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Simulated microgravity enhances cell growth, callus formation, antioxidant responses, and lignan biosynthesis in Phyllanthus amarus

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Abstract

Gravity is a fundamental environmental signal that regulates plant growth, development, and metabolism. In medicinal plants, altered gravity conditions may serve as a useful abiotic cue for modulating secondary metabolite biosynthesis. In this study, we investigated the effects of simulated microgravity generated by a 2D clinostat on growth, cellular morphology, antioxidant enzyme activity, regeneration capacity, and lignan accumulation in single cells of *Phyllanthus amarus*, an important medicinal species rich in hepatoprotective lignans. Cell suspension cultures were established from callus tissues and early logarithmic-phase single cells (20-day-old) were selected for treatment. Based on screening experiments, 5 rpm for 10 days produced the best response, resulting in the highest cell growth while maintaining relatively stable morphology. Simulated microgravity altered intracellular organization, including a more uniform distribution of statoliths, and significantly increased antioxidant enzyme activities, including superoxide dismutase, catalase, and ascorbate peroxidase. Under clinostat treatment, phyllanthin, which was undetectable under normal gravity, accumulated to 3.5 μg/g, whereas hypophyllanthin content increased approximately 3.1-fold relative to the control. After transfer back to normal gravity, previously treated cells showed markedly improved regeneration, with callus formation increasing from 38% in control to 90% in the simulated microgravity group. These findings indicate that simulated microgravity may be an effective physical elicitor for improving both regenerative competence and lignan biosynthesis in *P. amarus* cell cultures, with potential applications in medicinal plant biotechnology.

Key Message

A 2D clinostat treatment at 5 rpm for 10 days promoted growth, antioxidant defense, regeneration, and lignan accumulation in *Phyllanthus amarus* single cells.

Introduction

Gravity has continuously shaped the evolution, growth, and metabolism of terrestrial organisms and remains one of the most stable environmental cues perceived by plants (Hoson and Wakabayashi 2015). In plant cells, gravity sensing is closely linked to the sedimentation of statoliths (Chen et al. 2023), which initiates downstream signaling events affecting cell organization, growth orientation, and development (Bizet et al. 2018). When the gravity vector is continuously reoriented or effectively averaged, as under microgravity or simulated microgravity, plant cells experience altered physical signaling that may influence morphogenesis, oxidative balance, and metabolism (Hughes and Kiss 2022).

The space environment imposes multiple adverse conditions, particularly microgravity (MG) and radiation, both of which are known to trigger oxidative cellular damage, DNA lesions, protein oxidation, membrane instability, and oxidative stress, thereby increasing the risk of genotoxicity (Shelhamer et al. 2020). Bioactive compounds derived from medicinal plants have been reported to mitigate oxidative damage and may therefore help reduce the risk of cellular disorders in both plants and astronauts

exposed to space-related stressors (Halimeh 2022). Therefore, ground-based studies on medicinal plants have become increasingly important for supporting future space missions and advancing astrobiological research. However, real spaceflight experiments are limited by high cost and technical complexity, so ground-based systems such as clinostats are widely used to simulate microgravity (Herranz et al. 2013; Ferranti et al. 2021).

Clinostats continuously rotate biological samples, changing the direction of the gravity vector over time (Hasenstein and van Loon 2015). They are practical tools for long-term simulated microgravity studies and allow flexible control of rotation speed, usually from 1 to 100 rpm (Kiss et al. 2019). In cell suspension cultures, higher speeds, often 20–60 rpm, are commonly used to create microgravity-like conditions, although the optimal speed and duration depend on the biological system (Halimeh 2022).

Previous studies show that simulated microgravity affects plant tissue culture responses in a species and tissue specific manner. In strawberry, 2D clinostat treatment enhanced leaf morphogenesis, shoot regeneration, antioxidant enzyme activity, and hormone regulation (Bien et al. 2023). In Begonia, simulated microgravity influenced somatic embryogenesis, hormone balance, and energy metabolism (Khai et al. 2021). These findings suggest that clinostat treatment may regulate development, stress responses, and metabolism depending on genotype and tissue type.

Recently, simulated microgravity has gained attention for its potential to enhance secondary metabolite production in medicinal plants. Compounds such as alkaloids, flavonoids, and lignans are important for stress responses and therapeutic applications. In *Phyllanthus amarus*, the hepatoprotective lignans phyllanthin and hypophyllanthin can protect liver cells from toxin-induced damage (Bhattacharjee and Sil 2007). However, limited studies have examined how simulated microgravity affects isolated medicinal plant cells, especially in relation to oxidative stress, regeneration, and specialized metabolite accumulation.

Therefore, *P. amarus* is a suitable model for investigating the effects of simulated microgravity on medicinal plant cell cultures. This study aims to evaluate how 2D clinostat-induced simulated microgravity affects cell growth, cellular morphology, antioxidant enzyme activity, regeneration capacity, and lignan biosynthesis in *P. amarus* cell cultures. The findings may clarify how altered gravity regulates regeneration and improves bioactive compound production in medicinal plants.

Materials and methods

Plant material

40 days old *P. amarus* callus derived from the second longitudinal thin cell layer (ITCL) internode (Mai et al. 2024) was subcultured on MS medium without plant growth regulators for 7 days before being used to establish cell suspension cultures.

Establishment of cell suspension cultures

Callus was transferred from solid medium to 50 mL liquid MS medium and cultured on a 250 mL shaker to establish a uniform cell suspension. Explants were collected at regular intervals, transferred into microtubes, and loaded onto a hemocytometer for cell counting under a microscope. The data obtained were used to construct the plant cell growth curve and identify the main growth phases during the culture period (Fig. 1). The growth curve of the cultured cells was monitored from day 5 to day 40.

Single-cell isolation and simulated microgravity treatment

To obtain predominantly single cells, 20 days old cultures were sequentially filtered to remove large cell aggregates (Fig. 2). Cell suspensions were sequentially filtered through PTFE membranes with pore sizes of 100 µm, 70 µm, and 40 µm using a syringe filtration system. After each filtration step, the filtrate was collected in a sterile beaker. The final filtrate, containing cells of the desired size range, was transferred to a sterile culture flask for subsequent experiments.

Single cells were cultured in sterile 60 mm Petri dishes at a defined density and allowed to be attached briefly. To simulate microgravity, dishes were mounted on a clinostat and rotated continuously, maintaining cells in a free-floating configuration with minimal sedimentation. Under these conditions, cells are organized into a ring structure with an effective diameter of approximately 2–3 cm (Fig. 3). Following the culture period, cells were collected for subsequent analyses.

Simulated microgravity was generated using a 2D clinostat (Advanced Engineering Services Co., Ltd., Japan) with a horizontal axis perpendicular to the gravity vector. Different rotation speeds and exposure periods were screened, and 5 rpm for 10 days was selected as the optimal treatment condition based on cell growth and morphology stability. Control cells were maintained under stationary 1 g conditions. Cultures were maintained at 25 ± 2°C, 50–60% relative humidity, under an 8 h light/16 h dark photoperiod with fluorescent illumination at 40–45 $\mu mol.m^{-2}.s^{-1}$.

Biomass determination and microscopic observation

Cell material was collected from each treatment and gently dispersed to prepare a uniform single-cell suspension. The cell suspension was transferred into centrifuge tubes and centrifuged to separate the cellular fraction. After centrifugation, the supernatant was removed, and the remaining cell pellet was collected (Fig. 3). The fresh weight of the harvested cell material was then measured using an analytical balance.

For morphological observations, cultured single cells were stained with Lugol's iodine or acetocarmine and observed under a light microscope (Fig. 4). We observed that cell morphology, nuclear morphology, and statolith distribution were compared to normal gravity and clinostat-treated groups.

Antioxidant enzyme assays

Fresh explants (0.3 g) were ground in liquid nitrogen and homogenized with 2 mL of 0.1 M phosphate buffer (pH 7.4) containing 0.1 mM EDTA. The homogenate was centrifuged at 15,000 rpm for 20 min at 4°C, and the supernatant was collected on ice for antioxidant analyses.

SOD activity was determined based on the inhibition of pyrogallol autoxidation at 320 nm, following Marklund and Marklund (1974). One unit of SOD activity was defined as the amount of enzyme required to inhibit pyrogallol autoxidation by 50%.

CAT activity was measured according to Goth (1991) by reacting to the extract with H_2O_2 for 2 min, followed by measurement of the yellow ammonium molybdate- H_2O_2 complex at 405 nm. One unit of CAT activity was defined as the decomposition of 1 µmol H_2O_2 per min.

APX activity was assayed following Nakano and Asada (1981) by monitoring ascorbate oxidation at 290 nm in the presence of H_2O_2. One unit of APX activity was defined as the oxidation of 1 µmol ascorbate per min.

Lignan analysis

Ethanolic extracts (80%) 1 g of cell material collected from normal-gravity controls and from cells treated under the selected simulated-microgravity condition were analyzed using a Thermo Ultimate 3000 HPLC system (Thermo Scientific, USA), following the method described by Harikrishnan et al. (2018). Stock solutions of the extracts (20 mg/mL) were sonicated for 15 min and filtered through a 0.45 µm Millex PTFE membrane. Standard secondary metabolite solutions (1 mg/mL) were diluted to concentrations ranging from 1000 to 125 µg/mL and analyzed using an XBridge™ C18 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile (solvent A) and water containing 0.1% orthophosphoric acid (solvent B), with isocratic elution at a flow rate of 1.0 mL/min.

Recovery-phase regeneration assay

Following clinostat treatment, cells were returned to normal gravity conditions to evaluate recovery and regeneration. Callus formation frequency, cell aggregation, and biomass accumulation were assessed after transfer back to 1 g conditions. Single cells previously exposed to simulated microgravity formed cell clusters and multicellular aggregates more rapidly than control cells and subsequently regenerated callus tissues more efficiently.

Experimental design and statistical analysis

The experiments were arranged in a completely randomized design with three independent biological replicates, and each replicate consisted of 90 samples per treatment. Data were analyzed using two-way

ANOVA to evaluate the effects of rotation speed, exposure time, and their interaction. Mean comparisons among treatments were performed using Duncan's multiple range test at $p < 0.05$ (Duncan 1955). In addition, each clinostat treatment was compared with the stationary 1 g control using Dunnett's test, with significance indicated as ns, *, **, and ***, corresponding to not significant, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively (Dunnett 1955).

Results and discussion

Establishment of suspension culture and selection of responsive cells

Cell suspension cultures of *P. amarus* were successfully established from callus tissues (Figs. 5 and 6). The growth curve demonstrated a clear logarithmic phase between day 20 and day 30 (Fig. 5), with maximal biomass accumulation observed on day 30 (Fig. 5). Nevertheless, 20-day-old cells were selected for subsequent treatments because early log-phase cells were considered more physiologically responsive than older cells.

The successful establishment of *P. amarus* cell suspension cultures from callus tissues provided a suitable system for evaluating cellular responses to simulated microgravity. Suspension cultures are advantageous for gravity-related studies because they allow more direct exposure of individual cells or small cell aggregates to the rotating environment, thereby reducing the masking effects often observed in compact tissues or organs (Chebli and Geitmann 2011; Herranz et al. 2013). Although maximum biomass was recorded on day 30, 20-day-old cells were selected because early logarithmic-phase cultures provide actively growing and relatively homogeneous cells, which are suitable for evaluating elicitor- or stress-induced responses in plant cell suspension systems (Wang et al. 2015; Ramirez-Estrada et al. 2016).

Effect of simulated microgravity on cell growth

The present study demonstrates that simulated microgravity generated by a 2D clinostat can act as a controlled physical elicitor in *P. amarus* single-cell cultures. Among the screened treatments, 5 rpm for 10 days produced the most favorable response, enhancing cell growth while maintaining cellular integrity (Table 1, 2 and Fig. 7). This result indicates that the biological effect of clinostat treatment depends strongly on the balance between gravity-vector averaging and mechanical stress. At an appropriate rotation speed and exposure period, the continuously changing orientation of the gravity vector may reduce the persistence of a directional gravity stimulus without causing severe physical injury. In contrast, excessive rotation or prolonged exposure appeared detrimental, as shown by cell shrinkage and occasional rupture (Fig. 7). Therefore, simulated microgravity should not be interpreted simply as a growth-promoting condition, but as a stress-modulating environment whose effectiveness depends on the intensity and duration of treatment.

Table 1
Effect of rotation speed and exposure time on cell growth rate (%)

Time	Control	1 rpm	3 rpm	5 rpm	10 rpm	15 rpm	20 rpm	30 rpm
10 days	92^{a}	90$^{a\ ns}$	95$^{a\ ns}$	100$^{a\ *}$	85$^{b\ *}$	60$^{c\ ***}$	45$^{d\ ***}$	10$^{e\ ***}$
20 days	94^{a}	95$^{a\ ns}$	97$^{a\ ns}$	100$^{a\ *}$	85$^{b\ *}$	60$^{c\ ***}$	40$^{d\ ***}$	5$^{e\ ***}$
40 days	88^{b}	85$^{b\ ns}$	90$^{a\ ns}$	85$^{b\ ns}$	70$^{b\ **}$	50$^{c\ ***}$	30$^{d\ ***}$	0$^{e\ ***}$
60 days	8^{e}	10$^{e\ ns}$	10$^{e\ ns}$	5$^{e\ ns}$	5$^{e\ ns}$	0$^{e\ *}$	0$^{e\ *}$	0$^{e\ *}$

*ANOVA was performed followed by post-hoc multiple comparison tests. Different letters within the same row indicate statistically significant differences among treatments. The symbols ns, *, **, and *** indicate the statistical significance of each treatment compared with the Control group, where ns indicates no significant difference, and *, **, and *** indicate significance levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.*

Control: Normal gravity condition

Table 2
Effect of rotation speed and exposure time on morphology stability (%)

Time	Control	1 rpm	3 rpm	5 rpm	10 rpm	15 rpm	20 rpm	30 rpm
10 days	96^{a}	95$^{a\ ns}$	97$^{a\ ns}$	100$^{a\ ns}$	90$^{a\ *}$	70$^{b\ **}$	50$^{c\ ***}$	10$^{e\ ***}$
20 days	95^{a}	97$^{a\ ns}$	95$^{a\ ns}$	100$^{a\ *}$	85$^{b\ *}$	60$^{c\ ***}$	40$^{d\ ***}$	5$^{e\ ***}$
40 days	90^{a}	90$^{a\ ns}$	85$^{b\ *}$	90$^{a\ ns}$	60$^{c\ ***}$	50$^{c\ ***}$	30$^{d\ ***}$	0$^{e\ ***}$
60 days	10^{e}	10$^{e\ ns}$	10$^{e\ ns}$	5$^{e\ *}$	5$^{e\ *}$	0$^{e\ *}$	0$^{e\ *}$	0$^{e\ *}$

*ANOVA was performed followed by post-hoc multiple comparison tests. Different letters within the same row indicate statistically significant differences among treatments. The symbols ns, *, **, and *** indicate the statistical significance of each treatment compared with the Control group, where ns indicates no significant difference, and *, **, and *** indicate significance levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.*

Control: Normal gravity condition

This interpretation is consistent with the conceptual framework proposed by Chebli and Geitmann (2011), who emphasized that clinostats do not eliminate the magnitude of gravity; rather, they minimize the biological effect of a constant unidirectional gravity vector by continuously reorienting the sample. Such omnilateral stimulation can alter plant cellular functioning, metabolism, and morphogenesis, especially in systems where microscopic observation and environmental control are feasible. The use of *P. amarus* single cells is therefore appropriate because single-cell systems provide direct access to cellular responses, avoid masking effects from neighboring tissues, and permit more controlled interpretation of gravity-related changes (Chebli and Geitmann 2011).

Microscopic observations revealed that cells treated under the optimal clinostat condition remained structurally intact, whereas excessive rotation or prolonged exposure caused cell shrinkage and, in some cases, rupture. Under normal gravity, statoliths tended to accumulate asymmetrically on one side of the cell (Figs. 7 and 8). Under simulated microgravity, their distribution became more uniform, and the cells appeared more rounded and compact, indicating altered intracellular organization.

This redistribution of statoliths is one of the clearest cellular responses to simulated microgravity. According to the statolith-based model of plant gravisensing, statoliths are dense intracellular bodies, commonly starch-containing amyloplasts or crystals, that sediment toward the lower side of statocysts when the orientation of the cell changes relative to gravity (Perbal and Driss-Ecole 2003). Their displacement is thought to initiate mechanosensory signaling through interaction with subcellular structures such as the endoplasmic reticulum or plasma membrane. Although early models proposed that statolith movement affects actin arrays and mechanosensitive ion channels (Morita and Tasaka 2004), later studies showed that disruption of actin filaments can enhance, rather than inhibit, gravitropic responses in *Arabidopsis* and rice (Hou et al. 2003; 2004). More recent evidence suggests that direct contact between sedimenting amyloplasts and the cortical endoplasmic reticulum may be sufficient to trigger the gravity signal (Morita 2010). Similarly, magnetophoretic displacement of statoliths has been shown to induce gravitropic curvature in higher plants and *Chara* rhizoids, confirming the functional role of statolith positioning in gravity perception (Weise et al. 2000).

Therefore, the more uniform statolith distribution observed under clinostat treatment likely weakened or altered the normal gravity signal. This disruption may have affected downstream cellular processes, including membrane signaling, cytoskeletal organization, and cell morphology. The rounded and compact appearance of treated cells further supports the view that simulated microgravity influences not only statolith positioning but also broader intracellular architecture.

Antioxidant enzyme activities increase in clinostat-treated cells

Simulated microgravity triggered a measurable biochemical response. Activities of SOD, CAT, and APX increased in clinostat-treated cells compared with the normal-gravity control (Fig. 9), suggesting activation of antioxidant defense pathways in response to altered gravity stress.

The increased activities of SOD, CAT, and APX suggest that simulated microgravity induced a controlled oxidative challenge. In plant systems, such oxidative adjustments often function not only as defense responses but also as signaling events that reprogram secondary metabolism. This offers a plausible explanation for the substantial increase in lignan accumulation observed here. In particular, the de novo detection of phyllanthin in the clinostat-treated group and the marked increase in hypophyllanthin imply that the lignan pathway was strongly activated under simulated microgravity.

This result is consistent with previous plant tissue-culture studies. In strawberry leaf explants, 2D clinostat culture enhanced morphogenesis and increased antioxidant-related activities, including APX,

CAT, and DPPH, compared with control conditions (Bien et al. 2023). The same study associated these antioxidant changes with improved callus development, shoot regeneration, and biomass accumulation. In Hyoscyamus niger, clinorotation was also reported to enhance callogenesis through ROS-related signaling and to affect antioxidant enzymes and tropane alkaloid formation (Pourhabibian et al. 2024). These studies support the interpretation that ROS generated under simulated microgravity may function as a regulatory signal when maintained below the threshold of oxidative damage.

The antioxidant response observed in *P. amarus* therefore appears adaptive. Under the optimal 5 rpm for 10 days condition, increased SOD, CAT, and APX activities likely stabilized ROS homeostasis and protected membrane, protein, and metabolic functions. In contrast, higher rotational intensity or longer treatment duration probably exceeded this adaptive capacity, leading to shrinkage and rupture. This explains why simulated microgravity can produce beneficial growth and metabolic outcomes under moderate exposure but becomes detrimental under excessive exposure.

Simulated microgravity enhances lignan accumulation

Clinostat treatment strongly stimulated lignan production (Fig. 10). Phyllanthin was undetectable in control cells but accumulated to 3.5 μg/g under simulated microgravity (Fig. 10). Hypophyllanthin content increased approximately 3.1-fold relative to the control (Fig. 10). These findings indicate that simulated microgravity functioned as a potent elicitor of lignan biosynthesis in *P. amarus* single cells.

This finding agrees with the emerging view that altered gravity can modulate secondary metabolism in medicinal plants. In *Hibiscus sagittifolius*, 2D clinostat treatment increased the accumulation of secondary metabolites, including total saponins and total coumarins, despite having different effects on root and shoot growth (Nhut et al. 2018). In *Hyoscyamus niger* callus, simulated microgravity affected tropane alkaloid formation together with antioxidant responses and callogenesis (Pourhabibian et al. 2024). More recently, *Catharanthus roseus* grown under 3D clinostat simulated microgravity showed increased biomass and broad transcriptional reprogramming involving oxidative stress responses, auxin signaling, meristem regulation, and terpenoid indole alkaloid biosynthesis (Lyu et al. 2025). Together, these studies indicate that simulated microgravity can stimulate specialized metabolism, although the direction and magnitude of the response depend on species, tissue type, developmental stage, culture system, and treatment parameters.

The relationship between antioxidant activation and lignan accumulation is biologically plausible. Many secondary metabolites, including phenolics, lignans, flavonoids, alkaloids, and terpenoid-derived compounds, are associated with stress adaptation and redox regulation. Moderate ROS accumulation may act upstream of transcriptional and enzymatic changes in secondary metabolite pathways. Therefore, the increase in SOD, CAT, and APX activities in *P. amarus* can be interpreted not only as a defense response but also as part of a broader metabolic reprogramming process that favors lignan biosynthesis. The detection of phyllanthin only in clinostat-treated cells suggests that simulated microgravity may have activated otherwise weak or silent branches of the lignan pathway under the tested culture conditions.

Another possible explanation is energy redistribution. In tobacco cell cultures exposed to 2D clinorotation, increased biomass and total protein were interpreted as evidence that cells may save energy otherwise required for maintaining positional homeostasis against gravity and redirect it toward growth and biosynthetic processes (Soleimani et al. 2019). This hypothesis is consistent with the present results, where optimal clinostat treatment simultaneously increased cell growth, antioxidant capacity, and lignan accumulation. Thus, simulated microgravity may enhance secondary metabolism through a combination of altered mechanosensing, ROS-mediated signaling, and reallocation of cellular resources toward biosynthesis.

Regeneration capacity is improved during the recovery phase

After transferring back to normal gravity, clinostat-treated cells exhibited a markedly enhanced regenerative response (Figs. 11 and 12). Callus formation frequency increased from 38% in the control group to 90% in the simulated microgravity group (Fig. 10). Fresh and dry biomass were also higher in the treated cells (Fig. 11). Morphologically, these cells rapidly formed cell clusters and multicellular aggregates that subsequently developed into new callus tissue (Fig. 12).

The regeneration results add an important biotechnological dimension to the study. Cells exposed to simulated microgravity retained, and perhaps even improved, their competence to re-enter proliferation and callus formation after returning to 1 g conditions. This indicates that the treatment did not merely induce a transient metabolic change but also influenced cellular developmental potential in a manner that may be valuable for medicinal plant propagation and metabolite production platforms.

This result is consistent with reports showing positive effects of clinostat treatment on in vitro morphogenesis. In strawberry, leaf explants cultured under 2D clinostat conditions regenerated shoots one week earlier than controls and showed higher callus induction, shoot regeneration, fresh weight, and dry weight during culture (Bien et al. 2023). In Begonia, simulated microgravity was reported to affect somatic embryogenesis by modifying endogenous hormone balance and energy metabolism (Khai et al. 2021). These findings suggest that altered gravity can influence developmental reprogramming through hormonal, energetic, and redox pathways. The enhanced callus formation in *P. amarus* may therefore reflect a priming effect, in which cells exposed to moderate simulated microgravity acquired a physiological state more favorable for proliferation and dedifferentiation once returned to 1 g conditions.

Conclusion

Simulated microgravity generated by a 2D clinostat significantly affected the behavior of *P. amarus* single cells. Under the optimal treatment of 5 rpm for 10 days, cell growth was enhanced, antioxidant enzyme activities increased, lignan accumulation was stimulated, and regeneration capacity improved after transfer back to normal gravity.

These results suggest that simulated microgravity can be developed as a physical elicitation strategy for medicinal plant biotechnology, particularly for improving regenerative competence and the biosynthesis of valuable lignans such as phyllanthin and hypophyllanthin. Because this study used a ground-based simulator, future work should validate these responses under additional microgravity analogs or real spaceflight conditions and should examine the molecular regulation of lignan biosynthesis under altered-gravity stress.

Declarations

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Author contributions

DTN and NTNM conceptualized and designed the study. NTNM, DMC, and HHD performed experiments. NTNM, DMC, HHD, DTT, VQL, HHT, NVB, NBN, BVTV, and DTN participated in the interpretation of the data, and revision for intellectual content acquired data wrote the manuscript.

Compliance with Ethical Standards

Conflict of interest:

Duong Tan Nhut is the Associate Editor of the journal at the time of submission. This had no impact on the peer review process or the final decision.

***Ethical approval*:**

This article does not contain any studies involving human participants or animals performed by any authors.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Competing Interest

The authors declare no conflict of interest.

Research Involved Human and Animal Rights

This study did not involve experiments with human or animal participants.

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Figures

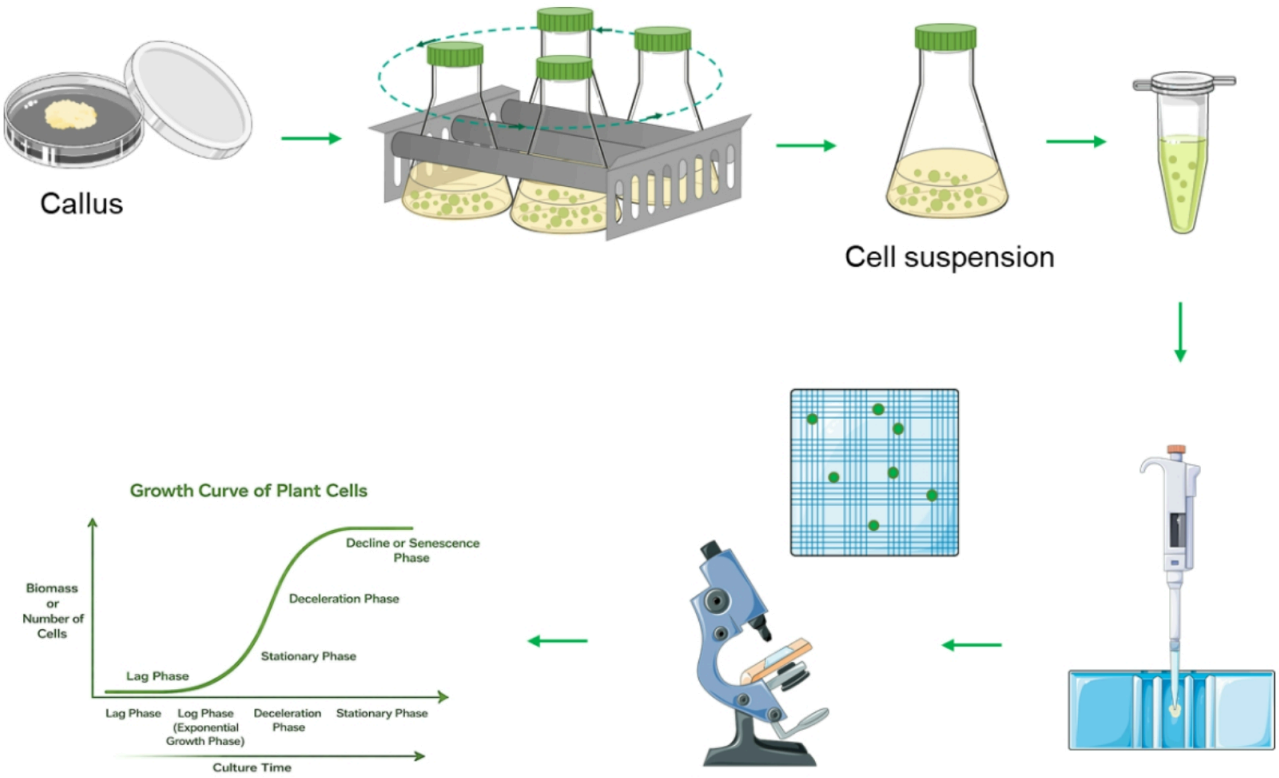


Figure 1

Establishment of *P. amarus* cell suspension cultures



Figure 2

Isolation of single cells from suspension cultures

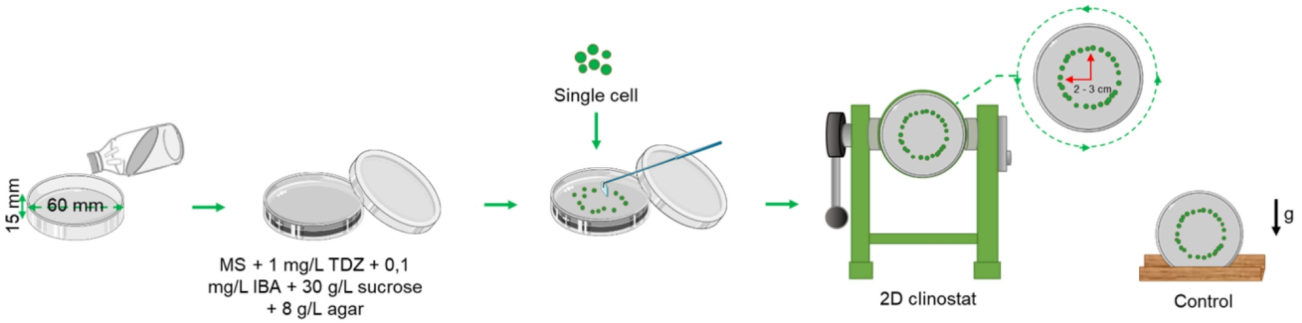


Figure 3

Single cell culture under simulated microgravity conditions using a 2D clinostat

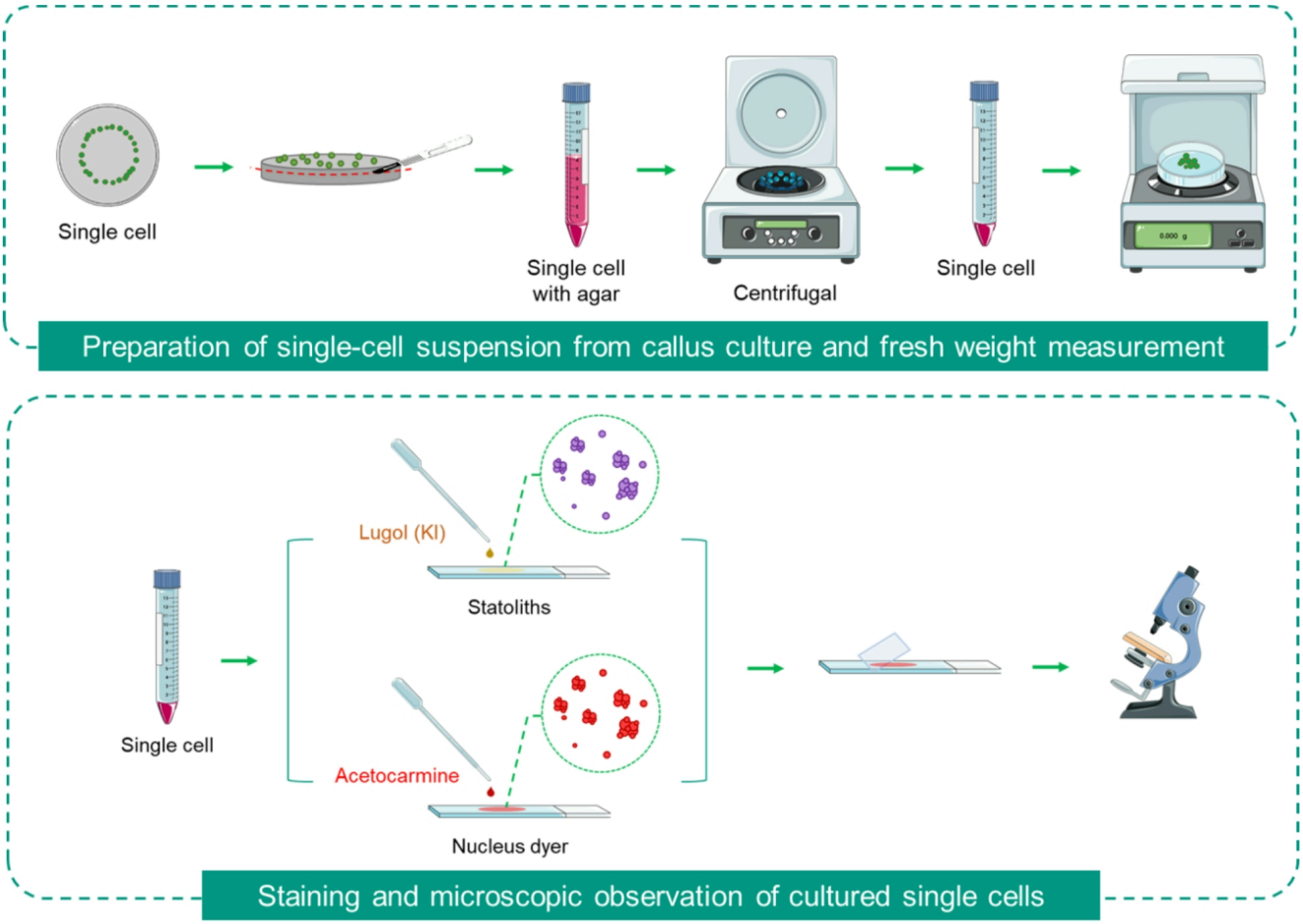


Figure 4

Recovery and staining of single cells after culture

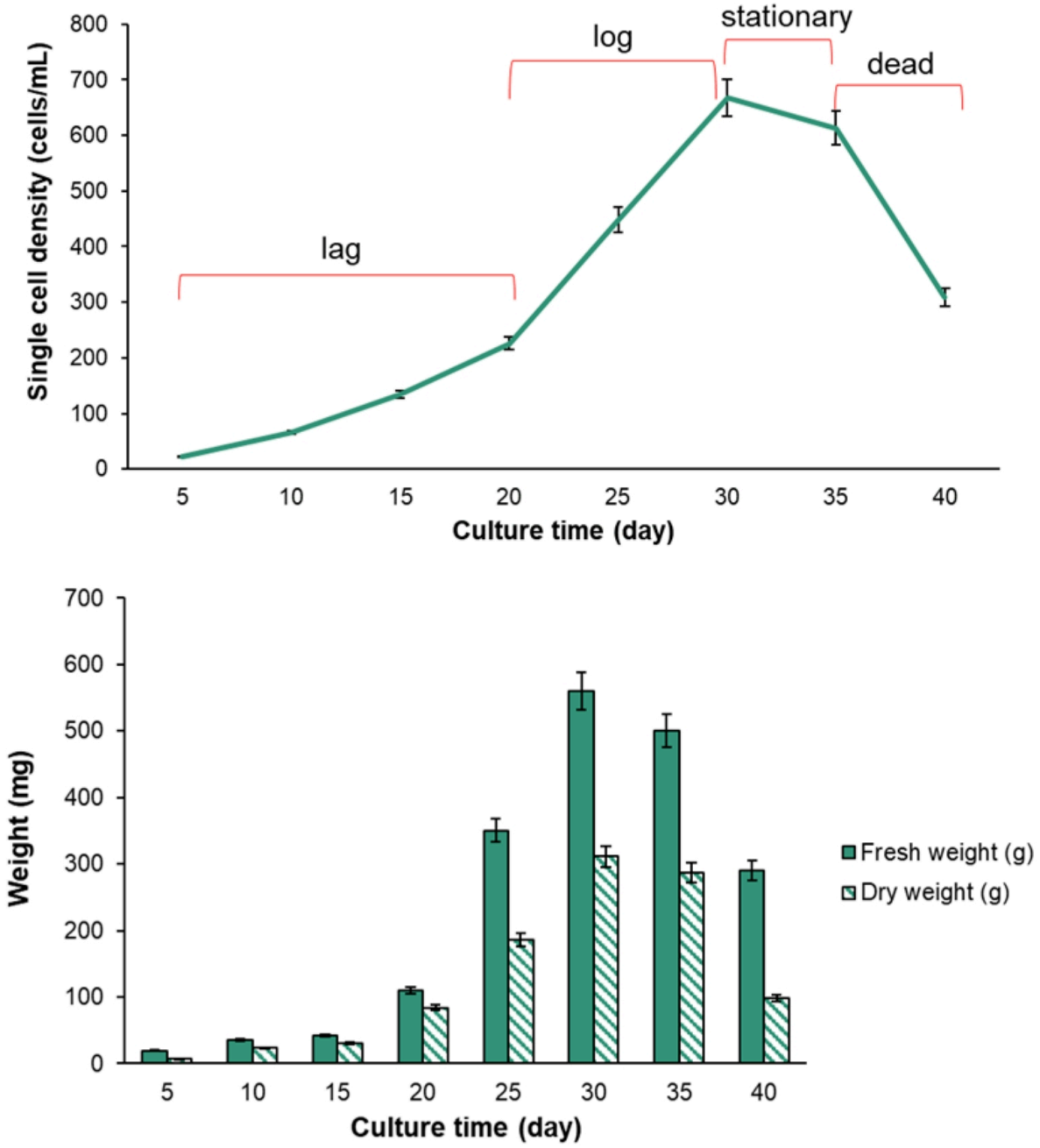


Figure 5

Growth dynamics of *P. amarus* cell suspension cultures, showing changes in single-cell density, fresh weight, and dry weight during the culture period.

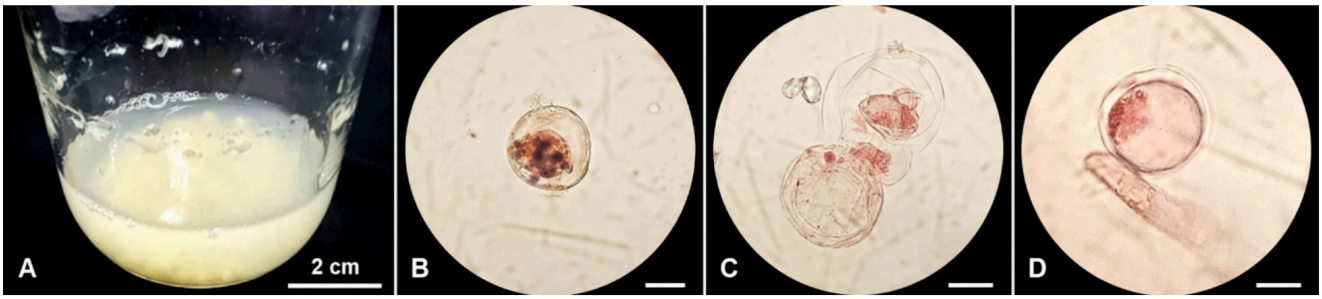


Figure 6

P. amarus cell suspension (A), single cells during the logarithmic phase (B), cell division (C), and cells in the death phase (D)

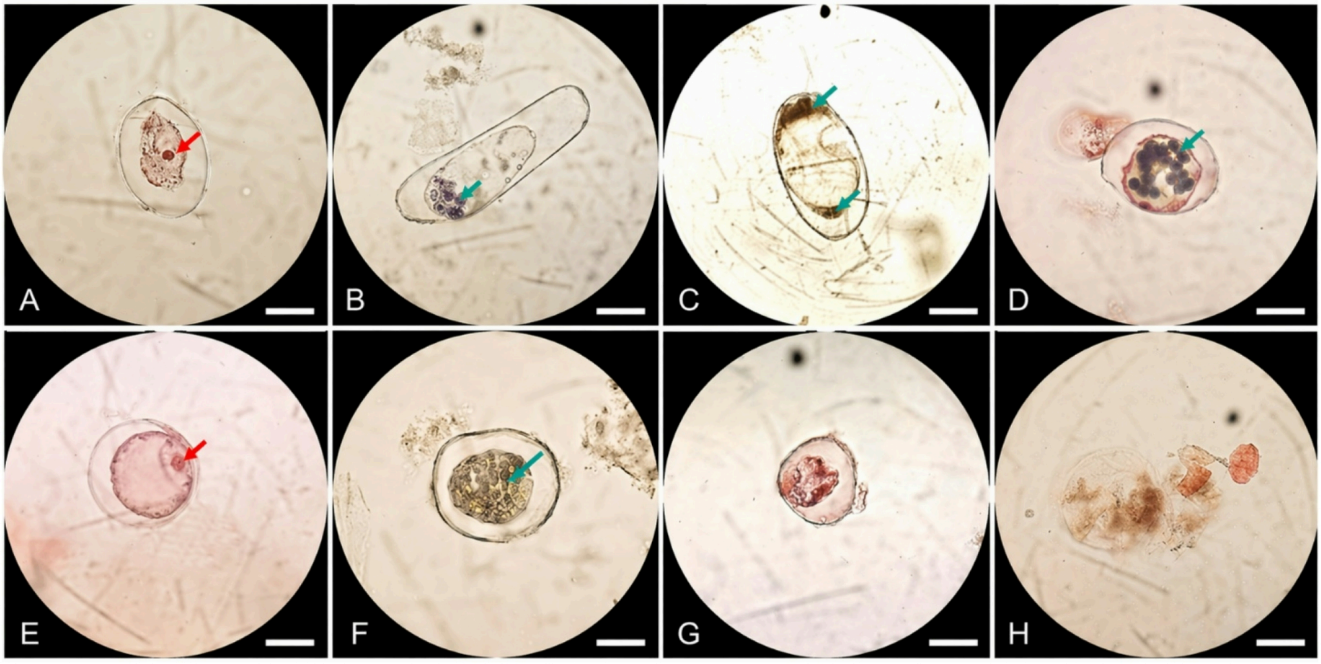


Figure 7

Morphology of P. amarus single cells under normal gravity and simulated microgravity. Bar = 20 μm.

A and B: Single cell in normal gravity.

C: Single cell in 2D clinostat system at 1 rpm for 10 days.

D: Single cell in 2D clinostat system at 3 rpm for 10 days.

E and F: Single cell in 2D clinostat system at 5 rpm for 10 days.

G: Single cell in 2D clinostat system at 5 rpm for 20 days.

H: Single cell in 2D clinostat system at 30 rpm for 10 days.

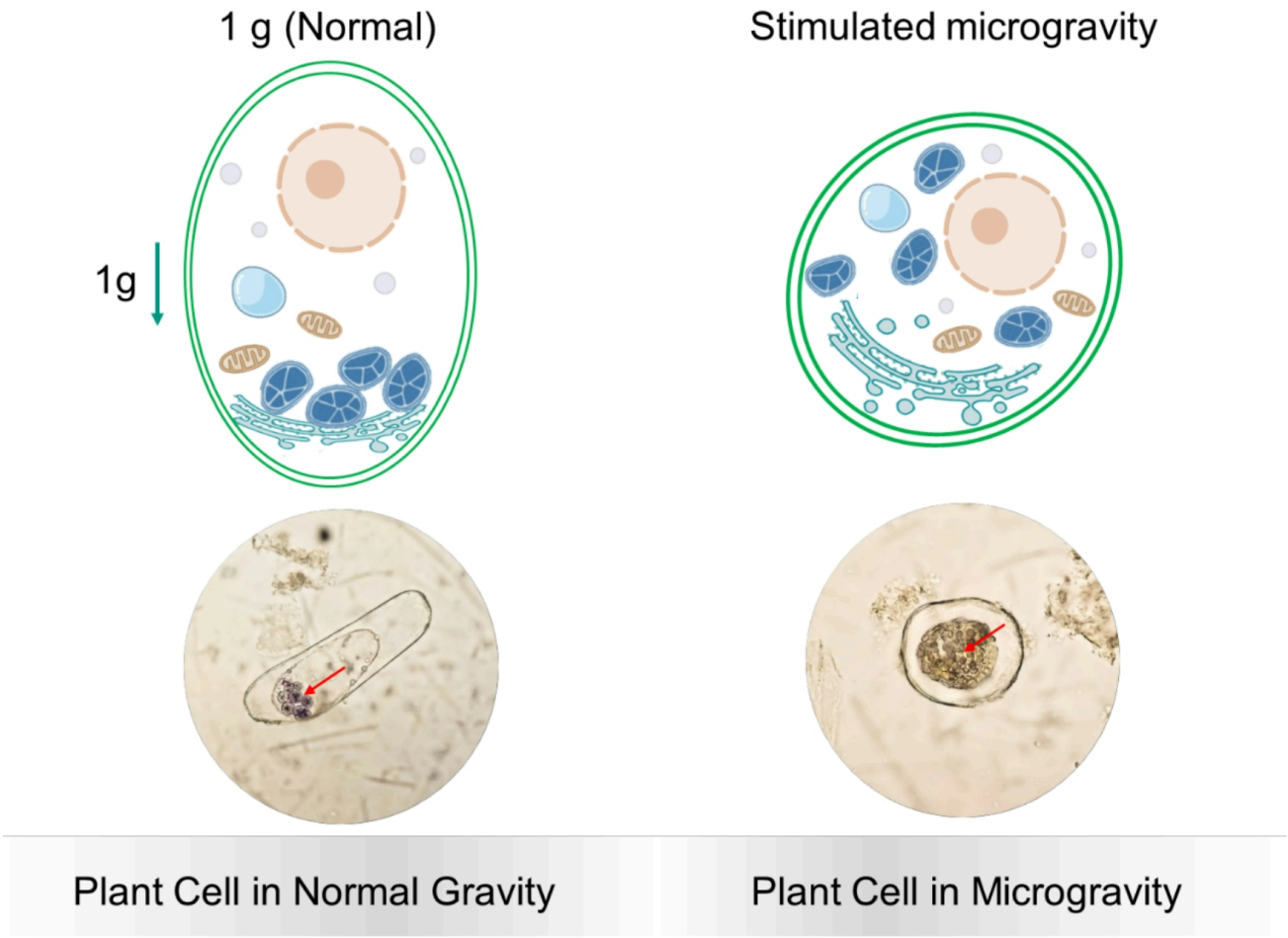


Figure 8

Cell viability and size distribution after filtration and culture, showing enrichment of single cells in the desired size range

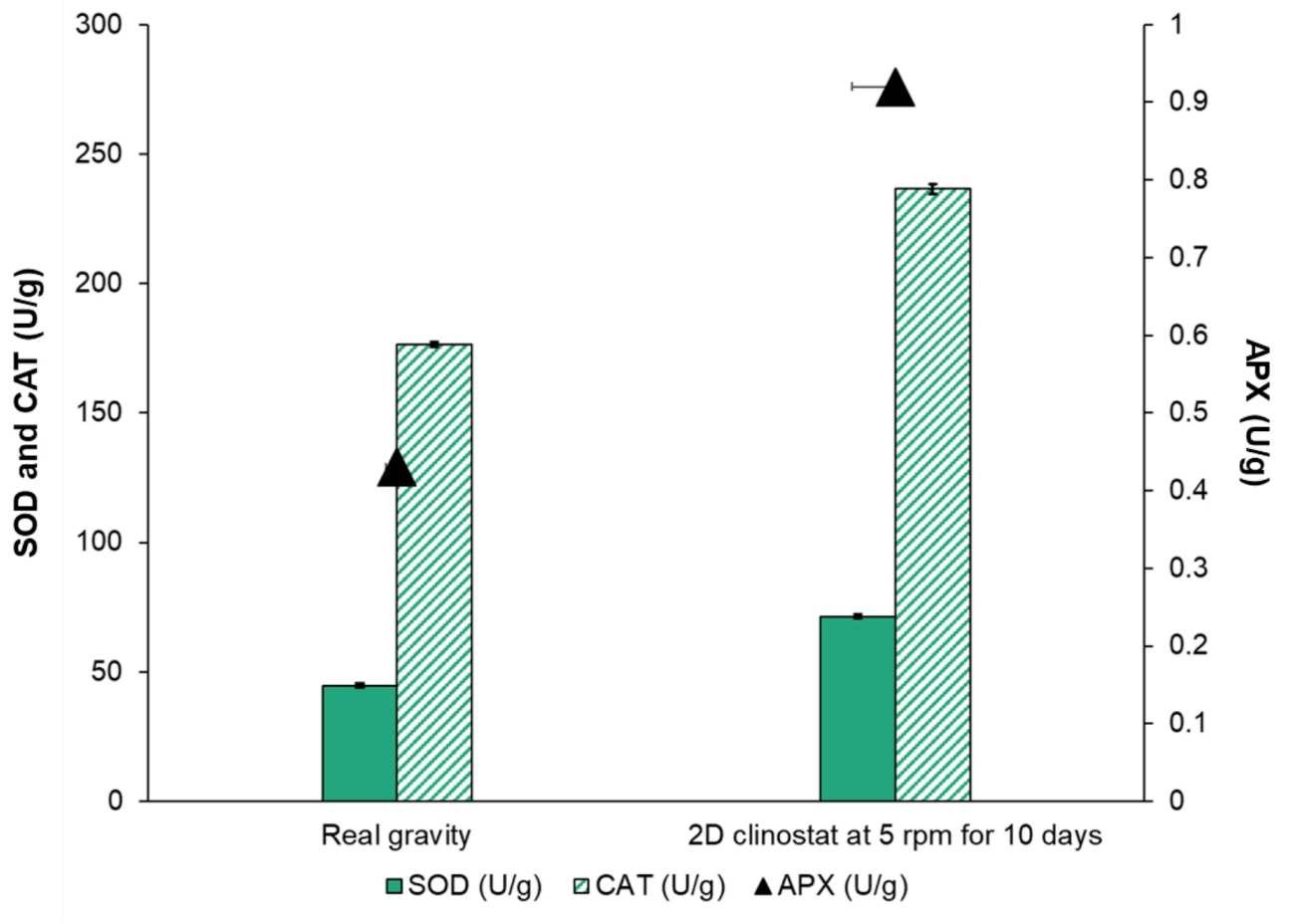


Figure 9

Antioxidant enzyme activities of *P. amarus* cells under real gravity and simulated microgravity conditions

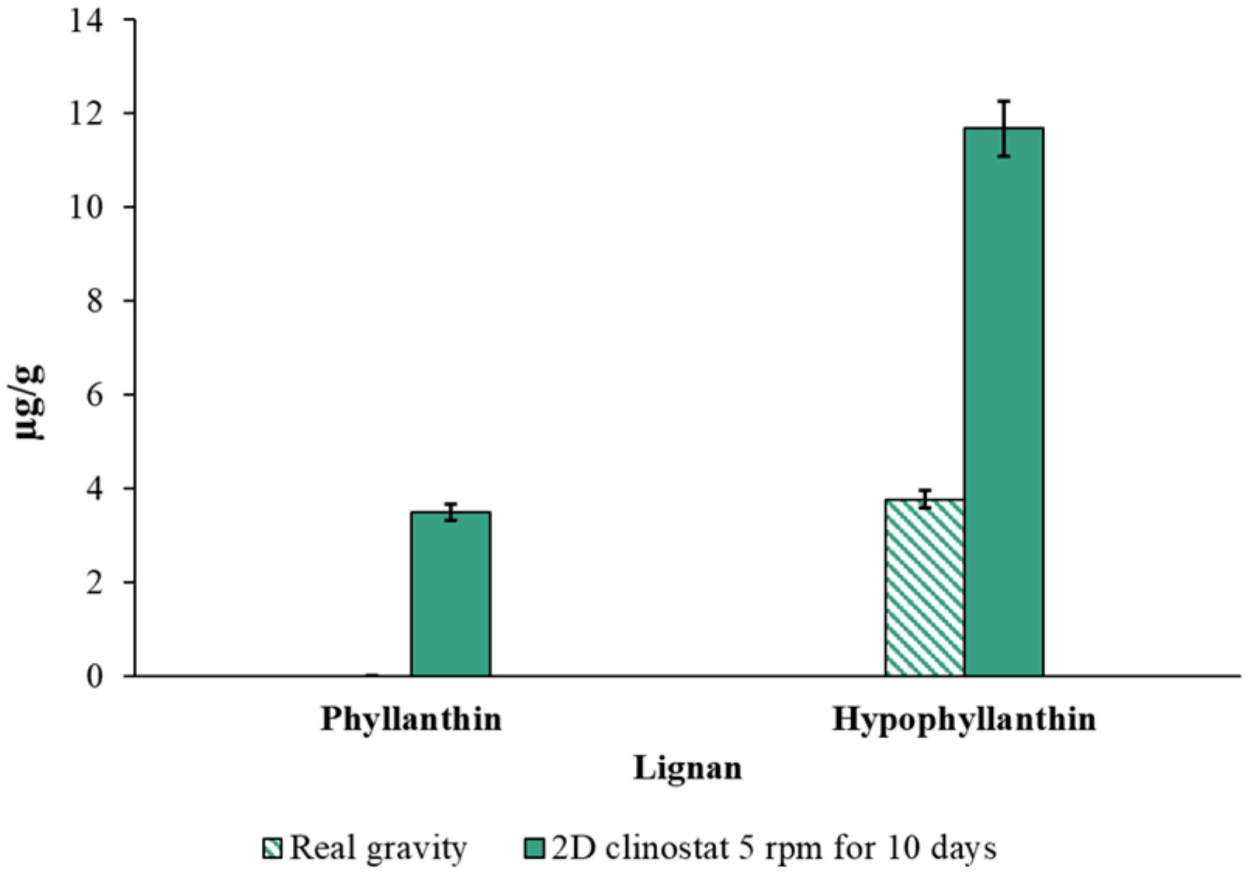


Figure 10

Lignan accumulation in *P. amarus* cells cultured under simulated microgravity conditions

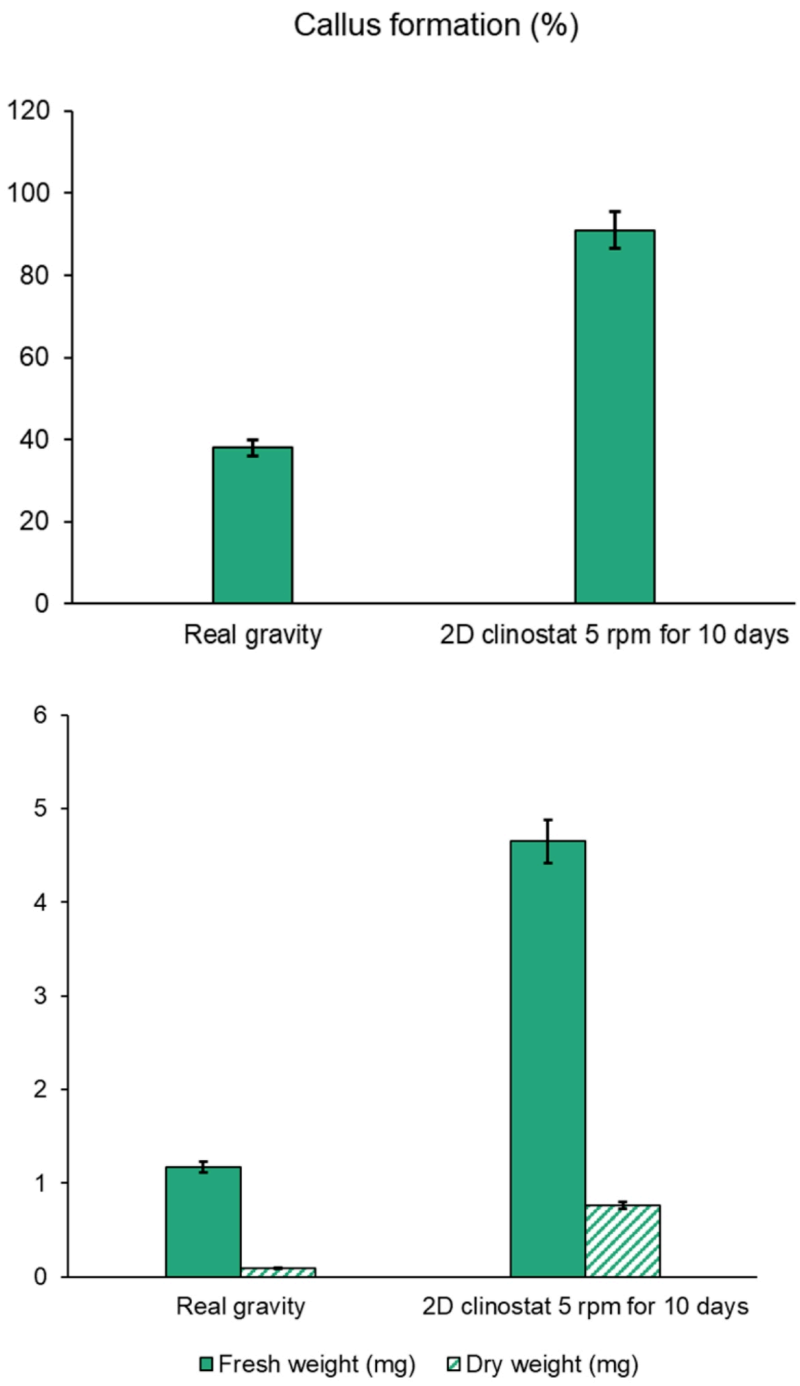


Figure 11

Regeneration of single cells after exposure to simulated microgravity

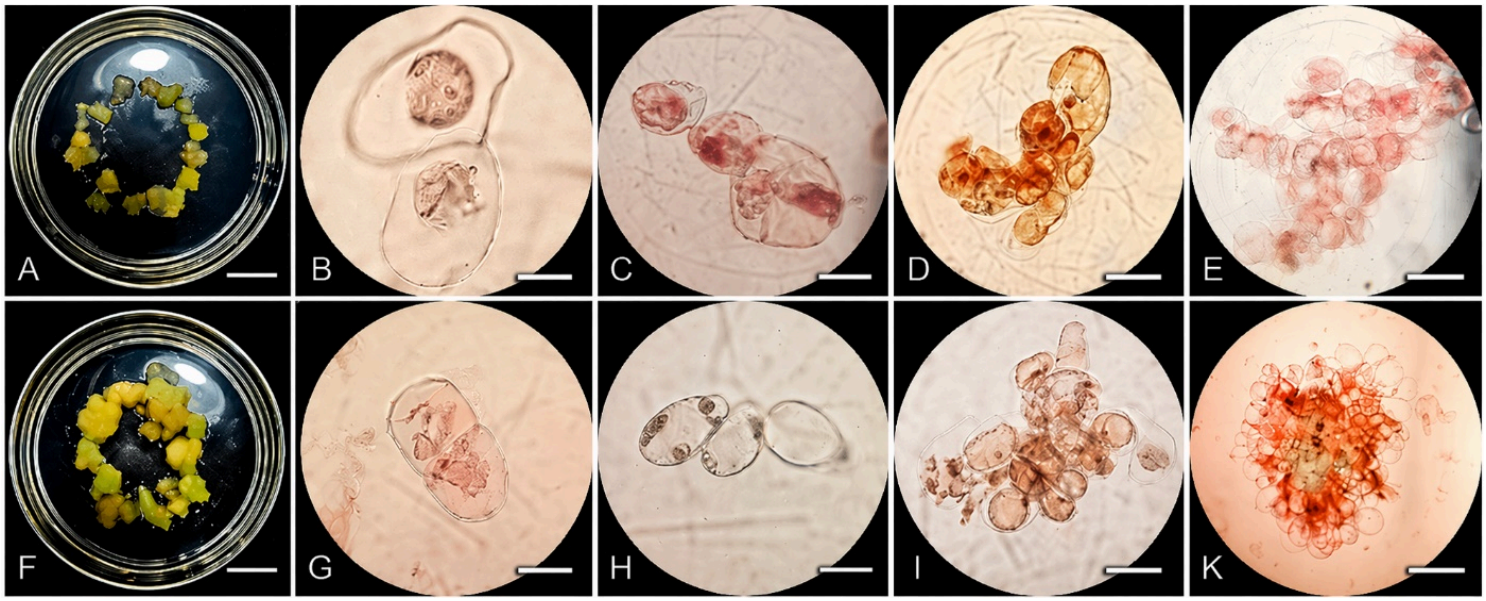


Figure 12

Regeneration ability of single cells of *P. amarus* derived from normal gravity and 2D clinostat system

A - E: Single cell derived from normal gravity condition

F - K: Single cell derived from 2D clinostat system at 5 rpm for 10 days after being transferred back to normal gravity condition

A, B: bar = 2 cm; B-D, G-I: bar = 50 µm; E=100 µm; K=300 µm