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INDUCTION OF GREEN GLOBULAR BODIES AND PLANTLET REGENERATION IN *ADIANTUM TRAPEZIFORME*

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ABSTRACT

The green globular body is a unique, organized tissue that emerges from explants in pteridophyte tissue cultures. This article describes the first comprehensive study of *in vitro* regeneration of *Adiantum trapeziforme via* green globular bodies. Green globular bodies were induced on the rhizomes of sterile sporophytes of *Adiantum trapeziforme*. The green globular bodies were used as explants for additional proliferation and regeneration. The study evaluated the concentrations of basal salts, sucrose, tryptone, and plant growth regulators. The results showed that the presence of 6-benzyladenine inhibited the proliferation and regeneration of green globular bodies, whereas while increasing the concentration of α-naphthaleneacetic acid to 5 $mg \cdot L^{-1}$ led to the highest proliferation and drastically impeded the development of green globular bodies. Hyponex No.1 fertilizer, especially at 3 $g \cdot L^{-1}$, with a proliferation rate up to 8.8 times, was a better salt source than Murashige and Skoog medium. In the sucrose concentration test, 30 $g \cdot L^{-1}$ sucrose resulted in the highest proliferation rate. Furthermore, medium with a higher tryptone concentration inhibited the proliferation of green globular bodies. Green globular body differentiation medium containing 2 $g \cdot L^{-1}$ Hyponex, 30 $g \cdot L^{-1}$ sucrose, 2 $g \cdot L^{-1}$ tryptone, and pH 5.8 resulted in the highest bud induction rate, averaging 18.6 buds. Paraffin sections were examined to observe the development of green globular bodies induced by the rhizome. In the soil containing perlite, 100% of regenerated shoots successfully formed roots during acclimation.

Key words: fern, micropropagation, plant tissue culture, plant growth regulators, rhizome

INTRODUCTION

Adiantum trapeziforme, commonly known as giant maidenhair or diamond maidenhair, is a species of fern belonging to the order Polypodiales and the family Pteridaceae. Native to southeastern Mexico and parts of Central America, it typically grows to a height of 60–120 cm. It thrives in shady, humid environments, particularly near waterfalls or streams where moisture availability is consistently high (McCarthy 2012). Species of the genus *Adiantum* have long been valued as ornamental plants and are also known for their potential medicinal uses. For example, *A. venustum* and *A. capillus-veneris* exhibit potent antimicrobial activity against *Escherichia coli*, *Trichophyton rubrum*, and *Aspergillus terreus* (Singh et al. 2008). Furthermore, methanolic extracts of *A. latifolium* have demonstrated antinociceptive and antiinflammatory effects in animal experiments (Nonato et al. 2011).

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For enhancing ornamental value or increasing the production of bioactive compounds for medicinal purposes, breeding is an important strategy. Conventional fern breeding relies primarily on sporeling and subsequent selection of sporophytes (Xiong et al. 2024), or on the accumulation of spontaneous mutations during repeated cycles of clonal propagation, followed by human selection for improved or novel traits (Schoen & Schultz 2019; Zheng et al. 2022). However, sporophyte-based selection is constrained by long generation times and the need to undergo alternation between gametophyte and sporophyte stages – a process that can take a long time in some fern species (Chou et al. 2007). Traditional clonal propagation also requires substantial time and labor, limiting its efficiency. Fern micropropagation, on the other hand, offers a promising alternative, enabling rapid sporophyte regeneration *via* the green globular body (GGB) regeneration system (Lin et al. 2024). When combined with mutagenic treatments such as gamma irradiation or chemical mutagens (e.g., sodium azide), micropropagation can generate phenotypic variation in a relatively short time, providing a variety of traits that may prove beneficial in fern breeding programs (Sajeev et al. 2018).

Green globular bodies (GGBs) are unique structures observed in pteridophytes under tissue culture conditions and are characterized by a high rate of sporophyte regeneration. GGBs were first discovered in *Nephrolepis cordifolia* by Higuchi et al. (1987). Despite their widespread use in fern micropropagation, the developmental mechanism underlying sporophyte regeneration from GGBs remains unclear (Wang et al. 2021). Studies in *A. capillus-veneris* have shown that somatic embryogenesis receptor kinase (SERK) genes are involved in GGB-mediated regeneration, suggesting that GGB development may be linked to the embryogenic pathway (Li et al. 2015). However, direct evidence for true somatic embryogenesis of GGBs is lacking, and the exact developmental pathway unknown. Higuchi et al. (1987) also compared GGBs with other morphogenetic tissues, such as orchid protocorm-like bodies and calli, and demonstrated distinct morphological and developmental distinctions between these structures. GGBs have since been described in many fern species and can be induced from various sporophytic tissues. Examples include runner tips of *Nephrolepis cordifolia* (Higuchi et al. 1987), leaf primordia of *Cibotium barometz* (Yu et al. 2017), and juvenile sporophytes of *Pteris aspericauli*s var. *tricolor* (Yu et al. 2021). Currently, GGB-based regeneration systems are widely used in the propagation of ornamental, medicinal, and endangered fern species due to their high proliferation efficiency and regenerative potential (Wang et al. 2021).

Traditionally, *A. trapeziforme* has been propagated primarily by spores, although vegetative propagation by division is also possible. However, to meet the demands of the ornamental plant market and support future breeding strategies, especially mutagenesis breeding, an effective tissue culture protocol for *A. trapeziforme* is essential. This study investigated the effect of culture medium components on the rate of GGB induction and proliferation and subsequent sporophyte regeneration efficiency. Specifically, the influence of basal salt composition, sucrose concentration, tryptone supplementation, and plant growth regulators (PGRs) was evaluated. Furthermore, an *in vitro* acclimation protocol for *A. trapeziforme* plantlets was developed to facilitate successful transition to *ex vitro* conditions.

MATERIALS AND METHODS

Plant material

A. trapeziforme sporangia and leaves were cut, washed with clean water, and then sterilized with 75% alcohol and 0.6% sodium hypochlorite. The sporangia were rinsed several times with sterile water

and dried on filter paper. Spores from sterilized sporangia were sown on Hyponex No. 1 (HYPONeX, USA) spore germination medium containing 7% total nitrogen (N), 6% available phosphate (P_2O_5), 19% soluble potassium (K_2O), 30 g·L^{-1} sucrose (Sigma Aldrich, USA), 2 g·L^{-1} Bacto Tryptone (Gibco, USA), and 4 g·L^{-1} Gelrite gellan gum (CP Kelco, USA), adjusted to pH 5.8. Gametophytes geminated from the spore within a month, and sporophytes were produced in the centers of several prothalli in the spore germination medium.

Rhizomes were isolated from *A. trapeziforme* sporophytes and then subcultured to a modified media from previous studies (Lin et al. 2024) containing 3 g·L^{-1} Hyponex, 15 g·L^{-1} sucrose, 2 g·L^{-1} tryptone, 4 g·L^{-1} gellan gum, 0.05 mg·L^{-1} 6-benzyladenine (6-BA), and 0.3 mg·L^{-1} α-naphthaleneacetic acid (NAA), with pH adjusted to 5.8 to induce GGBs. GGBs were induced from the rhizomes within 2 weeks and used in further experiments (Fig. 1a–c).

Proliferation of green globular bodies in different salt media

Approximately 0.008 g of GGBs were used as explants in this experiment. The control medium consisted of 2 g·L^{-1} Hyponex, 30 g·L^{-1} sucrose, 2 g·L^{-1} tryptone, and 4 g·L^{-1} gellan gum. Salt concentrations from MS (Murashige & Skoog 1962 medium, Duchefa Biochemie, the Netherlands) were adjusted to 1/2 (2.2 g·L^{-1}), full concentration (4.4 g·L^{-1}), and 3/2 (6.6 g·L^{-1}). Hyponex was tested at 1/2 (1 g·L^{-1}), full concentration (2 g·L^{-1}), and 3/2 (3 g·L^{-1}).

All media were adjusted to pH 5.8 with 1 M NaOH or 1 M HCl and then autoclaved. Each treatment consisted of five replicates. Cultures were incubated under a 10 h light/14 h dark photoperiod, with a photosynthetic photon flux density (PPFD) of 45 µmol·m^{-2}·s^{-1} at 24 ± 1 °C. After 30 days of culture, the total fresh weight of GGBs, the number of regenerated shoots, the shoot induction rate, and the proliferation rate of GGBs were assessed.

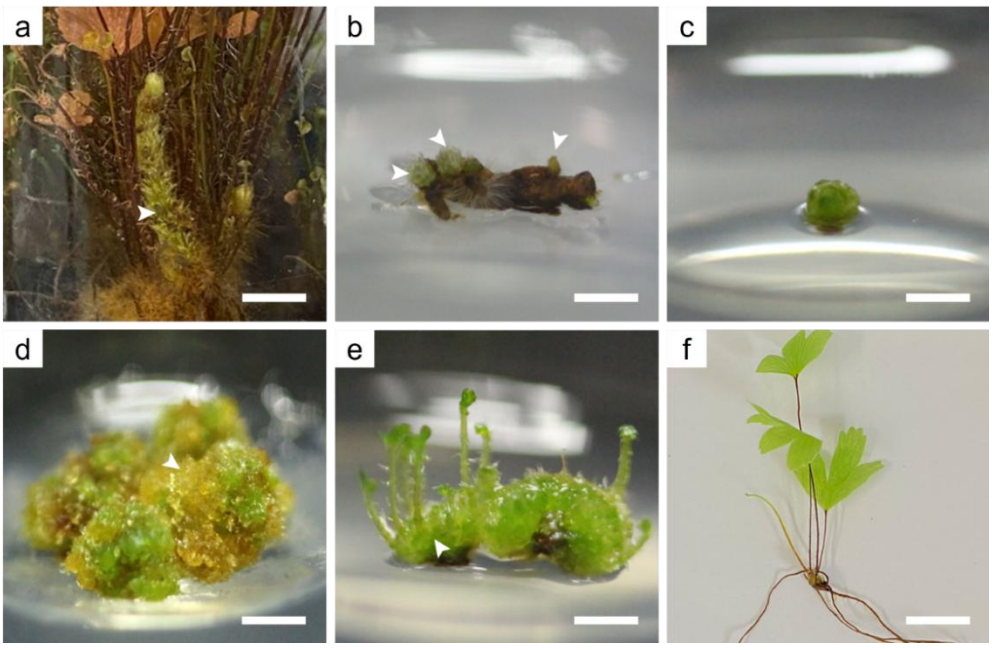

Figure 1. *In vitro Adiantum trapeziforme* regeneration by tissue culture of green globular bodies: sterile *Adiantum trapeziforme* sporophyte rhizomes (arrow), bar – 3 mm (a), several green globular bodies (arrows) produced for 10 days by isolated sterile rhizomes incubated in 3/2 Hyponex, 0.05 mg·L^{-1} 6-benzyladenine, and 0.3 mg·L^{-1} α-naphthaleneacetic acid, bar – 3 mm (b), green globular body isolated from sterile rhizome, bar – 3 mm (c), green globular bodies incubated in 3/2 Hyponex, 30 g·L^{-1} sucrose, and 5 mg·L^{-1} α-naphthaleneacetic acid proliferated for 4 weeks into groups of green globular body units with partial yellowing (arrow), bar – 3 mm (d), multiple shoots (arrow) produced by green globular bodies incubated in 2 g·L^{-1} Hyponex without plant growth regulators, bar – 3 mm (e), sporophyte regenerated from green globular bodies tissue culture after one month of acclimation, bar – 1 cm (f)

Proliferation of green globular bodies at different sucrose concentrations

GGBs, each weighing approximately 0.003 g, were used as explants. The sucrose concentration in the control media was adjusted to 0, 15, 30, and 45 $g \cdot L^{-1}$. Each treatment consisted of five replicates. After 30 days of culture, the total fresh weight of GGBs, the number of regenerated shoots, the rate of shoot induction, and the rate of GGB proliferation were assessed.

Proliferation of green globular bodies at different tryptone concentrations

GGBs, each weighing approximately 0.008 g, were used as explants. The tryptone concentration in the control media was adjusted to 0, 1, 2, 3, and 4 $g \cdot L^{-1}$. Each treatment consisted of five replicates. After 30 days of culture, the total fresh weight of GGBs, the number of regenerated shoots, the rate of shoot induction, and the rate of GGB proliferation were assessed.

Proliferation of green globular bodies under different plant growth regulators

GGBs with an average fresh weight of approximately 0.012 g were used as explants. Various types and concentrations of PGRs, including 6-BA and NAA, were added to the control medium at 0, 1, 5, and 10 $mg \cdot L^{-1}$. Medium without PGRs was used as a control. Each treatment consisted of five replicates. After 30 days of culture, the total fresh weight of GGBs, the number of regenerated shoots, the rate of shoot induction, and the rate of GGB proliferation were assessed.

Paraffin preparations from rhizomes and green globular bodies

A. trapeziforme plantlets with rhizomes 3–4 cm long were selected. After removing the apical meristem, leaves, and roots, rhizome segments were cut into approximately 1 cm long segments and placed horizontally in the medium to induce GGBs. Rhizome segments were selected every 2 days, each time 5 explants, until the first rhizome explants had induced GGBs approximately 0.1 cm wide. All explants were then removed from the medium and immersed in FPA fixative solution (5% formalin, 5% propionic acid, 90% ethyl alcohol). This was followed by vacuum infiltration for 3–7 days (depending on tissue size and density) until the explants completely sank to the bottom of the vials. A series of dehydration procedures were then performed using a mixture of tertiary butanol (t-butanol) and ethanol, and the samples were stained with 0.5% safranin. The dehydrated material was placed in an oven at 60–62 °C, gradually increasing the concentration of paraffin in the solution. After the paraffin had penetrated, the blocks were cut using a rotary microtome (Tominga, Taiwan). Thin 10–20 μm paraffin sections were spread on glass slides and air-dried for 2 days. Finally, they were dissolved in xylene, stained with 0.5% safranin and 0.1% Fast Green, mounted, and air-dried. The formation of GGBs from the rhizome and their proliferation were then examined histological under an optical microscope (Lin et al. 2024).

Statistical analysis

Data calculations were performed using Microsoft Excel 365. Statistical analyses were conducted using the Statistical Analysis System (SAS). Data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Mean values were compared to determine the significance of differences ($P < 0.05$).

RESULTS

The effect of basal salts, sucrose, and tryptone on the proliferation and differentiation of green globular bodies

In the basal salt experiment, Hyponex generally outperformed MS in most evaluated parameters (Table 1). The 3 $g \cdot L^{-1}$ Hyponex treatment resulted in the highest fresh weight (0.068 ± 0.013 g) of GGBs and GGB proliferation rate (8.8 ± 2.8), which were significantly greater than those observed with either MS treatment. For shoot induction, both 2 $g \cdot L^{-1}$ and 3 $g \cdot L^{-1}$ Hyponex resulted in a 100% induction rate. The highest average number of shoots was observed with 2 $g \cdot L^{-1}$ Hyponex (5.8 ± 4.1 shoots per explant). In contrast, the 3 $g \cdot L^{-1}$ Hyponex treatment showed a comparable but slightly lower number of shoots (5.0 ± 1.0 shoots per explant). Optimal proliferation of GGBs

on media containing MS salts was obtained using 4.4 g·L^{-1} MS (5.5 ± 1.4) and a fresh weight of 0.048 ± 0.007 g. Shoot induction under MS was most effective at 4.4 g·L^{-1} MS with an 80% shoot induction rate. At 6.6 g·L^{-1}, shoot induction dropped to 40% and the number of shoots to 2.8.

In the sucrose concentration experiment (Table 2), the highest proliferation rate was observed at 30 g·L^{-1} sucrose (7.2 ± 2.9), a shoot number of 4.5, and a shoot rate induction of 40%. Regarding shoot formation, the average number of shoots was highest at 30 g·L^{-1} sucrose (4.5 ± 3.5 shoots per explant), followed by 45 g·L^{-1} (1.3 ± 0.9 shoots). At 0 and 15 g·L^{-1} sucrose, no shoot induction was observed. Regarding shoot induction rate and fresh weight, treatment with 45 g·L^{-1} sucrose gave the highest values (60% induction rate and 0.028 ± 0.001 g fresh weight), followed by 30 g·L^{-1}.

Tryptone did not significantly affect the proliferation of GGBs, but inhibited the differentiation of GGB shoots (Table 3). The highest proliferation rates (4.2 ± 0.7 and 4.1) occurred in the control group without tryptone and at 1 g·L^{-1}. Under these conditions, the number of shoots was 15.4 and 10.5, respectively, and shoot induction was 100%. Higher tryptone concentrations caused a decrease in proliferation and shoot number, and at 4 g·L^{-1}, shoot formation was eliminated.

Table 1. Effect of basal salts on the proliferation and differentiation of green globular bodies in a medium (30 g·L^{-1} sucrose, 2 g·L^{-1} tryptone) without plant growth regulators after 30 days

Basal salt (g·L^{-1})		Green globular body fresh weight (g)	Green globular body proliferation rate	Number of shoots	Shoot induction rate (%)
MS	2.2	0.028±0.004 c	2.8±0.6 c	2.0±1.0 c	60
	4.4	0.048±0.007 b	5.5±1.4 b	2.8±1.5 bc	80
	6.6	0.041±0.012 bc	4.4±1.7 bc	1.0±0.0 c	40
Hyponex	1	0.036±0.004 bc	3.9±0.5 bc	3.0±2.2 bc	80
	2	0.046±0.014 b	5.7±1.9 b	5.8±4.1 a	100
	3	0.068±0.013 a*	8.8±2.8 a	5.0±1.0 ab	100

Proliferation rate = (Final fresh weight after 30 days of culture − Initial fresh weight on day 1)/Initial fresh weight on day 1; Means within the same column followed by different lowercase letters indicate significant differences between treatments according to Duncan's multiple range test ($P \leq 0.05$); n = 5

Table 2. Effect of sucrose concentration on the proliferation and differentiation of green globular bodies in a medium (2 g·L^{-1} Hyponex, 2 g·L^{-1} tryptone) without plant growth regulators after 30 days

Sucrose (g·L^{-1})	Green globular body fresh weight (g)	Green globular body proliferation rate	Number of shoots	Shoot induction rate (%)
0	0.005±0.001 c	0.6±0.4 b	0±0 a	0
15	0.018±0.003 b	4.8±1.9 a	0±0 a	0
30	0.022±0.006 ab	7.2±2.9 a	4.5±3.5 a	40
45	0.028±0.001 a*	6.7±2.7 a	1.3±0.9 a	60

Note: see Table 1

Table 3. Effect of tryptone concentration on the proliferation and differentiation of green globular bodies in a medium (2 g·L^{-1} Hyponex, 30 g·L^{-1} sucrose) without plant growth regulators after 30 days

Tryptone (g·L^{-1})	Green globular body fresh weight (g)	Green globular body proliferation rate	Number of shoots	Shoot induction rate (%)
0	0.038±0.005 a	4.2±0.7 a*	15.4±6.5 a	100
1	0.035±0.005 ab	4.1±0.9 ab	10±5.7 b	100
2	0.036±0.007 ab	3.8±0.9 abc	3±1.6 c	80
3	0.029±0.004 c	2.8±0.5 c	1±0 c	20
4	0.028±0.008 c	3.0±1.1 bc	0±0 c	0

Note: see Table 1

The effect of plant growth regulators on the proliferation and differentiation of green globular bodies

The data in Table 4 show that NAA significantly promoted the proliferation of GGBs, whereas 6-BA exerted an inhibitory effect. Increasing the concentration of NAA in the absence of 6-BA increased the proliferation rate of GGBs to 9.7 at 5 mg·L^{-1}. Shoots were induced only in medium without PGRs.

Microscopic analyses

In vertical histological sections of a rhizome-induced GGB of *A. trapeziforme* (Fig. 2), the GGB was seen protruding from the vascular bundles of the rhizome. Newly developed vascular strands in the GGBs were connected to existing vascular tissues in the rhizome. Clusters of actively dividing cells were observed at the top of the GGB, along with hair-like structures at the periphery. Successive transverse sections of rhizome-induced GGBs illustrate the development process of GGBs (Fig. 3). Initially, a single GGB in its early stage displayed a closed circular or oval vascular bundle structure (Fig. 3a). Subsequently, the inner ring-shaped vascular bundles extended irregularly and protruded outward, and tightly packed meristematic cells formed on the outermost extensions (Fig. 3c). As GGBs developed, the number of peripheral hair-like structures increased, eventually covering most of the GGB surface. Ultimately, the number of GGBs increased from one to more than five, which can explain the potential of the fern to reproduce in large quantities *via* tissue culture of GGBs.

Table 4. Effect of 6-benzyladenine (6-BA) and α-naphthaleneacetic acid (NAA) on the proliferation and differentiation of green globular bodies (GGBs) on a medium consisting of 2 g·L^{-1} Hyponex, 30 g·L^{-1} sucrose, 2 g·L^{-1} tryptone, and 4 g·L^{-1} gellan gum after 30 days

Plant growth regulator (mg·L^{-1})		Green globular body fresh weight (g)	Green globular body proliferation rate	Number of shoots	Shoot induction rate (%)
6-BA	NAA				
0	0	0.058±0.013 cd	3.4±0.8 cd	2.5±0.7 a	40
	1	0.100±0.020 b	7.1±1.5 b	0 b	0
	5	0.159±0.044 a*	9.7±0.1 a	0 b	0
	10	0.099±0.039 b	6.4±0.0 b	0 b	0
1	0	0.044±0.015 de	2.6±1.2 de	0 b	0
	1	0.068±0.013 cd	4.4±0.9 c	0 b	0
	5	0.116±0.017 b	8.0±1.3 b	0 b	0
	10	0.069±0.020 c	4.4±1.5 c	0 b	0
5	0	0.016±0.001 f	0.3±0.1 f	0 b	0
	1	0.021±0.001 ef	0.7±0.2 f	0 b	0
	5	0.025±0.003 ef	0.9±0.2 ef	0 b	0
	10	0.025±0.004 ef	0.9±0.2 ef	0 b	0
10	0	0.015±0.002 f	0.2±0.2 f	0 b	0
	1	0.018±0.003 ef	0.4±0.2 f	0 b	0
	5	0.015±0.002 f	0.2±0.2 f	0 b	0
	10	0.014±0.003 f	0.1±0.2 f	0 b	0

Note: see Table 1

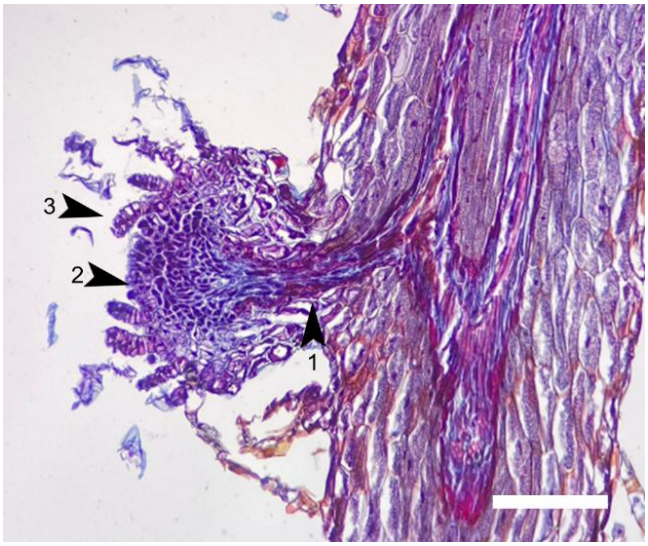

Figure 2. Morphology of paraffin sections of the green globular body of *Adiantum trapeziforme* developed from the rhizome (bar – 1 mm): the green globular body formed outside the vascular tissue inside the rhizome, new meristematic tissue constantly connected to the vascular tissue in the rhizome (arrow 1) formed inside the green globular body, the edge of the meristem of the green globular body forms a tightly arranged and vigorously dividing cluster of cells (arrow 2), numerous hair-like structures protruding outwards on the periphery of the green globular bodies (arrow 3)

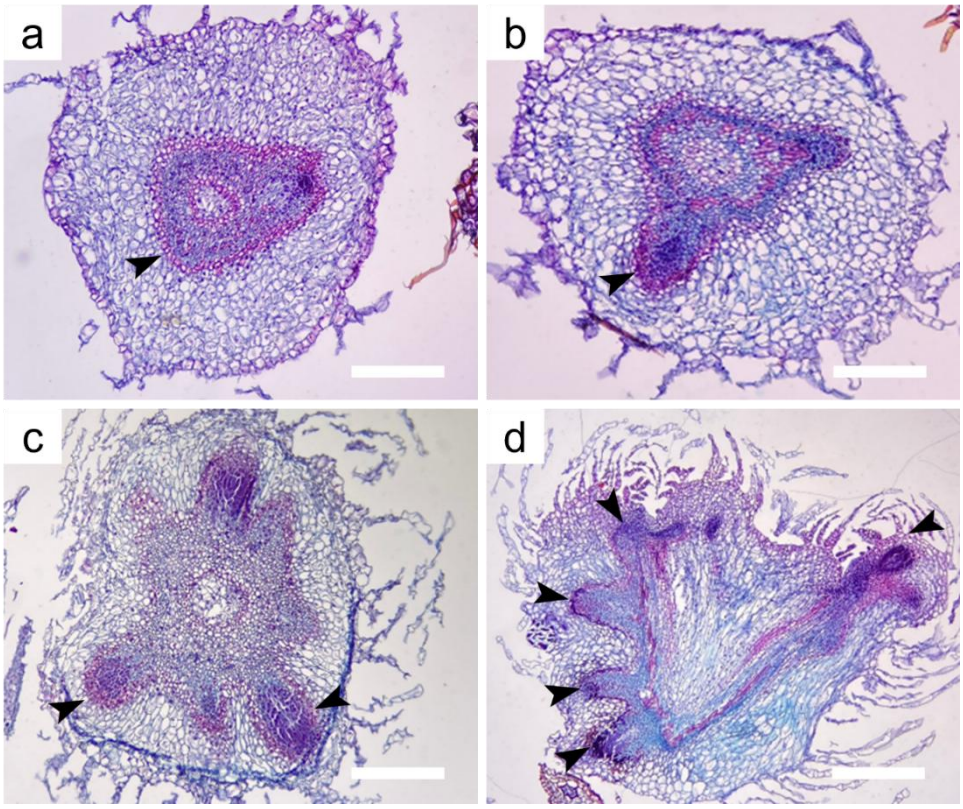

Figure 3. Morphology of paraffin transverse sections of proliferating green globular bodies of *Adiantum trapeziforme* (bar – 0.5 mm): a single green globular body at an early stage, a ring-shaped meristem (arrow) inside the green globular body (a), a meristem inside the green globular body starting to proliferate outwards (arrow) (b), a meristem starting to protrude towards the periphery and gradually increasing in size (arrows) and an increasing number of hair-like structures (c), a single green globular body proliferating at a late stage in all directions and forming several new green globular bodies (arrows) with a vascular ring extending through the entire green globular body (d)

DISCUSSION

In plant micropropagation, the base medium plays a key role in determining cell growth and differentiation by supplying essential micro- and macronutrients and regulating osmotic pressure, thereby supporting cell growth and development (Phillips & Garda 2019). MS is widely used in a wide range of crops, including ferns (Daniel 1993; Yu et al. 2021; Lo et al. 2022). The use of Hyponex-based media, however, has been reported primarily in orchid tissue culture, with relatively few documented cases in other crop groups. For example, studies on *Dendrobium moniliforme* showed that Hyponex-containing medium outperformed MS during the seedling growth stage (Hwang et al. 2024), and similar results were reported for *Gastrochilus fuscopunctatus* (Hwang et al. 2025). These findings explain why Hyponex is frequently adopted in orchid tissue culture systems. In fern tissue culture, MS remains the most commonly used basal salt formulation (Amaki & Kadokura 2009; Yu et al. 2017; Yu et al. 2021). However, our results comparing basal salts showed that Hyponex performed overall better than MS. Our previous study on *A. reniforme* var. *sinense* showed that Hyponex produced significantly higher fresh weight of GGBs and a greater number of regenerated shoots compared with MS (Lin et al. 2024). Together with the present results, these findings suggest that Hyponex may be an effective alternative to the basal salt formulation for *Adiantum* tissue culture, particularly for GGB proliferation and sporophyte regeneration.

In the sucrose concentration experiment, fresh weight increased with increasing sucrose concentration, reaching a maximum at 30 $g \cdot L^{-1}$, after which it declined. Sucrose serves multiple fundamental functions in plant tissue culture, acting not only as the primary carbon source for basal metabolism but also as a key regulator of osmotic pressure. These roles influence cell growth, biomass accumulation, and tissue differentiation (de Paiva Neto & Otoni 2003; Martínez et al. 2023). For example, in *Euonymus europaeus*, changes in osmotic pressure induced by sucrose concentration have been shown to affect the rate of somatic embryogenesis (Biahoua & Bonneau 1999). Similarly, in *Aquilaria malaccensis* callus culture, the highest fresh weight was observed at 15 $g \cdot L^{-1}$ sucrose, with biomass decreasing at higher concentrations (Jayaraman et al. 2014). These studies demonstrated that sucrose concentration has a species- and tissue-dependent effect on biomass production. In fern tissue culture, although several studies have evaluated the effects of sucrose concentration on the induction and proliferation of GGBs, fewer have emphasized its importance in optimizing culture protocols. Our previous work on *A. reniforme* var. *sinense* indicated that 15 $g \cdot L^{-1}$ sucrose was optimal for GGB proliferation (Lin et al. 2024). In contrast, the present study on *A. trapeziforme* showed that a concentration of 30 $g \cdot L^{-1}$ of sucrose was the most effective. Similar variability was reported by Amaki and Kadokura (2009), who showed that optimal sucrose concentrations in fern cultures are 15–30 $g \cdot L^{-1}$, depending on the species. Together, these results suggest that sucrose concentration is a key factor in fern tissue culture media and must be optimized depending on the species to maximize regeneration efficiency.

PGRs are essential in tissue culture, with cytokinins and auxins being the most widely used. In ferns, shoot, root, and GGB regeneration can be induced by using these PGRs alone or in combination (Rivera et al. 2018). Our results showed that 5 $mg \cdot L^{-1}$ NAA promoted the highest GGB proliferation (Fig. 1d, Table 4), similar to *A. reniforme* (Lin et al. 2024). However, unlike *A. reniforme*, where combined PGRs enhanced proliferation (Lin et al. 2024), NAA alone was the most effective in *A. trapeziforme*. These findings highlight the importance of selecting appropriate PGRs to maintain GGBs in a proliferative state. Shoot induction from GGBs in *A. trapeziforme* is similar to that of other ferns, occurring naturally without PGRs. However, the rate of induction varied depending on species and salt concentration (Yu et al. 2017; Pu et al. 2023).

Histological analysis of paraffin sections revealed that surface hair-like structures on GGBs correlated with active proliferation of meristematic tissue. The appearance of numerous hair-like structures indicated active division of the internal meristematic tissue, leading to the formation of multiple GGB units. Under optimal culture conditions, a single GGB could multiply to approximately 6 units (Fig. 3 d). These morphological features are consistent with previous findings demonstrating that GGBs contain multiple meristematic zones connected by vascular bundles, facilitating rapid proliferation (Higuchi et al. 1987).

CONCLUSIONS

This study evaluated the effect of medium components (including basal salts, sucrose, tryptone, and plant growth regulators) on the production and proliferation of green globular bodies and shoot differentiation in *Adiantum trapeziforme*. Based on the obtained results, the recommended medium for green globular body proliferation in *Adiantum trapeziforme* consists of 3 $g \cdot L^{-1}$ Hyponex, 30 $g \cdot L^{-1}$ sucrose, and 5 $mg \cdot L^{-1}$ α-naphthaleneacetic acid. The optimal medium for differentiation is 2 $g \cdot L^{-1}$ Hyponex without plant growth regulators. Histological observations confirmed that green globular bodies originate from the tissues of the vascular bundles of the rhizome and maintain internal meristematic connectivity during proliferation. This optimized system successfully developed a complete micropropagation protocol for *Adiantum trapeziforme*. Overall, this research provides a valuable foundation for commercial production and future breeding of *Adiantum trapeziforme* through rapid propagation using tissue culture based on green globular bodies.

Author contribution statement

WHC – A – Research concept and design, B – Collection and/or assembly of data, D – Writing the article
TSC – C – Data analysis and interpretation
SYL – B – Collection and/or assembly of data
CHS – D – Writing the article
CYK – E – Critical revision of the article, F – Final approval of the article

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