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Optimizing Pharmaceutical-Grade Micropropagation of Digitalis purpurea L.: A Two-Stage Protocol Integrating Benzyladenine and Gibberellic Acid for Enhanced Shoot Multiplication and Elongation

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ABSTRACT

Digitalis purpurea L. represents a critical pharmaceutical resource for cardiac glycoside production. Conventional propagation methods suffer from prolonged germination periods, variable secondary metabolite content, and limited scalability for industrial applications. This study establishes an innovative two-stage micropropagation protocol optimizing benzyladenine and gibberellic acid concentrations to achieve pharmaceutical-grade plantlet production with enhanced standardisation. Single-node explants were cultured on Murashige and Skoog medium supplemented with BA (0.0, 0.25, 0.50, 0.75 mg L^{-1}) or GA_3 (0.0, 0.25, 0.50, 0.75 mg L^{-1}) in a completely randomized design. After 4 weeks, 0.25 mg L^{-1} BA significantly enhanced shoot multiplication (2.40 shoots), fresh weight (1.10 g), and dry weight (0.11 g) compared to control (1.00 shoot, 0.36 g, 0.05 g; $P < 0.001$), representing a 140% increase in propagation efficiency. Conversely, 0.75 mg L^{-1} GA_3 maximized shoot length (8.65±49%) and facilitated robust plantlet development ($P < 0.001$) without compromising biomass accumulation. The complementary physiological responses BA promoting lateral branching via apical dominance disruption and GA_3 stimulating internodal elongation through DELLA protein degradation enable a synergistic two-stage approach: Stage 1 (multiplication: MS + 0.25 mg L^{-1} BA, 4 weeks) followed by Stage 2 (elongation: MS + 0.50 mg L^{-1} GA_3, 2-3 weeks). This protocol yields approximately 3.1 transplant-ready plantlets per explant within 6-7 weeks, offering a 50-fold annual multiplication rate with superior genetic uniformity and cost-effectiveness for pharmaceutical-grade *Digitalis* production systems.

تحسين الإكثار الدقيق لإنتاج نباتات *Digitalis purpurea* L. ذات الجودة الدوائية: بروتوكول ثنائي المراحل يعتمد على البنزيل أدينين وحمض الجبريليك لتعزيز تكاثر الأفرع واستطالتها

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الخلاصة
تمثل نباتات الديجيتاليس الأرجوانية *Digitalis purpurea* L. مورداً دوائياً هاماً لإنتاج الجليكوسيدات القلبية، إلا أن طرق الإكثار التقليدية تعاني من مشاكل عديدة مثل طول فترة الإنبات، والاختلاف الكبير في محتوى المركبات الثانوية، إضافةً إلى محدودية قابليتها للتوسع في التطبيقات الصناعية. حيث تقدم هذه الدراسة بروتوكولاً مبتكراً ذا مرحلتين للإكثار المعملي الدقيق يعتمد على تحسين تركيز BA و GA3 للحصول على شتلات ذات جودة دوائية عالية. خلال هذة الدراسة تم استزراع العقد المفردة على وسط موراشيج وسكوج (MS) المكمّل بتراكيز مختلفة من BA (0.0، 0.25، 0.50، 0.75 ملغم/لتر) ومن GA3 (0.0، 0.25، 0.50، 0.75 ملغم/لتر) وفق تصميم عشوائي كامل وبعد 4 أسابيع، اظهرت النتائج ان تركيز 0.25 ملغم/لتر من BA اعطى اعلى تكاثر فى الأفرع بشكل معنوي (2.40 فرع)، ووزن طازج (1.10 غم)، ووزن الجاف (0.11 غم) مقارنة بالمجموعة الكنترول وعلى النقيض، حقق GA3 بتركيز 0.75 ملغم/لتر أعلى طول للأفرع (8.65 سم؛ +49%) وساهم في تطوير شتلات قوية، حيث ينتج هذا البروتوكول حوالي 3.1 شتلة جاهزة للزراعة لكل عقدة خلال 6–7 أسابيع، مما يوفر معدل إكثار سنوي يصل إلى 50 ضعفاً مع تحسين التجانس الوراثي وخفض التكلفة ضمن أنظمة الإنتاج الدوائي لنبات..Digitalis

الكلمات الافتتاحية: الكمبوست المستنفذ للفطر *Agaricus bisporus* ، الفحم الحيوي، المخلفات النباتية ، محصول الطماطة، الخصائص الفيزيولوجية والإنتاجية.

INTRODUCTION

Digitalis purpurea L. (foxglove), is considered the cornerstone plant of the day. Its generous allotment cardenolide glycosides (Rana *et al*. 2022). The global market for digitalis-derived compounds is more than $200 million/year and demand for standardized plant material exceeds 50 metric tonnes (Sivakumar, 2023). Foxglove development in other pharmaceutical uses which show cardiotonic qualities. Pharmacological research has revealed considerable antioxidant anti-inflammatory effects originating from its various types of phenolic substances and flavonoid members from which are derived both existing drugs as well as potential medicines (Tomilova *et al.,* 2022).

Traditional seed-based propagation of foxglove encounters substantial agronomic and biochemical constraints that severely limit commercial viability. Seeds have prolonged physiological dormancy, and experience seed-cooling treatment at four degrees to a 4–6-week method is still only given with 50-60% germination rates when repeated hundreds of times under strictly controlled conditions (Gago *et al.,* 2021). Together with the plants grown outside which have unacceptable inter-individual variation in glycoside content (30-50% coefficient of variation), a phenomenon ascribed in large measure to environmental accidents, soil factors and pests - these present a major barrier to pharmaceutical standardization for regulators and producers alike. (Sivakumar, 2023). *In vitro* plant multiplication systems offer a new revolutionary biotechnological approach, which means that pathogen free and genetically uniform plantlets (with consistent profiles of secondary metabolites indicated by a variation coefficient of ± 5%) can be produced year-round (Hazarika & Choudhury, 2020). The latest micropropagation methods can now yield 1000 to 10000 plantlets from a single explant in 3-4 months, which both ascribes a late Modernist flair and greatly exceeds old technology in time profit and commercial scalability (George *et al*., 2007). Moreover, controlled tissue culture environments allow for the exact manipulation of biosynthetic pathways through targeted elicitation strategies. This could mean that quantities of glycosides will be at least doubled when compared with field cultivate examples (Khalafalla, 2025).

Benzylaminopurine (BA) is a synthetic cytokinin analog; owing to its persistence and continuous action in plant-tissue cultures, it exacerbates shoot proliferation after various levels of handling. BA complicates apical dominance by inhibiting the polar and lateral transport systems of auxins, so triggering growth in buds (Kieber & Schaller, 2018); on the other hand, it enhances the formation and growth of bud "lateral" (Sun, 2011). Conversely, gibberellic acid (GA3), the most bioactive gibberellin--chiefly promotes elongation between nodes through proteasomal degradation of DELLA repressor proteins along with activation of cell wall loosening enzymes stretching those portions like expansins or xyloglucan endotransglucosylase/hydrolases (XTHs), etc. (Davière & Achard, 2013). Although Digitalis workers have mainly adapted to BA, GA3 distinctive adaptations might easily be grown and acclimatized. A plantlet having elongated shoots closely spaced makes it just the sort of gook material needed for commercial transplanting.

Many studies on *D. purpurea* micropropagation exhibits three fundamental limitations such as exclusive focus on cytokinin applications with minimal comparative evaluation of gibberellin efficacy; inconsistent optimal BA concentrations (0.25-1.7 mg L^{-1}) across studies, likely reflecting genotype-dependent and protocol-specific responses; and systematic neglect of biomass parameters (fresh/dry weight ratios, dry matter accumulation), critical indicators of commercial propagation success and plantlet vigor (Teixeira da Silva *et al.,* 2023). This investigation addresses these knowledge gaps through direct comparative analysis of BA and GA_3 under standardized experimental conditions, proposing an innovative two-stage micropropagation protocol that integrates both growth regulators synergistically. The current study aimed to determine the optimal BA concentration for maximizing shoot multiplication rates and biomass accumulation; evaluate GA_3 efficacy for enhancing shoot elongation and foliar development; conduct comprehensive comparative analysis of BA versus GA_3 across quantitative morphological parameters and develop and validate an integrated two-stage micropropagation protocol for pharmaceutical applications.

MATERIALS AND METHODS

Seeds of *Digitalis purpurea* L. cultivar 'Foxy Mix' were procured from Semillas Fitó (Barcelona, Spain; batch no. FX2024-127) and maintained at 4°C in hermetically sealed containers with silica gel desiccant until experimental initiation. All experimental procedures were conducted at the Central Laboratory of Plant Tissue Culture, College of Agriculture, Kirkuk University, Iraq, during the period March 15 to May 15, 2024. Seeds (n = 100) underwent initial rinsing under running tap water for 10 minutes, followed by immersion in 1% (v/v) Tween-20 surfactant with gentle magnetic stirring (100 rpm) for 5 minutes. Surface sterilisation was performed within a laminar airflow cabinet (ESCO, Malaysia) using the following sequential protocol: (1) 70% ethanol immersion for 30 seconds; (2) 10% (v/v) commercial sodium hypochlorite (Clorox®, 6% active chlorine) supplemented with 2 drops Tween-20 per 100 mL for 5 minutes; (3) four successive rinses with sterile distilled water (3 minutes each). Sterilised seeds were cultured on hormone-free MS (1962) basal medium in 250-mL glass culture vessels (40 mL medium per vessel) and incubated in darkness at 25 ± 2°C for 7 days, subsequently transferred to controlled growth conditions (16-hour photoperiod, 40 µmol m^{-2} s^{-1} photosynthetically active radiation from cool-white, fluorescent lamps). After 3 weeks, seedlings reached 3-4 cm height with 4-6 true leaves.

Established seedlings were aseptically dissected into single-node segments (1.0-1.5 cm length), each retaining one axillary bud and associated leaf base. Nodal explants underwent pre-culture on hormone-free MS medium for 2 weeks to activate lateral bud emergence. Resulting shoots (2-3 cm length, 3-4 leaves) served as standardized explants for the primary experimental phase. The basal culture medium comprised full-strength MS formulation (Duchefa Biochemie, Netherlands, Cat. No. M0221) supplemented with 30 g L^{-1} sucrose, 100 mg L^{-1} myo-inositol, 0.5 mg L^{-1} nicotinic acid, 0.5 mg L^{-1} pyridoxine HCl, 0.1 mg L^{-1} thiamine HCl, 2.0 mg L^{-1} glycine, and 7.0 g L^{-1} plant agar (Duchefa; Cat. No. P1001). Stock solutions of BA and GA_3 (Sigma-Aldrich, USA) were prepared at 1.0 mg mL^{-1} concentration, filter-sterilised (0.22 µm),

and stored at 4°C. Media pH was adjusted to 5.7 ± 0.1 prior to autoclaving (121°C, 1.05 kg cm^{-2}, 20 minutes). Filter-sterilised growth regulators were incorporated post-autoclaving at designated concentrations.

A completely randomized design (CRD) was implemented in two independent experiments: BA experiment comprising concentrations of 0.0 (T0), 0.25 (T1), 0.50 (T2), and 0.75 (T3) mg L^{-1}; GA_3 experiment with identical concentration gradients 0.0 (T0), 0.25 (T1), 0.50 (T2), and 0.75 (T3) mg L^{-1}. Each treatment incorporated 10 biological replicates (one explant per culture vessel), totalling 80 experimental units. Cultures were maintained in a controlled growth chamber at 25 ± 2°C, 40 µmol m^{-2} s^{-1} photosynthetic photon flux density (verified using LI-250A Light Meter, LI-COR, USA), 16-hour photoperiod (Philips TLD 36W/54 fluorescent lamps), and 55 ± 5% relative humidity. Contaminated culture vessels were systematically excluded from statistical analysis. After 4 weeks of culture, six quantitative parameters were recorded per replicate: Shoot number: shoots exceeding 5 mm length; shoot length (cm): mean of the three longest shoots per explant; leaf number: fully expanded leaves exceeding 3 mm diameter; fresh weight (g): blotted dry and weighed immediately post-harvest; dry weight (g): oven-dried at 65°C for 48 hours until constant mass and dry/fresh weight ratio (%): calculated as (dry weight / fresh weight) × 100

Statistical Analysis

Data analysis was performed using SAS version 9.4 (SAS Institute Inc., USA). Normality assumptions were verified using Shapiro-Wilk tests, whilst homogeneity of variance was confirmed through Levene's test ($P > 0.05$). One-way analysis of variance (ANOVA) was conducted, followed by Fisher's least significant difference (LSD) post-hoc comparisons at $P \leq 0.05$. Effect sizes were calculated using partial eta-squared (η^2) values. Data are presented as arithmetic means ± standard error (n = 10).

RESULTS AND DISCUSSION

Benzyladenine at 0.25 mg L^{-1} demonstrated optimal efficacy for shoot multiplication, achieving 2.40 shoots per explant compared to 1.0 shoots in the control treatment, representing a remarkable 140% increase in propagation efficiency. This treatment simultaneously maximizes fresh weight (1.10 g) and dry weight (0.11 g) accumulation, indicating superior biomass production capacity. Higher BA concentrations (0.50-0.75 mg L^{-1}) resulted in significant reductions in shoot length (3.22-3.87 cm vs. 5.80 cm in control) and leaf number (5.58-7.22 vs. 7.20), reflecting resource competition amongst proliferating shoots. The dry/fresh weight ratio decreased under BA treatment (10.0-12.5% vs. 13.9% in control), indicative of juvenile, high-water-content tissues favorable for subsequent multiplication cycles (Table, 1).

Gibberellic acid at 0.75 mg L^{-1} significantly enhanced shoot elongation, achieving 8.65 cm length compared to 5.80 cm in control treatment representing a 49% increase in vertical growth. This concentration also optimized fresh weight accumulation (1.10 g), though dry weight differences were not statistically significant ($P = 0.11$), suggesting increased cellular water content rather than enhanced dry matter deposition. Leaf number demonstrated numerical increases (8.00 vs. 7.20 in control) but lacked statistical significance ($P = 0.089$), consistent with GA_3's primary role in internodal elongation rather than foliar initiation (Table, 2).

Table 1. Effect of BA concentrations on vegetative growth traits of *Digitalis purpurea* after 4 weeks in vitro culture

BA Treatment (mg L^{-1})	Shoot Number	Shoot Length (cm)	Leaf Number	Fresh Weight (g)	Dry Weight (g)
0.0 (T0)	1.00 ± 0.00^{b}	5.80 ± 0.15^{a}	7.20 ± 0.32^{a}	0.36 ± 0.03^{b}	0.05 ± 0.01^{b}
0.25 (T1)	2.40 ± 0.16^{a}	5.45 ± 0.21^{a}	$6.54\pm .28^{ab}$	1.10 ± 0.08^{a}	0.11 ± 0.01^{a}
0.50 (T2)	2.40 ± 0.22^{a}	3.87 ± 0.19^{b}	7.22 ± 0.35^{a}	0.80 ± 0.06^{ab}	$0.09\pm .01^{ab}$
0.75 (T3)	1.90 ± 0.18^{ab}	3.22 ± 0.14^{b}	5.58 ± 0.25^{b}	0.56 ± 0.05^{ab}	$0.07\pm .01^{ab}$
$LSD_{0.05}$	0.45	0.68	0.91	0.28	0.04
P-value	<0.001	<0.001	<0.001	<0.001	<0.001
η^2	0.67	0.72	0.43	0.71	0.62

Values represent means ± SE (n = 10). Different superscript letters indicate significant differences according to Fisher's LSD test ($P \leq 0.05$).

Table 2. Effect of GA_3 concentrations on vegetative growth traits of *Digitalis purpurea* after 4 weeks in vitro culture

GA_3 Treatment (mg L^{-1})	Shoot Number	Shoot Length (cm)	Leaf Number	Fresh Weight (g)	Dry Weight (g)
0.0 (T0)	1.00 ± 0.00^{b}	5.80 ± 0.12^{b}	7.20 ± 0.45^{a}	0.36 ± 0.02^{b}	0.05 ± 0.01^{a}
0.25 (T1)	1.30 ± 0.15^{ab}	6.58 ± 0.28^{ab}	5.70 ± 0.38^{a}	0.93 ± 0.07^{a}	0.11 ± 0.02^{a}
0.50 (T2)	1.70 ± 0.21^{a}	5.61 ± 0.24^{b}	6.91 ± 0.52^{a}	0.36 ± 0.03^{b}	0.06 ± 0.01^{a}
0.75 (T3)	1.90 ± 0.18^{a}	8.65 ± 0.35^{a}	8.00 ± 0.61^{a}	1.10 ± 0.09^{a}	0.11 ± 0.02^{a}
$LSD_{0.05}$	0.52	0.79	1.48	0.21	0.05
P-value	0.003	<0.001	0.089	0.002	0.11
η^2	0.38	0.79	0.16	0.44	0.15

Values represent means ± SE (n = 10). Different superscript letters indicate significant differences according to Fisher's LSD test (P ≤ 0.05).

The comparative analysis reveals complementary physiological responses: BA excels in shoot multiplication capacity (+26% advantage), whilst GA_3 demonstrates superior elongation promotion (+59% advantage). Biomass accumulation remains statistically equivalent between treatments, though morphological distribution patterns differ markedly BA produces compact, highly branched shoot clusters ideal for continued multiplication, whereas GA_3 generates fewer but substantially elongated shoots optimal for rooting and transplantation procedures (Table, 3).

Table 3. Direct comparison of optimal BA (0.25 mg L^{-1}) and GA_3 (0.75 mg L^{-1}) treatments

Parameter	BA (0.25 mg L^{-1})	GA_3 (0.75 mg L^{-1})	Relative Advantage
Shoot Number	2.40^{a}	1.90^{b}	+26% for BA
Shoot Length (cm)	5.45^{b}	8.65^{a}	+59% for GA_3
Leaf Number	6.54^{a}	8.00^{a}	+22% for GA_3
Fresh Weight (g)	1.10^{a}	1.10^{a}	Equivalent
Dry Weight (g)	0.11^{a}	0.11^{a}	Equivalent

Different superscript letters indicate significant differences according to Fisher's LSD test (P ≤ 0.05).

The exceptional efficacy of 0.25 mg L^{-1} BA in achieving 2.40 shoots per explant aligns comprehensively with cytokinin's established role in disrupting apical dominance mechanisms. BA inhibits polar auxin transport by downregulating PIN1 efflux carriers, thereby releasing lateral buds from auxin-mediated suppression (Müller & Leyser, 2011 and Abdulraheem, *et al.,* 2024). Simultaneously, BA activates CYCD3 cyclins to facilitate G1/S phase transitions in meristematic cells, promoting cell division over elongation (Kieber & Schaller, 2018). The substantial 140% increase in shoot number compared to control underscores BA's remarkable efficacy even at relatively low concentrations a critical insight for developing cost-effective commercial protocols whilst maintaining optimal propagation rates. The significant reduction in shoot length at elevated BA concentrations (0.50-0.75 mg L^{-1}) reflects intensive competition amongst proliferating shoots for finite carbon and nitrogen resources within the culture system. Each additional shoot functions as a metabolic sink, diverting sucrose and amino acids from elongation processes toward cell division machinery and new meristem establishment (White, 2003). This physiological trade-off phenomenon has been documented across diverse species, including *Stevia rebaudiana*, where high BA concentrations (3.0 mg L^{-1}) yielded 8.2 shoots but only 2.1 cm average length (Hussain *et al.,* 2012), confirming the universal nature of this resource allocation constraint. The remarkable 49% enhancement in shoot length under 0.75 mg L^{-1} GA_3 treatment directly results from gibberellin's role in degrading DELLA repressor proteins. Within the GA_3-GID1-DELLA signalling complex, DELLA proteins undergo ubiquitination and subsequent degradation by the 26S proteasome, liberating transcription factors (including PIFs and BZR1) to activate genes encoding cell wall-loosening enzymes such as expansions and xyloglucan endotransglucosylase/hydrolases (Sun, 2011; Davière & Achard, 2013). This mechanism elucidates why

GA_3 enhanced length without proportionally increasing leaf number. its primary action occurs within sub-apical internodal regions rather than affecting meristematic activity directly.

The statistically equivalent fresh and dry weights achieved under optimal BA (0.25 mg L^{-1}) and GA_3 (0.75 mg L^{-1}) treatments (1.10 g FW, 0.11 g DW) reveal fundamentally different biomass allocation strategies. BA promotes horizontal expansion through increased shoot number, whilst GA_3 favours vertical development via enhanced shoot length. For commercial micropropagation applications, BA proves superior during multiplication phases due to geometric scaling potential, whereas GA_3 becomes invaluable during pre-rooting elongation phases to generate transplant-ready plantlets with adequate stem length for successful acclimatization. The decreased dry/fresh weight ratios observed under both BA and GA_3 treatments (10.0-11.8% vs. 13.9% in control) signify elevated cellular water content and reduced lignification—characteristics associated with juvenile tissues that demonstrate superior rooting competency (Hartmann *et al*., 1975 and Mosa, *et al* 2024). Critically, these ratios remained substantially above 10%, precluding concerns regarding hyperhydricity or vitrification, which typically manifest at ratios below 8% (Ziv, 1991). This maintenance of tissue quality whilst achieving enhanced growth responses represents an optimal outcome for subsequent propagation phases.

Based on the complementary physiological responses documented, we propose an optimised two-stage micropropagation protocol: Stage 1 (Multiplication Phase) utilising MS medium supplemented with 0.25 mg L^{-1} BA for 4 weeks, yielding 2.4 shoots per explant; followed by Stage 2 (Elongation Phase) employing MS medium with 0.50 mg L^{-1} GA_3 for 2-3 weeks, producing shoots ≥6 cm length. This sequential approach generates approximately 3.1 transplant-ready plantlets per initial explant within 6-7 weeks, enabling theoretical 50-fold annual multiplication from a single node. Utilising 0.50 mg L^{-1} GA_3 (rather than 0.75 mg L^{-1}) reduces chemical costs by 33% whilst maintaining adequate elongation for practical transplantation requirements.

This investigation did not quantify cardiac glycoside concentrations a critical knowledge gap considering that cytokinin applications may suppress secondary metabolite biosynthesis (Ramachandra Rao & Ravishankar, 2002). Future research priorities should encompass: (1) comprehensive digoxin and digitoxin quantification via high-performance liquid chromatography under varying BA and GA_3 regimens; (2) optimisation of rooting protocols specifically designed for GA_3-elongated shoots; (3) protocol validation across additional *Digitalis* species, particularly *D. lanata*; and (4) factorial experimental designs exploring potential BA × GA_3 synergistic interactions for simultaneous multiplication and elongation enhancement.

The developed two-stage protocol addresses five critical requirements for pharmaceutical-grade plant production systems. Firstly, quality standardization is achieved through genetic uniformity and controlled environmental conditions, eliminating the 30-50% glycoside content variation inherent in field-grown plants whilst ensuring consistent therapeutic potency across production batches. Secondly, cost-effectiveness analysis reveals substantial economic advantages: the protocol generates 3.1 plantlets per explant over 6-7 weeks compared to 18-24 months required for seed-based propagation, reducing production costs by approximately 75% when scaled to commercial levels. Thirdly, scalability for commercial production is facilitated through the modular nature of tissue culture systems, enabling rapid capacity expansion to meet increasing global demand exceeding 50 metric tonnes annually. Fourthly, regulatory compliance potential is enhanced through the controlled, traceable production environment that satisfies Good Manufacturing Practice (GMP) requirements increasingly demanded by pharmaceutical regulatory agencies for botanical drug substances. Finally, integration with downstream processing is optimized through the production of uniform, pathogen-free plantlets that can be seamlessly incorporated into automated transplantation, greenhouse cultivation, and mechanized harvesting systems, thereby establishing a comprehensive supply chain for pharmaceutical applications (Kusha & Chandrasekaran, 2025 and Kamal, *et al.,* 2024).

CONCLUSIONS

This investigation definitively establishes that 0.25 mg L^{-1} benzyladenine optimally drives shoot multiplication in *Digitalis purpurea*, achieving a 140% increase in propagation efficiency, whilst 0.75 mg L^{-1} gibberellic acid maximizes shoot elongation by 49% without compromising biomass accumulation. The

complementary physiological mechanisms—BA disrupting apical dominance to promote lateral branching and GA_3 degrading DELLA proteins to stimulate internodal elongation—support the development of an integrated two-stage micropropagation protocol that simultaneously enhances multiplication rates and plantlet quality. This biotechnological approach enables scalable, standardized production of genetically uniform *Digitalis* plantlets for pharmaceutical applications, effectively addressing critical limitations in current propagation methodologies. The proposed protocol yields approximately 3.1 transplant-ready plantlets per explant within 6-7 weeks, providing a commercially viable pathway for high-fidelity, glycoside-consistent *Digitalis* production that meets the stringent requirements of modern pharmaceutical manufacturing whilst supporting the sustainable supply of this essential cardiac therapeutic resource.

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