



RESEARCH ARTICLE

An optimised *in vitro* propagation protocol for scarlet banana (*Musa coccinea* Andrews) promoting efficient shoot multiplication, rooting and acclimatisation

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Abstract

Scarlet banana (*Musa coccinea* Andrews) is a valuable ornamental species, but its commercial production remains constrained by low multiplication rates, non-uniform planting material and limited availability of species-specific propagation protocols. This study developed a comprehensive *in vitro* regeneration protocol for *M. coccinea* by sequentially optimising basal medium, cytokinin and auxin concentrations, rooting conditions and acclimatisation substrates. To our knowledge, this is the first integrated protocol for *M. coccinea* that covers shoot initiation, shoot multiplication, root induction and *ex vitro* acclimatisation in a single regeneration system. Among the basal media tested, Murashige and Skoog (MS) medium produced the highest survival rate (88.88 %), the highest shoot initiation percentage (88.88 %) and the greatest shoot height (4.20 cm). During shoot multiplication, 2.0 mg L⁻¹ 6-benzylaminopurine (BAP) produced the highest number of shoots per explant, while the combination of 2.0 mg L⁻¹ BAP and 0.3 mg L⁻¹ α -naphthaleneacetic acid (α -NAA) further enhanced shoot proliferation and produced vigorous, compact shoots. Root induction was most effective on medium supplemented with 0.5 mg L⁻¹ α -NAA, which produced the greatest root number and root length. During acclimatisation, the substrate composed of alluvial soil, burnt rice husk and sand supported the highest survival rate and strongest vegetative growth. The study demonstrated that optimised nutrient composition, hormonal balance and substrate selection are critical for efficient regeneration. The optimised protocol provides a practical and reproducible system for large-scale production of uniform and high-quality *M. coccinea* plantlets for ornamental horticulture.

Keywords: acclimatisation; alpha-naphthaleneacetic acid; basal media; micropropagation; *Musa coccinea*; root induction; shoot proliferation

Introduction

Scarlet banana (*Musa coccinea* Andrews) is considered one of the most attractive ornamental species of the family Musaceae. This species is native to tropical and subtropical regions of Southern China and Northern Vietnam (1). Natural populations occur mainly in moist lowland forests and riverbank habitats in Guangxi and Guangdong, as well as in some areas of Northern Indochina (2). *Musa coccinea* possesses distinctive inflorescences composed of bright red to orange bracts arranged in overlapping layers that form a compact conical structure. Small tubular flowers remain enclosed within the bracts, while the vivid bract colour, symmetrical arrangement and long-lasting inflorescence confer strong ornamental value on the species (3). In landscape design, *M. coccinea* is frequently used in resorts, recreational parks, tropical gardens and architectural plantings that require bold visual presence. The species contributes to overall plant diversity in ornamental collections and provides nectar and shelter for pollinating insects. In several Asian cultures, its red inflorescences are

associated with prosperity, vitality and good fortune (4). Extracts of *M. coccinea* have been reported to contain bioactive compounds with antimicrobial and antioxidant properties, which expands interest in the species beyond strictly ornamental use (5).

Despite these advantages, commercial production of *M. coccinea* remains constrained by the limited availability of uniform and disease-free planting material. Conventional propagation through sucker division produces a low multiplication rate, generates non-uniform plantlets and increases the risk of transmitting fungal and viral pathogens from field-grown mother plants (6). These limitations reduce growers' ability to supply consistent planting materials for commercial landscaping and nursery production. Tissue culture offers a practical solution because it enables rapid multiplication of genetically uniform and pathogen-free plantlets under controlled conditions. In edible banana, micropropagation has been widely optimised through adjustment of basal media, cytokinin concentration, auxin supplementation, rooting treatments and acclimatisation procedures (7–9). However, protocols developed for edible *Musa* cultivars cannot be directly

applied to ornamental *Musa* species because regeneration response is highly genotype dependent. Differences in meristem activity, endogenous hormone balance, shoot proliferation capacity, rooting behaviour and sensitivity to plant growth regulators can strongly influence regeneration efficiency (10). Although several studies have described micropropagation approaches for edible banana, information on ornamental *Musa* species remains limited and the specific requirements of *M. coccinea* have not been systematically defined (7–9, 11). The current knowledge gap is not limited to shoot multiplication alone. Available information lacks an integrated protocol that combines shoot initiation, shoot proliferation, root induction and *ex vitro* acclimatisation in a single reproducible system. In particular, rooting efficiency and acclimatisation success are critical stages for commercial application, yet these steps are often less emphasised than shoot multiplication in previous *Musa* micropropagation studies (11, 12). The absence of species-specific optimisation can result in poor regeneration efficiency, weak root development, low survival during acclimatisation and inconsistent plantlet quality. Thus, a complete protocol for *M. coccinea* must evaluate nutrient composition, cytokinin and auxin balance, rooting conditions and substrate properties as connected stages of the regeneration process.

This study aims to establish an efficient and reproducible *in vitro* propagation system for *M. coccinea*. The study examined the effects of different basal media on shoot initiation, the influence of 6-benzylaminopurine (BAP) on shoot multiplication, the interaction between BAP and alpha-naphthaleneacetic acid (α -NAA), the role of α -NAA in root formation and the influence of substrates on *ex vitro* acclimatisation. The goal was to provide an integrated protocol that can support large-scale production of high-quality *M. coccinea* plantlets for landscape use and commercial horticulture.

Materials and Methods

Plant material and explant preparation

Shoot tip explants of *M. coccinea* were obtained from uniform, disease-free donor plants supplied by the Saigon Seedling Company (Vietnam) and processed immediately after arrival at the laboratory. Actively growing apical shoots were excised to include a 0.5–1.0 cm meristematic region with 1–2 leaf primordia. The explants were washed under running tap water for 20 min to remove surface debris, immersed in 1 % (v/v) Tween-20 solution for 5 min and then rinsed three times with distilled water. Surface sterilisation was performed in a Class II laminar flow cabinet. Explants were first immersed in 70 % (v/v) ethanol (C_2H_5OH) for 30 sec and then treated with 10 % (v/v) commercial sodium hypochlorite (NaOCl) solution supplemented with two drops of Tween-20 for 12 min with gentle agitation. After disinfection, explants were rinsed five times with sterile distilled water to remove residual chlorine. Outer sheathing leaves were aseptically removed with sterile forceps and a scalpel to expose a clean meristematic dome. Excess moisture was blotted on sterile, autoclaved filter paper before explants were inoculated into culture vessels containing the designated basal media. All manipulations were performed under aseptic conditions to maintain sterility during the initiation phase.

Experimental design and general culture conditions

All experiments followed a completely randomised design (CRD) under controlled *in vitro* conditions, as previously reported (13).

Experiments 1–4 included three replicates per treatment. Each replicate contained five culture vessels and each vessel held five explants, yielding a total of 75 explants per treatment. Experiment 5 included 3 replicates, with 30 plantlets per replicate during acclimatisation. Unless otherwise stated, all *in vitro* cultures were maintained at 25 ± 2 °C under a 16 hr light and 8 hr dark photoperiod at 2000–2500 lux using cool-white fluorescent lamps. All culture media contained 30 g L⁻¹ sucrose and 7 g L⁻¹ agar. The pH was adjusted to 5.8 before autoclaving at 121 °C for 20 min (14).

Effects of basal media on shoot initiation

Experiment 1 evaluated the influence of three basal media, including Murashige and Skoog (MS) medium (15), White (WH) medium (16) and Knudson C (KC) medium (17), with distinct nutrient compositions, on the shoot initiation of *M. coccinea*. The media were prepared according to their original formulations and supplemented with sucrose and agar as described previously (15, 17). Each medium received 75 explants. After 6 weeks of culture, the survival rate, shoot initiation percentage and shoot height were recorded. Survival rate was calculated as the percentage of viable explants relative to the total number of cultured explants. Shoot initiation percentage was calculated as the percentage of explants that produced at least one visible shoot apex. Shoot height was measured from the base of the explant to the tip of the tallest shoot using a digital ruler.

Effects of 6-benzylaminopurine on shoot multiplication

Experiment 2 evaluated the influence of BAP on shoot multiplication. MS medium was supplemented with 0.0, 1.0, 1.5, 2.0, 2.5 and 3.0 mg L⁻¹ BAP. Each treatment included 75 explants. After 6 weeks of culture, the number of shoots per explant (shoots explant⁻¹) and shoot height was recorded. Individual shoots were counted when they possessed distinct apical meristems. Shoot height was measured from the base of the explant to the apex of the tallest shoot.

Effects of combined 6-benzylaminopurine and alpha-naphthaleneacetic acid on shoot proliferation

Experiment 3 examined the combined influence of BAP and α -NAA on shoot proliferation. MS medium was supplemented with a fixed concentration of 2.0 mg L⁻¹ BAP and three concentrations of α -NAA, including 0.1, 0.3 and 0.5 mg L⁻¹. Each treatment included 75 explants. After 6 weeks of culture, shoot number per explant and shoot height were recorded as described above.

Effects of alpha-naphthaleneacetic acid on root induction

Experiment 4 evaluated the effect of α -NAA on root induction. Shoots obtained from the multiplication stage were transferred to MS medium supplemented with 0.3, 0.5 or 0.7 mg L⁻¹ α -NAA. Each treatment included 75 shoots. After 4 weeks of culture, root induction percentage, root number per shoot, root length and root quality were recorded. Root induction percentage was calculated as the percentage of shoots that formed at least one root. The root number was determined by counting all visible primary roots. Root length was measured from the root base to the root tip using a digital ruler. Root quality was assessed visually based on thickness, branching and the presence of healthy white root tissue.

Effects of substrate composition on acclimatisation

Experiment 5 assessed the effect of substrate composition on acclimatisation and early *ex vitro* growth of *M. coccinea* plantlets.

Plantlets selected for transplanting had an average height of approximately 8 cm, at least four fully expanded leaves and a minimum of three well-developed roots. Before transplanting, plantlets were gently removed from culture vessels, washed with sterile distilled water to remove adhering agar and maintained under nursery conditions for hardening. Three substrate formulations were tested: alluvial soil and burnt rice husk at a 1 : 1 ratio; alluvial soil, burnt rice husk and sand at a 1 : 1 : 1 ratio; and alluvial soil, burnt rice husk and coconut coir dust at a 1 : 1 : 1 ratio. The substrates were placed in plastic trays before transplanting. Plantlets were maintained under a 50 % shade net at 28 ± 2 °C and 70–80 % relative humidity. During the first week, plantlets were misted twice daily to reduce transplanting stress and maintain humidity around the foliage. Irrigation was applied manually using a fine-spray watering system once or twice daily, depending on substrate moisture. No fertiliser was applied during the first two weeks after transfer. After initial establishment, plantlets received a diluted balanced nutrient solution once per week. Nursery light intensity was maintained at approximately 3000–4000 lux under natural photoperiod conditions. After 6 weeks of acclimatisation, survival rate, plant height, leaf number, leaf length, leaf width, canopy diameter and root development were recorded. Survival rate was calculated as the percentage of living plantlets relative to the initial number of transplanted plantlets. Plant height was measured from the crown to the youngest leaf tip. Leaf number was determined by counting all leaves longer than 3 mm. Leaf length and leaf width were measured along the lamina using a digital ruler. Canopy diameter was measured in two perpendicular directions and averaged. Root development was assessed visually based on new root formation, branching and root colouration.

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA). Before ANOVA, the data were examined for normality and homogeneity of variance. Percentage data, including survival rate, shoot initiation percentage and root induction percentage were subjected to arcsine square-root transformation before statistical analysis to stabilise variance. When treatment effects were significant at $p < 0.05$, mean separation was performed using the least significant difference (LSD) test. Treatment means followed by different letters were considered significantly different. All statistical analyses were performed using IRRISTAT version 5.0 and data organisation and table preparation were completed using Microsoft Excel.

Results

Effects of basal media on shoot initiation of *Musa coccinea*

Basal medium significantly affected shoot initiation and early shoot growth of *M. coccinea* after 6 weeks of culture ($p < 0.05$) (Table 1). MS medium produced the highest survival rate and shoot initiation percentage, both reaching 88.88 % and these values were significantly higher than those recorded on KC and WH media. KC medium produced intermediate survival and shoot initiation values of 75.54 %, while WH medium produced the lowest values at 37.80 %. Shoot height also differed significantly among media. The greatest shoot height was recorded on MS medium at 4.20 cm, followed by KC medium at 3.50 cm and WH medium at 2.10 cm. Shoots formed on MS medium were vigorous, dark green and structurally firm, while shoots on WH medium were fewer, weaker and yellow-green.

Table 1. Effects of basal media on shoot initiation of *Musa coccinea* after 6 weeks of culture

#	Basal medium	Survival rate (%)	Shoot initiation (%)	Shoot height (cm)	Morphological quality
1	Murashige and Skoog	88.88 ^a	88.88 ^a	4.20 ^a	Numerous shoots, vigorous, dark green
2	White	37.80 ^c	37.80 ^c	2.10 ^c	Few shoots, weak, yellow-green leaves
3	Knudson C	75.54 ^b	75.54 ^b	3.50 ^b	Several shoots, healthy, light green

Means with different superscript letters within a column differ significantly at $p < 0.05$ according to the least significant difference test.

Effects of 6-benzylaminopurine concentration on shoot multiplication

6-benzylaminopurine (BAP) concentration significantly influenced shoot multiplication and shoot height of *M. coccinea* after 6 weeks of culture ($p < 0.05$) (Fig. 1, Table 2). The control treatment without BAP produced the lowest shoot number, with 6.60 shoots per explant. Shoot number increased with BAP supplementation and reached the highest numerical value at 2.0 mg L⁻¹ BAP, with 12.53 shoots per explant. This value was statistically comparable to that obtained at 1.5 mg L⁻¹ BAP but significantly higher than those obtained at 0.0, 1.0, 2.5 and 3.0 mg L⁻¹ BAP. Shoot height was also significantly affected by BAP concentration. The highest shoot height was recorded at 2.0 mg L⁻¹ BAP, reaching 4.45 cm and was significantly higher than all other treatments. Treatments with 2.5 and 3.0 mg L⁻¹ BAP produced lower shoot numbers and shorter shoots than the 2.0 mg L⁻¹ BAP treatment.

Table 2. Effects of 6-benzylaminopurine (BAP) concentration on shoot multiplication of *Musa coccinea* after 6 weeks

#	BAP concentration (mg L ⁻¹)	Shoot height (cm)	Shoot number (shoots explant ⁻¹)	Morphological quality
1	0.0	3.50 ^c	6.60 ^c	Yellow-green shoots, weak growth
2	1.0	4.01 ^b	8.35 ^b	Green leaves, moderate vigour
3	1.5	3.89 ^b	11.80 ^a	Healthy shoots, uniform green colour
4	2.0	4.45 ^a	12.53 ^a	Thick shoots, dark green leaves, strong vigour
5	2.5	4.00 ^b	9.86 ^b	Green shoots, slight growth reduction
6	3.0	3.87 ^b	9.07 ^b	Green shoots, reduced elongation

Means with different superscript letters within a column differ significantly at $p < 0.05$ according to the least significant difference test.

Interactive effects of 6-benzylaminopurine and alpha-naphthaleneacetic acid on shoot proliferation

The combination of BAP and α -NAA significantly affected shoot proliferation of *M. coccinea* ($p < 0.05$) (Fig. 2, Table 3). All treatments contained 2.0 mg L⁻¹ BAP, while α -NAA concentration varied. The combination of 2.0 mg L⁻¹ BAP and 0.3 mg L⁻¹ α -NAA produced the highest shoot number, with 14.33 shoots per explant, which was significantly higher than the values recorded at 0.1 and 0.5 mg L⁻¹ α -NAA. The same treatment also produced the highest shoot height, at 4.68 cm. Shoot height at 0.3 mg L⁻¹ α -NAA was significantly higher than that at 0.5 mg L⁻¹ α -NAA, while the 0.1 mg L⁻¹ treatment showed an intermediate value. The lowest shoot number was recorded at 0.5 mg L⁻¹ α -NAA, with 11.60 shoots per explant.



Fig. 1. Effect of 6-benzylaminopurine (BAP) concentrations on shoot multiplication of *Musa coccinea* after 6 weeks of culture. Representative culture vessels show shoot proliferation at 0.0, 1.0, 1.5, 2.0, 2.5 and 3.0 mg L⁻¹ BAP.

Table 3. Effects of 6-benzylaminopurine (BAP) and alpha-naphthaleneacetic acid (α-NAA) combinations on shoot proliferation of *Musa coccinea* after 6 weeks

#	BAP concentration (mg L ⁻¹)	α-NAA concentration (mg L ⁻¹)	Shoot height (cm)	Shoot number (shoots explant ⁻¹)	Morphological quality
1	2.0	0.1	4.37 ^{ab}	12.40 ^b	Green shoots, moderate vigour
2	2.0	0.3	4.68 ^a	14.33 ^a	Thick shoots, dark green, vigorous, many shoots
3	2.0	0.5	4.25 ^b	11.60 ^c	Light green shoots, weaker structure

Means with different superscript letters within a column differ significantly at $p < 0.05$ according to the least significant difference test.



Fig. 2. Interactive effects of 6-benzylaminopurine (BAP) and α-naphthaleneacetic acid (α-NAA) on shoot proliferation of *Musa coccinea* after 6 weeks of culture. Representative vessels show shoot development under 2.0 mg L⁻¹ BAP combined with 0.1, 0.3 or 0.5 mg L⁻¹ α-NAA.

Effects of alpha-naphthaleneacetic acid on root induction

Alpha-naphthaleneacetic acid (α-NAA) concentration significantly affected root development of *M. coccinea* shoots after 4 weeks of culture ($p < 0.05$) (Fig. 3, Table 4). The treatment with 0.5 mg L⁻¹ α-NAA produced the longest roots, with a mean root length of 14.99 cm and this value was significantly higher than those recorded at 0.3 and 0.7 mg L⁻¹ α-NAA. Root number also differed significantly among treatments. The highest root number was obtained at 0.5 mg L⁻¹ α-NAA, with 13.53 roots per shoot, while the 0.3 and 0.7 mg L⁻¹ treatments produced 11.27 and 10.40 roots per shoot, respectively. Root quality observations showed that plantlets cultured on 0.5 mg L⁻¹ α-NAA produced thick white roots with strong elongation and abundant root hairs.

Table 4. Effects of alpha-naphthaleneacetic acid (α-NAA) concentration on root induction of *Musa coccinea* after 4 weeks

#	α-NAA (mg L ⁻¹)	Root length (cm)	Root number (roots shoot ⁻¹)	Root quality
1	0.3	10.56 ^b	11.27 ^b	Medium thickness, moderate branching, many root hairs
2	0.5	14.99 ^a	13.53 ^a	Thick white roots, strong elongation, abundant root hairs
3	0.7	9.08 ^c	10.40 ^b	Thin roots, limited branching, fewer root hairs

Means with different superscript letters within a column differ significantly at $p < 0.05$ according to the least significant difference test.

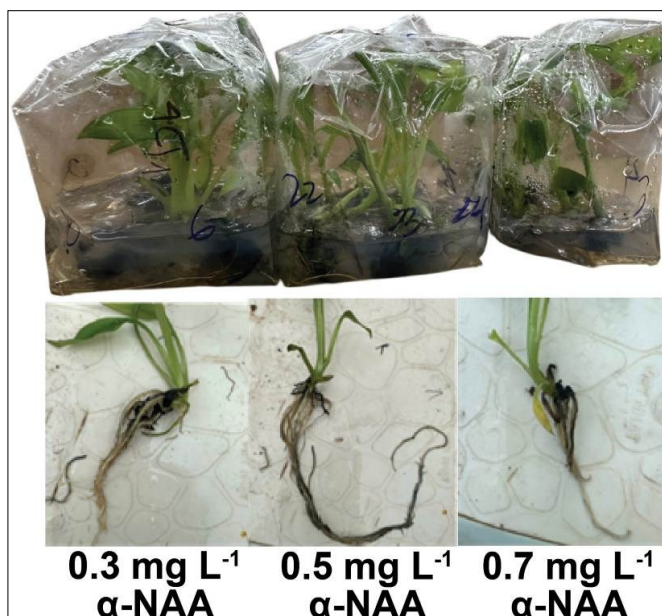


Fig. 3. Effects of α-naphthaleneacetic acid (α-NAA) concentrations on root induction of *Musa coccinea* after 4 weeks of culture. Representative culture vessels show shoot and root development at 0.3, 0.5 and 0.7 mg L⁻¹ α-NAA.

Effects of substrate composition on acclimatisation and *ex vitro* growth

Substrate composition significantly influenced survival and early *ex vitro* growth of *M. coccinea* plantlets after 6 weeks of acclimatisation ($p < 0.05$) (Fig. 4, Table 5). The substrate composed of alluvial soil, burnt rice husk and sand at a 1 : 1 : 1 ratio produced the highest survival rate at 95.57 %, which was significantly higher than the survival rates recorded in the other two substrates. This treatment also produced the greatest plant height at 15.01 cm, the highest leaf number at 10.47 leaves per plant, the greatest leaf length at 10.66 cm, the greatest leaf width at 4.67 cm and the widest canopy diameter at 15.07 cm. The substrate composed of alluvial soil and burnt rice husk at a 1 : 1 ratio produced intermediate values. The substrate containing alluvial soil, burnt rice husk and coconut coir dust produced the lowest survival rate and the weakest vegetative growth. These results suggest that the addition of sand to alluvial soil and burnt rice husk may have created more favourable substrate conditions for plantlet establishment, although substrate porosity and aeration were not directly measured in this experiment.

Discussion

The regeneration performance of *M. coccinea* was governed by coordinated effects of nutrient composition, plant growth regulators and acclimatisation substrate. Murashige and Skoog (MS) medium produced the highest survival rate, shoot initiation percentage and shoot height. These results indicate that early morphogenesis in this species requires a nutrient-rich basal medium as previously reviewed (12). The strong response on MS medium agrees with previous reports on banana and other monocot tissue culture systems, where inorganic nitrogen (N), potassium (K), calcium (Ca),



Fig. 4. Acclimatisation and *ex vitro* growth of *in vitro*-derived *Musa coccinea* plantlets under different substrate compositions after 6 weeks under nursery conditions. NL1: alluvial soil + burnt rice husk at a 1 : 1 ratio; NL2: alluvial soil + burnt rice husk + sand at a 1 : 1 : 1 ratio; NL3: alluvial soil + burnt rice husk + coconut coir dust at a 1 : 1 : 1 ratio.

magnesium (Mg) and phosphate support meristem activity, chlorophyll formation and early shoot growth (7–9, 15). However, responses to basal medium composition vary among *Musa* genotypes. Some genotypes tolerate reduced salt strength or modified MS formulations, whereas others show weak regeneration under media with lower mineral availability (11). In the present study, KC medium supported moderate initiation and WH medium produced poor survival and weak shoot growth. This pattern indicates that *M. coccinea* has a relatively high nutrient requirement during the initiation phase.

Shoot multiplication depended strongly on cytokinin concentration (18). 6-benzylaminopurine (BAP) increased shoot proliferation up to 2.0 mg L⁻¹, whereas higher concentrations reduced shoot number and shoot height. This result agrees with earlier studies on *Musa* micropropagation, where moderate BAP levels promoted axillary shoot formation and excessive cytokinin reduced shoot elongation or shoot quality (19). The response also highlights the genotype-dependent nature of cytokinin sensitivity. Different edible banana cultivars have shown different optimal BAP concentrations due to differences in explant type, endogenous hormone status and culture conditions (7–9). For this reason, protocols developed for edible bananas cannot be directly transferred to ornamental *Musa* species. Although hyperhydricity was not observed in the present cultures, high cytokinin levels are a common cause of hyperhydric or glassy shoots in *Musa* micropropagation. This concern supports the selection of 2.0 mg L⁻¹ BAP as the optimum concentration for high multiplication and acceptable shoot quality. The interaction between BAP and α -NAA showed that shoot proliferation in *M. coccinea* depends on hormonal balance instead of cytokinin concentration alone. The combination of 2.0 mg L⁻¹ BAP and 0.3 mg L⁻¹ α -NAA produced the highest shoot number and the most vigorous shoot morphology. This result is consistent with the role of cytokinin in shoot meristem activation and the role of low auxin levels in coordinated tissue expansion (20, 21). However, 0.5 mg L⁻¹ α -NAA reduced shoot proliferation. This decrease indicates that excess auxin can interfere with cytokinin-driven shoot formation (22). Similar inhibitory effects have been reported in other *Musa* systems when auxin levels exceed the requirement for organogenic balance (7–9). These findings show that the optimal auxin-cytokinin ratio is specific to species and developmental stage.

Root induction required a hormonal condition different from shoot proliferation. The strongest rooting response occurred at 0.5 mg L⁻¹ α -NAA, which produced the longest and most numerous roots. This result confirms the central role of auxin in the initiation and elongation of adventitious roots (23). However, 0.7 mg L⁻¹ α -NAA reduced both root length and root number. This decline may be associated with inhibitory effects of excessive auxin

Table 5. Effects of substrate composition on acclimatisation and early growth of *Musa coccinea* after 6 weeks

#	Substrate composition	Survival rate (%)	Plant height (cm)	Leaf number (leaves plant ⁻¹)	Leaf length (cm)	Leaf width (cm)	Canopy diameter (cm)	Growth characteristics
1	Alluvial soil + burnt rice husk (1 : 1)	84.43 ^b	12.82 ^b	8.53 ^b	8.35 ^b	3.54 ^b	12.51 ^b	Green leaves, stable growth
2	Alluvial soil + burnt rice husk + sand (1 : 1 : 1)	95.57 ^a	15.01 ^a	10.47 ^a	10.66 ^a	4.67 ^a	15.07 ^a	Strong growth, tall plants, wide canopy
3	Alluvial soil + burnt rice husk + coconut coir dust (1 : 1 : 1)	77.77 ^c	11.32 ^c	8.00 ^c	8.09 ^b	3.15 ^c	10.12 ^c	Low vigour, smaller leaves, reduced canopy spread

Means with different superscript letters within a column differ significantly at $p < 0.05$ according to the least significant difference test.

on root elongation and cellular expansion (24). The contrast between shoot proliferation and rooting responses shows that *M. coccinea* requires stage-specific hormonal adjustment. A cytokinin-dominant medium is suitable for multiplication, whereas a moderate auxin supply is required for root induction.

Acclimatisation success was highest in the substrate composed of alluvial soil, burnt rice husk and sand. This treatment produced the highest survival rate and the strongest vegetative growth among the tested substrates. Because substrate porosity, water-holding capacity and aeration were not directly measured, the role of physical substrate properties should be interpreted with caution. The observed plantlet performance suggests that this substrate mixture may have provided a more favourable balance between moisture retention and drainage during *ex vitro* establishment. Burnt rice husk and sand are commonly used to improve substrate structure (25), but their specific effects in the present experiment were inferred from plant growth responses. The lower performance of the coconut coir dust treatment may also exhibit moisture-related differences, but this interpretation requires further confirmation through measurements of substrate porosity, water retention, bulk density and oxygen availability (26). The findings demonstrate that efficient regeneration of *M. coccinea* depends on coordinated optimisation across all propagation stages. The study also highlights the limitations of directly applying edible banana protocols to ornamental *Musa* species. Regeneration efficiency varies among *Musa* genotypes due to differences in nutrient demand, endogenous hormone status, cytokinin sensitivity, rooting capacity and tolerance to *ex vitro* stress. Acclimatisation success also depends on environmental and nursery conditions such as shading level, humidity, irrigation frequency, substrate structure and light intensity. These sources of variation should be considered when the present protocol is adapted to other genotypes or production systems.

Conclusion

This study developed an integrated *in vitro* propagation protocol for *Musa coccinea* through the sequential optimisation of basal medium, plant growth regulators, rooting treatment and acclimatisation substrate. Murashige and Skoog (MS) medium supported effective shoot initiation, while 2.0 mg L⁻¹ 6-benzylaminopurine (BAP) promoted shoot multiplication. The combination of 2.0 mg L⁻¹ BAP and 0.3 mg L⁻¹ alpha-naphthaleneacetic (α -NAA) improved shoot proliferation and 0.5 mg L⁻¹ α -NAA supported root development. During acclimatisation, the substrate composed of alluvial soil, burnt rice husk and sand provided favourable conditions for plantlet survival and early growth. These findings indicate that efficient regeneration of *Musa coccinea* depends on coordinated optimisation across both *in vitro* and *ex vitro* stages. The protocol provides a practical basis for the production of uniform ornamental planting material and can support future studies on genetic stability, field performance and large-scale nursery application of *Musa coccinea*.

Authors' contributions

LNV conducted the experiments, collected data and drafted the manuscript. BTV assisted with laboratory work, data collection and figure preparation. HVT performed statistical analysis and

contributed to data interpretation. DPTN supported culture maintenance and data recording. HDC designed and supervised the study, interpreted the results, finalised and reviewed the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflicts of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Grammarly and Quillbot to edit grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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