 150 mg/L kanamycin and incubated in darkness for an additional two months to facilitate somatic embryo development and maturation. No subculturing was performed during this stage.

Once mature embryos had formed, they were gently removed from the filter paper and placed onto germination medium (GM), consisting of half-strength Murashige and Skoog (½ MS) salts supplemented with 0.05 mg/L benzylaminopurine (BAP) and 0.1 mg/L indole-3-acetic acid (IAA), but without kanamycin. Cultures were kept in the dark for two weeks, after which they were transferred to low light conditions to promote further development. Germinated embryos were subsequently cultured on agar-solidified ½ MS medium containing 150 mg/L kanamycin to encourage shoot and root formation and facilitate the recovery of whole transgenic plants.

2.10. Molecular Characterization: Genomic DNA Isolation and PCR Analysis

The CTAB extraction technique was employed with minor adjustments to isolate genomic DNA from suspected transformants [19]. RNase H was added at the final stage of the extraction to remove RNA contamination. DNA concentration and purity were determined using a Nano Drop spectrophotometer (Thermo Fisher Scientific, USA).

The NPTII and GFP genes were amplified in separate PCR reactions utilizing GOTAQ Green Master Mix (Promega, USA). Each 20 µL reaction contained 10 ng of genomic DNA and 10 ρmol of each gene-specific primer pair. The plasmid was used as a positive control and DNase-free water as a negative control.

The PCR cycling conditions for both genes were: initial denaturation at 94°C for 2 min; followed by 35 cycles of denaturation at 94°C for 20 s, annealing at 60°C for 20 s, and extension at 72°C for 1 min; with a final extension at 72°C for 2 min. Despite slight differences in primer melting temperatures, a common annealing temperature of 60°C was used for both reactions.

PCR products were separated on 1.5% (w/v) agarose gels containing SYBR® Green and electrophoresed at 240 V for 45 min. Visualization and imaging of the amplified DNA bands were carried out using the GelDoc system from Bio-Rad (USA). Primer sequences, melting temperatures (Tm), and orientation used for PCR amplification of GFP and NPTII genes are listed in Table 1.

Table 1: Primer details for PCR detection of *GFP* and *NPTII* genes

Target Gene	Primer Direction	Sequence (5'→3')	Tm (°C)
gfp	Forward	ACC CTC GTG ACC ACC CTG ACC TA	61
	Reverse	GAC CAT GTG ATC GCG CTT CTC GTT	59
nptII	Forward	CCT TAT CCG CAA CTT CTT TAC CTA	54
	Reverse	ACA CCC AGC CGG CCA CAG TCG	62

2.11. Statistical Analysis

Experimental data were subjected to statistical evaluation using analysis of variance (ANOVA) and Student's t-tests, as appropriate, at a 95% confidence level. When significant differences were detected ($p < 0.05$), treatment means were compared using Fisher's Least Significant Difference (LSD) test to determine pairwise significance. Statistical analyses were conducted using SPSS software (version 11, Windows platform).

3. Results & Discussion

3.1. Kill Curve Analysis

To determine suitable antibiotic concentrations for selection, the sensitivity of non-transformed taro suspension cultures to kanamycin and hygromycin was assessed (Figure 2A–F). Kanamycin at 100 mg/L was the minimum concentration that visibly suppressed cell growth, with gradual necrosis leading to complete cell death by the seventh week.

Higher kanamycin levels (150, 200, and 250 mg/L) were also lethal, but concentrations above 150 mg/L caused more rapid necrosis, with cultures completely dying by week five. This shortened exposure period may reduce the likelihood of transformed cells establishing themselves before non-transformed cells are eliminated. As a result, 150 mg/L kanamycin was identified as the most appropriate concentration that was effectively selective yet still allowing a sufficient window for transformed cell recovery.

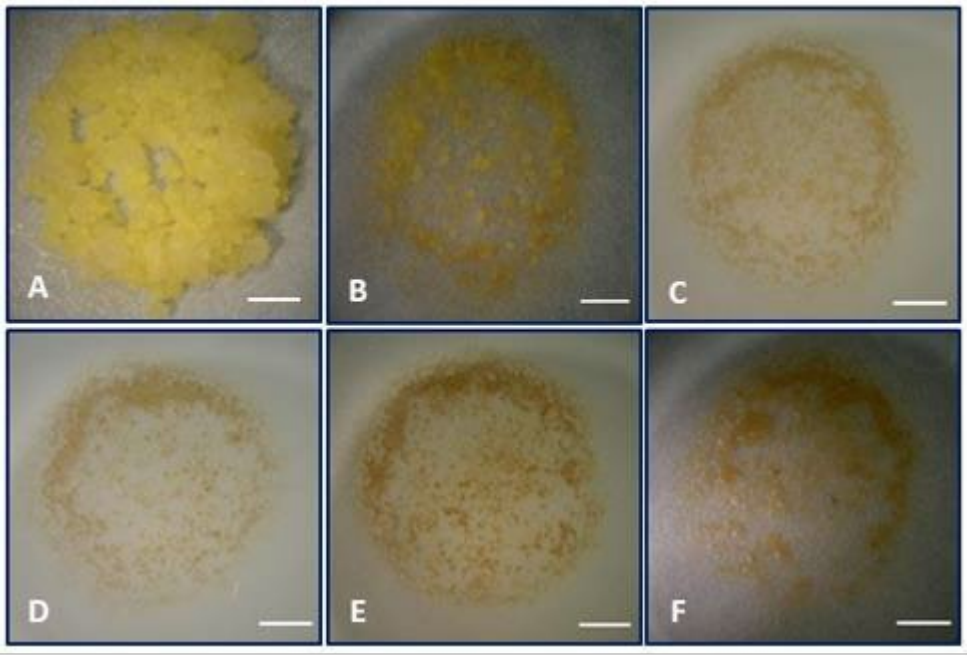


Figure 2: Regeneration response of non-transformed taro suspension cultures (cv. CPUK) under varying Kanamycin concentrations

Cells were cultured on selection medium supplemented with increasing concentrations of Kanamycin: (A) 0 mg/L, (B) 50 mg/L, (C) 100 mg/L, (D) 150 mg/L, (E) 200 mg/L, and (F) 250 mg/L. Images were captured five weeks post-plating. Scale bar = 5 mm.

In contrast, hygromycin exhibited extreme cytotoxicity across all tested concentrations (10–50 mg/L), with complete necrosis observed within two weeks in all samples. This suggests that taro suspension cells are particularly sensitive to hygromycin, rendering it unsuitable for use in this transformation system.

These findings align with earlier reports, working with callus from C. *esculenta var. antiquorum*, tolerated kanamycin up to 200 mg/L but were completely inhibited by hygromycin at just 20 mg/L. Similarly, used 50 mg/L geneticin in *C . esculenta* var. *esculenta*, but reported the presence of non-transformed "escapes," indicating suboptimal selection pressure [9,20].

The sensitivity to kanamycin observed in the current study could be attributed to the use of suspension cells, which form a thinner and more evenly distributed layer than callus tissues. This likely increased the surface area exposed to the antibiotic, improving selection efficiency. These results emphasize the importance of tissue type and morphology in defining effective selection conditions for plant transformation protocols.

3.2. Transgene Expression, Selection and Regeneration of Plants

A total of 30 independent transformation events were conducted for each taro cultivar (THA-07 and CPUK) using gold microprojectiles coated with the binary vector pART-TEST7 (Figure 1). Transient expression of the gfp reporter gene was evident 72 hours after bombardment, with each Petri dish displaying over 100 green fluorescent foci (Figure 3A, B). No fluorescence was observed in the untransformed control samples. The high frequency of GFP expression confirmed effective DNA delivery and supported the progression to selection for stable integration.

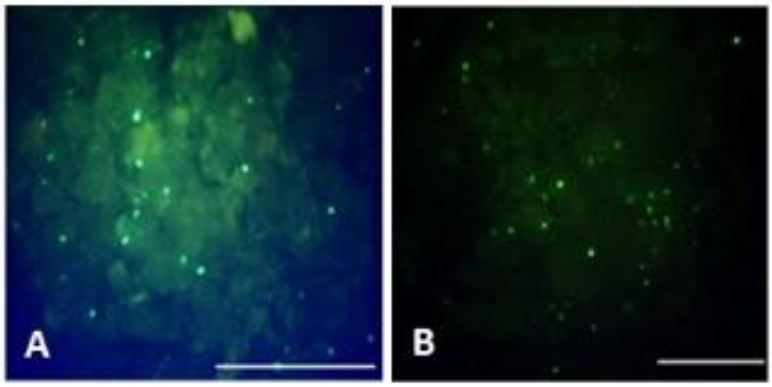


Figure 3: Visualization of green fluorescent protein (GFP) transient expression in taro embryogenic suspension cultures, 72 hours following biolistic delivery. Panels show (A) CPUK and (B) and THA-07 Cultivars. Fluorescent foci denote effective gene expression. Scale bar = 1 mm.

Three days post-bombardment, cells were transferred to selection medium containing 150 mg/L kanamycin and maintained in darkness for two months, with subcultures performed at monthly intervals. By the fourth week, non-transformed cells exhibited necrosis, whereas GFP-expressing cells remained viable, proliferated, and formed fluorescent callus, suggesting successful selection and potential stable transgene integration.

This GFP-positive callus was transferred to embryogenesis medium (EM) supplemented with 100 mg/L kanamycin to support somatic embryo development while maintaining selective pressure. Somatic embryos expressing GFP began to emerge within 3–4 weeks and continued to mature over the following two months. No somatic embryos formed from non-transformed controls, further validating the efficacy of both the transformation and selection processes. Both cultivars showed comparable embryogenic responses (Figure 4A, B; Table 2), indicating that the protocol is broadly applicable across genotypes under the tested conditions.

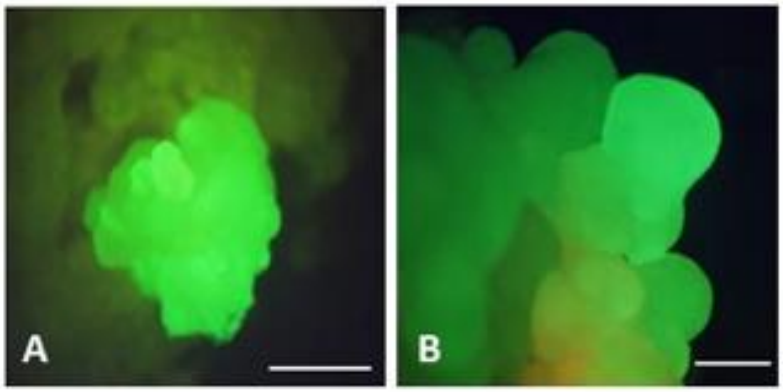


Figure 4: Selection and somatic embryo development in transgenic taro cultures following particle bombardment. Embryogenic suspension cells were cultured on CMM_S medium containing 150 mg/L Kanamycin, where fluorescing, putatively transformed cells gradually proliferated and formed small aggregates. Upon transfer to embryogenesis medium (EM), these cell clusters developed into fluorescing pro-embryogenic masses (PEMs) and somatic embryos (SEs) in both cultivars: CPUK (A) and THA-07 (B). Scale bar = 1 mm.

Table 2: Number of putative transgenic pro-embryogenic masses (PEMs) formed on embryogenesis medium. Following microprojectile-mediated transformation, taro suspension cells were maintained on selective medium for two months before being transferred to embryogenesis medium to induce PEM formation

Cultivar	Total number of GFP fluorescent PEMs/SEs	Mean number of GFP fluorescent PEMs/SEs per plate	Mean number of GFP fluorescent PEMs/SEs per mL SCV
CPUK	276	14.5 ± 1.2[a]	290
THA-07	174	10.9 ± 1.5[a]	218

Values represent means ± standard error of the mean (SEM), based on data from 16–19 bombarded plates per cultivar, each receiving 50 µL of settled cell volume (SCV). Means sharing the same letter do not differ significantly at $p < 0.05$, based on Fisher's LSD test.

Embryos were then transferred to germination medium (GM). Germination success was poor when kanamycin was included in the medium. However, when kanamycin was excluded, embryo conversion significantly improved, implying that kanamycin may negatively impact the germination phase. Similar effects have been noted in other species, including Alstroemeria and Panicum maximum [21,22]. Future research could explore alternative selection approaches, such as reduced antibiotic concentrations, different selection agents, or positive selection systems, to enhance regeneration without compromising selection efficiency.

From the THA-07 genotype, five embryos exhibiting GFP expression were recovered, but none successfully germinated. This may be due to residual stress or genotype-specific sensitivity, highlighting that high transient expression does not necessarily lead to stable plant regeneration. In contrast, several GFP-positive embryos derived from CPUK cultures germinated on ½ MS medium containing 150 mg/L kanamycin and progressed into whole plants. Ultimately, six independent transgenic lines were successfully regenerated from the CPUK background. All regenerated plants exhibited strong, uniform GFP fluorescence (Figure 5).

PCR analysis confirmed stable integration of both GFP and NPTII genes. Genomic DNA extracted from putative transformants yielded specific amplicons of approximately 500 bp for both GFP and NPTII gene, consistent with the plasmid positive controls (Figure 6). These results verify the success of stable transformation using this protocol.

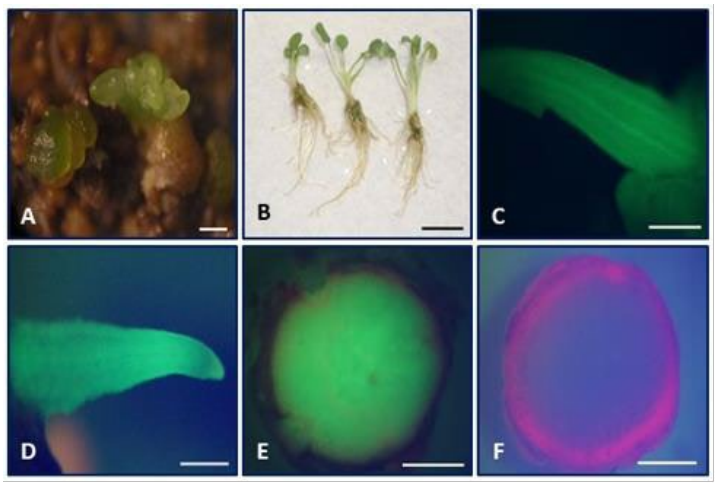


Figure 5: Selection and regeneration of transgenic taro plants (cultivar CPUK). (A) Putatively transformed somatic embryos cultured on germination medium (GM). (B) Fully regenerated transgenic taro plant. Green fluorescent protein (GFP) expression observed in various tissues of transgenic plants: (C) Petiole, (D), Root, and (E) Corm. (F) Corm section from a non-transformed control plant, showing no detectable GFP fluorescence. Scale bars: 2 mm (A), 1 cm (B), 1 mm (C–F).

The parameters for this transformation method were initially adapted from a protocol developed for banana suspension cultures, which produced high transient expression levels in taro [23]. One important modification involved increasing the pre-bombardment incubation period on CMM_S from four to six days. This likely allowed better cellular acclimation and enhanced proliferation, leading to a higher proportion of actively dividing, transformation-competent cells. Comparable improvements in transformation efficiency have been reported in Zea mays and Triticum aestivum [5,24]. Since actively dividing cells are more receptive to foreign DNA integration and better tolerate physical stress from bombardment, this optimization proved critical for enhancing transformation outcomes [25,26].

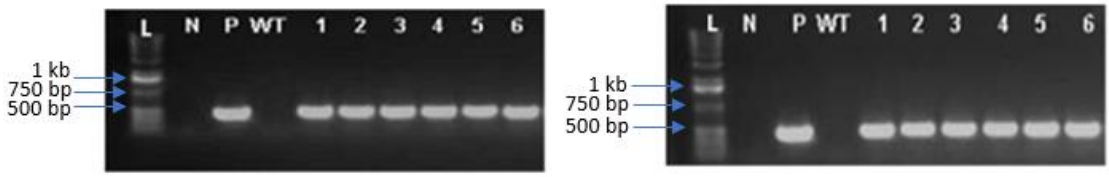


Figure 6: Molecular confirmation of transgenic taro plants (cv. CPUK) by PCR analysis. Agarose gel electrophoresis showing amplification of the GFP gene (left panel) and the nptII selectable marker gene (right panel) from genomic DNA of putative transgenic lines. Lanes: L – DNA ladder (1 KB Plus), N – negative control (no template), P – positive control (plasmid DNA), WT – wild-type (non-transformed taro), 1–6 – PCR products from independent transgenic taro plants

3.3. Effect of Osmotic Conditioning on Transgene Expression

Application of an osmotic treatment, consisting of a two-hour exposure both prior to and immediately after particle bombardment, led to a significant enhancement in transient GFP expression in embryogenic suspension cultures of both taro cultivars, CPUK and THA-07 (Table 3). On average, treated cells exhibited a 1.4-fold increase in the number of fluorescent foci compared to untreated controls, suggesting that osmotic preconditioning improved either the delivery efficiency of plasmid DNA or the expression of the introduced gene.

Table 3: Effect of osmoticum treatment on transient GFP expression in *Colocasia esculenta var. esculenta* suspension cells, assessed three days after microprojectile bombardment. Cells were incubated on osmotic medium (0.2M mannitol + 0.2M sorbitol) for two hours both before and after bombardment. Following treatment, cells were cultured on CMM_S for three days prior to transfer on to selection medium.

Cultivar	Mean total number of GFP foci per replicate	
	Osmoticum	Without Osmoticum
CPUK	423 ± 18.1^{b}	301 ± 15.2^{d}
THA-07	502 ± 18.3^{a}	361 ± 14.3^{c}

Values are presented as means ± standard error of the mean (SEM), based on nine independent bombardment plates per treatment, each containing 50 µL of settled cell volume (SCV) of embryogenic suspension cells. Means with identical letters are not significantly different at $p < 0.05$, as determined by Fisher's LSD test.

In addition to the osmotic effect, a notable genotypic difference was observed. Cultivar THA-07 consistently produced a greater number of GFP-positive foci following bombardment, regardless of osmotic treatment. This finding implies that intrinsic factors such as cellular physiology, transformation competence, or response to mechanical stress may play a role in determining transformation efficiency across different taro genotypes.

These results are in agreement with similar findings reported in a range of plant species. Enhanced transgene expression following osmotic treatment has been observed in *Picea abies, Asparagus officinalis, Elaeis guineensis, Solanum tuberosum Triticum aestivum-Zea mays, Bouteloua gracilis, Allium sativum, and Cynodon dactylon* [13] [27-33].

Osmotic conditioning is believed to temporarily reduce cellular turgor pressure, thereby minimizing physical damage during particle entry and limiting protoplasmic leakage [25,27,34,35]. These protective effects likely contribute to the improved gene expression observed in pretreated cells.

It is important to note, however, that in certain species such as banana, high levels of transgene expression have been achieved without any osmotic pre-treatment [23]. This may be due to differences in cellular structure, stress tolerance, or inherent

physical properties of the suspension cultures. These observations highlight the necessity of tailoring transformation protocols to specific species or genotypes to achieve optimal results.

3.4. Transient Promoter Activity in Taro Suspension Cells

Transient expression of the GUS reporter gene driven by different promoters was quantified in taro suspension cultures using a fluorometric assay. The results, averaged from three independent experiments each with five replicates per promoter construct, revealed significant variation in promoter strength. Among the promoters tested, the maize polyubiquitin-1 (UBI-1) promoter exhibited the highest transient GUS activity, followed by the cauliflower mosaic virus 35S (CaMV-35S) promoter, while the taro bacilliform virus (TaBV-600) promoter showed the lowest expression levels (Figure 7A).

Histochemical staining of transformed cells corroborated these quantitative findings, showing consistent expression patterns with the fluorometric data (Figure 7B). This dual confirmation reinforces the relative promoter strengths observed in taro embryogenic suspension cells.

Previous work by reported comparatively higher activity of the TaBV-600 promoter in transient assays using detached taro leaves, implying possible tissue-specific differences in promoter efficacy [16]. Our findings suggest that in undifferentiated suspension cells, TaBV-600 drives weaker expression, whereas the UBI-1 and CaMV-35S promoters are more robust. These observations highlight the importance of selecting promoters suited to the target tissue, particularly in early-stage transformation experiments where promoter strength can impact transformation success. Future studies involving stable transgenic lines will be important to assess the persistence and tissue specificity of these promoters in *Colocasia esculenta*.

Utilizing embryogenic suspension cultures in our transformation system appears to enhance transformation efficiency compared to previous reports. For instance, we successfully generated six independent transgenic lines from the CPUK cultivar after 30 bombardment events, whereas obtained only a single stable transformant using callus tissue [8]. This improvement underscores the advantage of using embryogenic suspensions, likely due to their high regenerative capacity and more uniform cell population.

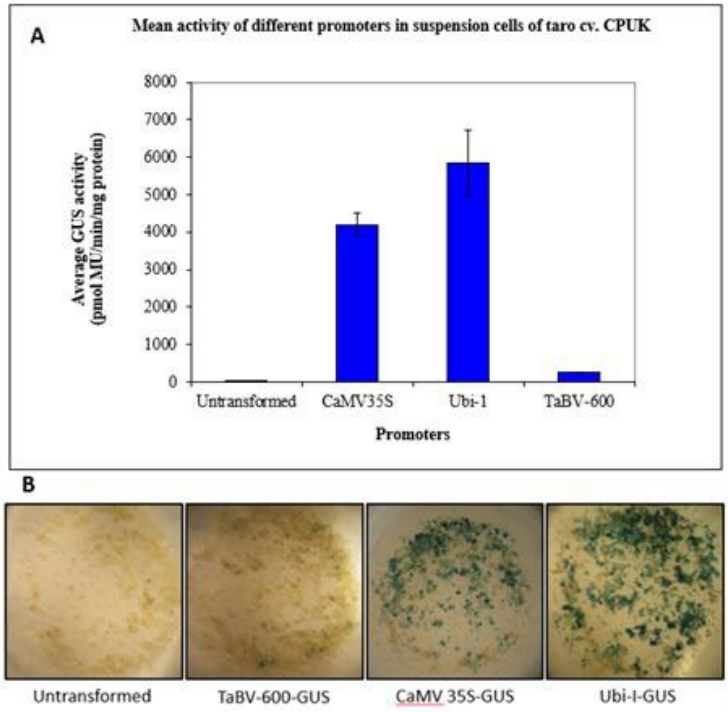


Figure 7: Transient promoter activities in taro embryogenic suspension cells measured using the *uid A* (GUS) reporter gene. Suspension cultures were bombarded with plasmids carrying different promoter-GUS constructs, and GUS activity was assessed fluorometrically 72 hours post-bombardment. Data represent means ± standard error of the mean (SEM) from three independent experiments, each with five replicates per promoter. GUS activity is expressed as picomoles of 4-methylumbelliferone (MU) produced per minute per milligram of total soluble protein.

(A) Quantitative fluorometric assay comparing GUS expression driven by the maize polyubiquitin-1 (*ubI*-1), cauliflower mosaic virus 35S (CaMV-35S), and taro bacilliform virus (TaBV-600) promoters. Untransformed cells served as negative controls to account for background activity.

(B) Representative histochemical staining images of cells transformed with each promoter construct, illustrating spatial patterns of GUS expression.

However, the use of kanamycin as a selective agent during regeneration may have contributed to slower recovery of transformed plants. He employed geneticin for selection, but their study noted occurrences of escapes, indicating potential limitations of this agent's stringency [8]. A thorough comparative analysis of selective agents and regeneration protocols could further optimize transformation outcomes.

These findings contribute to the development of an efficient biolistic transformation method for taro. It could be emphasized that optimization of tissue type, selection conditions, and promoter choice are essential for successful genetic engineering of this important but understudied crop.

Acknowledgments

The authors gratefully acknowledge financial support from New Zealand's International Aid and Development Agency, the University Research Committee of The University of the South Pacific, and the Faculty of Science, Technology and Environment at The University of the South Pacific. Technical assistance was kindly provided by the Centre for Tropical Crops and Bio commodities at Queensland University of Technology, the Secretariat of the Pacific Community, and The University of the South Pacific. PCD was a PhD candidate at The University of the South Pacific during this project.

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