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RESEARCH ARTICLE

DEVELOPMENT OF MICROPROPAGATION PROTOCOL FOR LITTLE GOURD (*COCCINIA GRANDIS* L.) UNDER IN VITRO CONDITIONS

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Abstract: An efficient protocol was developed for plant regeneration, multiplication and rooting under in vitro condition in *Coccinia grandis*. Seed setting is low and seeds are pseudo due to parthenocarpic nature. So far, farmers in India have been cultivating tindora traditionally through the age-old asexual cuttings, which are often infected by diseases and pests such as thrips and aphids, and therefore give low yields. Micro propagated tindora offers clean disease-free planting material and thereby giving as much as 10 times higher yield than traditional propagules. The present experiment was conducted at Center for Advance Research in Plant Tissue Culture, Anand Agricultural University, Anand during 2018 - 2019. Combination of bavistin (2000 ppm) for 18 min., cefotaxime (1000 ppm) for 14 min., kanamycin (1000 ppm) for 14 min., streptocyclin (1000 ppm) for 18 min. and 0.1% $HgCl_2$ for 8 min.for hard node explants, bavistin for 16 min., cefotaxime for 12 min., kanamycin for 12 min., streptocycline for 16 min. and 0.1% $HgCl_2$ for 7 min. for soft nodal explants and bavistin for 10 min., cefotaxime for 6 min., kanamycin for 6 min., streptocyclin for 8 min. and 0.1% $HgCl_2$ for 2 min. was found best for surface sterilization in the term of fungal contamination, bacterial contamination and drying of the all explants. Half strength of MS was the best initiation medium for all the explants. Treatment T_6 took lesser number of days to sprouting and gave higher number of sprouted explants and response percentage at 7, 14 and 21 days after initiation. Half strength of MS supplemented with 0.1 BA mgl^{-1} was optimum for maximum multiplication of shoots. Half strength of MS supplemented with 0.1 mgl^{-1}BA+1.0 $mgl^{-1}GA_3$ was optimum for maximum length of shoots. Half strength of MS supplemented with 1.0 IBA mgl^{-1} was optimum for maximum root length. Half strength of MS supplemented with 2.0 IBA mgl^{-1} was optimum for maximum number of roots. Coco peat was best suited for overall development of plants during primary hardening.

Keywords: Micro propagation, Parthenocarpic, Hardening

INTRODUCTION

Coccinia grandis, commonly known as ivy gourd, baby watermelon, or little gourd, belongs to the family Cucurbitaceae, which comprises nearly 960 species. Most cucurbits are annual climbing vines, and *C. grandis* is an aggressive perennial climber capable of forming dense mats that readily smother shrubs and young trees. The leaves are simple and alternately arranged, variable in shape from heart-shaped to pentagonal, and may grow up to 10 cm in length. The upper leaf surface is green and glabrous, whereas the lower surface is pale green with fine hairs. Tendrils are simple. The plant bears solitary white flowers, with a calyx of five recurved lobes, ovate corolla lobes, three stamens, and an inferior ovary. The fruit is slimy, pulpy and varies from ovoid to ellipsoid or cylindrical, typically exhibiting about ten white longitudinal stripes on the posterior region. It is green when immature and turns scarlet red upon ripening, measuring 2.5–6.0 cm in length and containing numerous oblong seeds (6–7 mm) (Pekamwar et al., 2013).

The chemical composition of *C. grandis* includes 12.62% carbohydrates, 15% total protein, 11.25% water-soluble protein, 4.0% lipids, 61.92 mg/100 g total phenols, 25.55 mg/100 g vitamin C, 70.05 mg/100 g β-carotene, and appreciable amounts of minerals such as potassium (3.38 mg/100 g), phosphorus (1.15 mg/100 g), sodium (0.95 mg/100 g), iron (2.23 mg/100 g), and calcium (3.79 mg/100 g). The fruits contain a diverse range of bioactive compounds including steroids, saponins, ellagic acid, terpenoids, lignin, alkaloids, tannins, flavonoids, glycosides, phenols, β-amyrin acetate, lupeol, taraxerol, carotenoids, lycopene, cryptoxanthin, xyloglucan and β-sitosterol (Khatun et al., 2012).

Phytochemical analyses indicate the presence of saponins, flavonoids and polyphenols that contribute to the antioxidant and anti-inflammatory properties of the leaves (Umamaheswari & Chatterjee, 2008). Traditionally, *C. grandis* is

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widely used in indigenous medicine, particularly for the management of diabetes, and has been described as the "Indian substitute for insulin."

Reproductive biology of *C. grandis* poses challenges for large-scale cultivation. Seed setting is extremely low, and most seeds formed are pseudo-seeds due to its parthenocarpic nature. In India, farmers with limited resources continue to multiply the crop through age-old vegetative methods using vine cuttings, which are often infected with pests such as thrips and aphids as well as systemic diseases, resulting in reduced yield. Micro propagated plants, in contrast, provide disease-free planting material and have been reported to yield nearly ten times more than traditional propagules. Although *C. grandis* is dioecious, parthenocarpy leads to the cultivation of female plants only under field conditions. This selective bias has contributed to a gradual reduction in natural populations of little gourd.

Considering the declining natural population, poor seed fertility, and the urgent need for clean planting material, development of an efficient in vitro micropropagation protocol is essential for large-scale multiplication and conservation of this important medicinal cucurbit. Therefore, the present study was undertaken to standardize a reliable protocol for mass propagation of *C. grandis* using shoot tip and nodal explants.

MATERIALS AND METHODS

The present investigation was conducted at the Center for Advanced Research in Plant Tissue Culture, Anand Agricultural University, Anand. The experimental site is located at 22.35° N latitude and 72.55° E longitude, at an elevation of 45.1 m above mean sea level. Explants were collected from elite *Coccinia grandis* plants maintained in the farmer's field and in the greenhouse of the Center. All experiments were laid out in a Completely Randomized Design (CRD).

The basal medium used for culture was Murashige and Skoog (MS) medium supplemented with 3% (w/v) sucrose, 0.8% (w/v) agar, and appropriate concentrations of plant growth regulators (Murashige and Skoog, 1962). The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 minutes at 15 psi.

Nodal segments and shoot tips were excised from healthy plants and initially washed under running tap water to remove surface debris and microorganisms. They were then treated with 2–3 drops of Tween-20 for 7–8 minutes with continuous swirling, followed by thorough rinsing with sterile distilled water. Surface sterilization was carried out using a combination of antibiotics (Streptomycin, Kanamycin, Cefotaxime), a fungicide (Bavistin), and a heavy metal disinfectant ($HgCl_2$) for varying durations. After treatment, explants were rinsed several times with sterile distilled water. All procedures were performed aseptically inside a laminar airflow cabinet.

For culture establishment, explants showing initial bud break were transferred to MS medium containing different combinations of auxins and cytokinins after 21 days of incubation. Multiple shoot induction was achieved on MS medium supplemented with varying concentrations of BA, GA_3 and other growth regulators. The most effective medium for shoot multiplication was identified through repeated subculturing and observation of shoot proliferation. All culture vessels were handled under aseptic conditions and incubated in the growth room under controlled environmental conditions.

For rooting, individual healthy shoots measuring approximately 4–6 cm in length were excised and transferred to half-strength MS medium containing suitable concentrations of root-promoting auxins, primarily indole-3-butyric acid (IBA).

Hardening of tissue culture–raised plantlets was carried out in the greenhouse using different hardening substrates such as cocopeat alone and in combination with soil. Plantlets were regularly supplemented with a nutrient solution and protected from infections using mild antifungal applications until acclimatization was completed.

RESULTS AND DISCUSSION

The effect of different surface sterilization treatments (T_1–T_6) on fungal contamination percentages in hard node, soft node, and shoot tip explants of *Coccinia grandis* is presented in Table 1. Each treatment consisted of a sequential combination of fungicide (Bavistin), antibiotics (Cefotaxime, Kanamycin, Streptocycline), and a disinfectant ($HgCl_2$), with variations in exposure time. The results clearly indicate that the duration and combination of sterilants significantly influenced contamination levels across all explant types.

Across treatments, fungal contamination ranged from 0.0 to 10% in hard nodes**,** 1.0 to 9.0% in soft nodes**,** and 1.0 to 10% in shoot tips**.** The highest fungal contamination was recorded in T_1**,** with 10% in hard nodes, 9% in soft nodes, and 8% in shoot tips. This suggests that the exposure durations applied in T_1 were insufficient to eliminate fungal spores, particularly in explants with higher surface microbial load such as hard nodes.

In contrast, T_6 was the most effective sterilization treatment**,** yielding 0% contamination in hard nodes**,** and only 1% in both soft node and shoot tip explants. This treatment involved the longest cumulative exposure to Bavistin (18 min), Streptocycline (18 min), and slightly extended exposure to $HgCl_2$ compared to other treatments. The improved sterilization efficiency in T_6 can be

attributed to the synergistic action of the fungicide and antibiotics, combined with adequate $HgCl_2$ exposure, which collectively inhibit a wide spectrum of fungal contaminants.

Intermediate treatments (T_2, T_3, T_4, and T_5) showed progressively reduced contamination as exposure durations increased, but none were as effective as T_6. For example, T_5 recorded low contamination levels (2% in hard nodes, 2% in soft nodes, 1% in shoot tips)**,** indicating partial effectiveness, but still higher fungal presence compared to T_6. Notably, shoot tips consistently showed slightly lower contamination than hard nodes in all treatments except T_1, likely due to their younger, less lignified tissue which harbors fewer fungal propagules.

Overall, the data demonstrate that longer and sequential exposure to combined Bavistin–antibiotic–$HgCl_2$ treatment (T_6) is essential for achieving near-complete elimination of fungal contaminants in *C. grandis* explants. These findings align with previous studies in cucurbit tissue culture, where extended sterilant exposure significantly improved aseptic culture establishment (Devi et al. 2017).

Overall Response of Explants to Sterilization Treatments

Table 2 summarizes the influence of six sterilization treatments on bacterial contamination in hard node, soft node and shoot tip explants of *Coccinia grandis*. The results show that the percentage of bacterial contamination varied considerably among treatments, indicating that the duration and combination of fungicides, antibiotics and disinfectants had a significant impact on the efficiency of surface sterilization. Hard and soft node explants generally exhibited higher levels of bacterial contamination compared to shoot tips, which is expected because nodes are physiologically older tissues with more crevices and internal microbial load.

Performance of Lower-Duration Treatments (T_1–T_3)

The first three treatments, which involved comparatively shorter exposure times to sterilants, were less effective in controlling bacterial contamination. In treatment T_1, bacterial contamination reached 4.0% in hard nodes, 6.0% in soft nodes and 2.0% in shoot tips. These values suggest inadequate penetration and microbial suppression by antibiotics, likely because of insufficient exposure to Cefotaxime, Kanamycin and Streptocycline. Treatment T_2 showed slight improvement, with contamination levels decreasing to 1.0% in hard nodes, 3.0% in soft nodes and 1.0% in shoot tips. However, T_3 again exhibited increased bacterial contamination in hard nodes (6.0%) and in shoot tips (3.0%), indicating that the moderate variation in exposure time in T_2 and T_3 was not uniformly effective. Similar variations in contamination with shorter sterilization durations have been reported in cucurbits such as *Momordica dioica* and *Momordica cymbalaria*, where inadequate sterilant exposure leads to partial survival of endophytic bacteria (Devi et al., 2017).

Performance of Intermediate-Duration Treatments (T_4 and T_5)

The intermediate treatments, T_4 and T_5, showed considerably improved control of bacterial contamination. In T_4, contamination levels were reduced to 5.0% in hard nodes, 3.0% in soft nodes and 4.0% in shoot tips. The improved performance is mainly attributable to the extended exposure to Bavistin, Cefotaxime and Streptocycline, which appeared to suppress surface-residing bacteria more effectively. Treatment T_5 performed even better, with contamination levels of 2.0% in hard nodes and only 1.0% in soft nodes and shoot tips. The higher efficiency in T_5 can be linked to the balanced combination of moderate $HgCl_2$ exposure and extended antibiotic treatment. Comparable improvements with prolonged sterilant exposure have been documented in *Trichosanthes dioica* (Kumar et al., 2003), demonstrating that intermediate exposure times often achieve a suitable balance between microbial suppression and tissue viability.

Effectiveness of the Longest-Duration Treatment (T_6)

Treatment T_6 was the most effective sterilization regime, yielding only 1.0% bacterial contamination in both hard and soft node explants and complete elimination (0.0%) of bacterial contamination in shoot tip explants. The superior performance of T_6 is attributed to the longest exposure durations to Bavistin (18 min), Streptocycline (18 min) and slight extension in $HgCl_2$ treatment, along with consistent exposure to Cefotaxime and Kanamycin. This extended and synergistic antibiotic-fungicide-disinfectant combination appears to neutralize both surface-associated and endophytic bacteria. Shoot tips, being physiologically young tissues with smoother epidermal surfaces, responded exceptionally well to T_6, resulting in zero contamination. These results strongly agree with earlier reports in cucurbits where prolonged antibiotic/sterilant exposure yielded near-sterile explants (Devi et al., 2017).

Comparative Trend Across Explant Types

A consistent trend is clearly visible across explant types. Hard nodes showed the highest bacterial contamination in treatments with shorter exposure durations, due to their higher microbial load and thicker tissues. Soft nodes exhibited intermediate contamination levels, while shoot tips consistently showed the lowest contamination across treatments. This pattern matches observations in other cucurbitaceous species where meristematic tissues are more responsive to sterilization than nodal tissues (Sarowar et al., 2003). The gradual decrease in contamination from T_1 to T_6 reflects the

cumulative effectiveness of longer sterilization durations and stronger combinations of antibiotics and disinfectants.

General Response of Explants to Sterilization Treatments

Table 3 presents the drying (desiccation) percentage observed in hard node, soft node and shoot tip explants of *Coccinia grandis* subjected to six surface sterilization treatments. Drying of explants is a common physiological response to chemical stress induced by sterilants, particularly heavy metals such as mercuric chloride ($HgCl_2$), detergents and high concentrations of antibiotics. The percentage of drying across treatments ranged from 0.0% to 6.0%, indicating differential sensitivity of the explants depending on their tissue type and the intensity of sterilization. Shoot tips consistently showed higher drying percentages than hard and soft nodes due to their delicate meristematic nature, a trend also reported in cucurbit tissue cultures by Devi et al. (2017).

Response Under Lower Sterilization Exposure

In treatment T_1, drying was highest across explants, with 2.0% in hard nodes, 3.0% in soft nodes and 6.0% in shoot tips. This heightened dryness can be attributed to the rapid osmotic shock and membrane injury produced by shorter but more abrupt exposure to multiple sterilants, particularly $HgCl_2$. The initial shock of sterilant exposure often leads to dehydration of explants, especially in species where epidermal tissues are thin. Treatment T_2 showed reduced drying, with only 1.0% in hard and soft nodes and 3.0% in shoot tips, suggesting that the slight increase in Streptocycline exposure provided better microbial control without markedly increasing tissue stress.

Response Under Moderate Sterilization Durations

Treatments T_3 and T_4 maintained relatively low drying percentages in hard and soft nodes (0–1.0%), demonstrating that these tissues tolerated moderate exposure durations without significant desiccation. Soft nodes exhibited slightly higher drying (1.0%) compared to hard nodes, likely due to a thinner epidermis and higher water content, making them more sensitive to osmotic stress. Shoot tips, however, consistently showed drying around 3.0% in T_3, T_4 and T_5, indicating that they remained the most vulnerable tissue type. Research by Kumar et al. (2003) in *Trichosanthes dioica* confirms that shoot meristems are highly sensitive to $HgCl_2$ and prolonged antibiotic treatments, which disrupt membrane integrity and accelerate dehydration.

Treatment T_5 demonstrated improved tolerance in soft nodes (0.0% drying), which suggests that the balance between antibiotic exposure and $HgCl_2$ treatment was optimal in minimizing tissue stress. However, shoot tips still exhibited 3.0% drying, reflecting their inherent sensitivity.

Best Performance Under Longest Sterilization Duration

Treatment T_6 was the most effective in minimizing drying across all explant types. Both hard nodes and soft nodes recorded 0.0% drying, indicating that these tissues tolerated the extended sterilization duration without physiological dehydration. Shoot tips also showed minimum drying at 1.0%, demonstrating that the combination of prolonged antibiotic exposure and slightly extended $HgCl_2$ treatment provided sterilization efficiency while minimizing tissue stress. These results align with the findings of Devi et al. (2017), who reported that optimized exposure to Bavistin and antibiotics reduced drying and browning in *Momordica cymbalaria* explants. Similarly, Sarowar et al. (2003) noted that controlled exposure to $HgCl_2$ prevents excessive plasmolysis and dehydration in cucurbits, supporting the present findings.

Comparative Sensitivity of Explant Types

A clear pattern emerges from the data: shoot tips exhibited the highest drying across treatments, followed by soft nodes and hard nodes. Meristematic shoot tips contain higher water content, thinner cell walls and more tender tissues, making them more susceptible to osmotic injury, as noted in earlier studies on cucurbit micropropagation. Hard nodes, being more lignified, consistently showed the least drying across all treatments. The almost negligible drying observed in T_6 suggests that the sequential sterilization strategy successfully minimized physiological stress in all explant types.

Overview of Shoot Multiplication Response at Second Subculture

Table 6 summarizes the influence of different MS-based media combinations supplemented with varying concentrations of BA, GA_3, NAA, IAA and kinetin on shoot multiplication in *Coccinia grandis* at 42 days after culture initiation. All media supported shoot induction, as indicated by 100% shooting percentage, demonstrating that the species responds well to cytokinin-rich medium even at half-strength MS. Similar universal shoot induction in cucurbits has been reported in *Momordica cymbalaria* (Devi et al., 2017) and *Trichosanthes dioica* (Kumar et al., 2003).

Effect of BA-Supplemented Media on Shoot Number

The treatments containing BA alone (M1 to M5) exhibited marked differences in shoot multiplication. The highest number of shoots (2.75) was observed under medium **M4 (½ MS + 0.1 mg L^{-1} BA)**, indicating that BA at moderate concentrations promotes optimal cytokinin-mediated cell division and axillary bud break. Lower or higher concentrations of BA (e.g., 0.01 or 0.2 mg L^{-1}) produced fewer shoots, suggesting a threshold beyond which BA does not further stimulate axillary proliferation due to hormonal

imbalance. Similar concentration-dependent responses to BA have been documented in *Momordica dioica* and *Coccinia abyssinica* (Bekele et al., 2013).

Effect of BA and GA_3 Combination on Shoot Multiplication and Elongation

Media containing combinations of BA with GA_3 (M7–M10) showed varied responses. Moderate proliferation occurred in M_8 (½ MS + 0.05 mg L^{-1} BA + 1.0 mg L^{-1} GA_3) with two shoots per explant, whereas the highest shoot length (5.35 cm) was recorded in M9 (½ MS + 0.1 mg L^{-1} BA + 1.0 mg L^{-1} GA_3), indicating the synergistic role of gibberellin in promoting cell elongation. The increased elongation in BA + GA_3 combinations aligns with reports in *Momordica charantia* (Verma et al., 2014), where GA_3 enhanced internode elongation without substantially increasing shoot number. Higher BA combined with GA_3 (M10) reduced shoot length (3.45 cm), suggesting possible hormonal antagonism at elevated cytokinin levels.

Effect of GA_3 Alone on Shoot Development

Media M11 and M12 contained GA_3 either alone or combined with very low BA. These treatments resulted in poor shoot multiplication (1.00 shoot per explant) but moderate shoot elongation (3.37–4.50 cm). This supports the physiological role of GA_3 as primarily an elongation promoter rather than a shoot multiplication regulator. Similar findings were reported by Devi et al. (2017), where GA_3 increased shoot height but did not substantially influence axillary bud proliferation.

Effect of BA + NAA Combination on Shoot Response

Treatments M_{16} to M_{18}, containing BA with low NAA (0.05 mg L^{-1}), resulted in minimal shoot proliferation (1.00–1.75 shoots) and short shoot length (1.52–2.15 cm). The presence of auxin along with cytokinin likely influenced apical dominance and callus induction at the basal region, thereby reducing shoot multiplication efficiency. Similar inhibitory effects of BA–NAA combinations on shoot proliferation have been reported in *Cucumis sativus* and *Momordica cymbalaria* (Devi et al., 2017).

Effect of IAA + Kinetin Combination on Shoot Characteristics

In media M19–M25, IAA was combined with kinetin (Kn), producing generally low shoot numbers (1.00–2.00) and short shoot lengths (1.32–2.17 cm). This cytokinin type was less effective than BA for shoot proliferation, confirming earlier reports that kinetin is comparatively weaker than BA in cucurbits for stimulating axillary bud activity (Kumar et al., 2003). The minimum shoot length (1.32 cm) in M25 supports the observation that high kinetin relative to IAA does not enhance elongation. Treatments such as M19 and M23 produced moderate multiplication (2 shoots), but overall, the IAA + kinetin combination was significantly inferior to BA-based treatments.

Overall Performance of Media in Second Subculture

Two media formulations exhibited clear and complementary advantages during the multiplication phase. Medium M4 (½ MS supplemented with 0.1 mg L^{-1} BA) proved most effective for maximizing shoot proliferation, producing an average of 3.00 shoots per explant. In contrast, Medium M9 (½ MS containing 0.1 mg L^{-1} BA and 1.0 mg L^{-1} GA_3) supported superior shoot elongation, with shoots reaching an average length of 4.57 cm. These results reinforce the distinct functional roles of the growth regulators: BA serves as the primary cytokinin responsible for inducing multiple shoot buds, while GA_3 promotes internodal elongation and overall shoot extension. Similar synergistic responses between BA and GA_3 have been reported in related cucurbit micropropagation systems, further validating the observed patterns (Devi et al., 2017; Verma et al., 2014).

Overall Response During the Third Subculture

Table 7 presents the performance of different MS-based media formulations on the number of shoots, shoot elongation and shooting percentage in *Coccinia grandis* at the 63-day stage (third subculture). All treatments recorded 100% shooting response, indicating that once established, *C. grandis* maintains a stable regeneration capacity through successive subcultures. A similar high survival percentage in subsequent subcultures has been reported in *Momordica cymbalaria* (Devi et al., 2017) and *Trichosanthes dioica* (Kumar et al., 2003), confirming that cucurbits are resilient during in vitro proliferation.

Effect of BA-Based Media on Shoot Multiplication

The BA-only treatments (M1–M5) again displayed a clear concentration-dependent response. Among these, **M4 (½ MS + 0.1 mg L^{-1} BA)** produced the highest number of shoots (3.00), maintaining its superiority from the second subculture. This demonstrates that 0.1 mg L^{-1} BA is the optimum concentration for repeated axillary bud induction in *C. grandis*. Lower (0.01 mg L^{-1}) or higher (0.2 mg L^{-1}) BA concentrations produced only one shoot per explant, suggesting that either hormonal insufficiency or supra-optimal cytokinin levels inhibited shoot proliferation. A similar optimum for BA (0.1–0.5 mg L^{-1}) has been documented for shoot multiplication in *Momordica charantia* (Verma et al., 2014) and *Coccinia abyssinica* (Bekele et al., 2013).

Effect of BA + GA_3 Combination on Shoot Numbers and Elongation

In treatments with BA + GA_3 (M7–M10), shoot numbers remained moderate (1.00–2.00), with M_7 and M_{10} producing two shoots per explant. Meanwhile, shoot elongation was pronounced in M_9

(4.57 cm) and M_{14} (4.25 cm), indicating that GA_3 showed a strong synergistic effect with BA for promoting internodal elongation. The role of GA_3 in enhancing shoot length is consistent with studies in *Momordica cymbalaria* (Devi et al., 2017), where GA_3 significantly improved shoot extension without substantially increasing multiplication.

Effect of GA_3 Alone and Combined with Low BA

GA_3 alone (M_{11}) produced fewer shoots (1.25) but reasonable shoot elongation (2.97 cm), confirming its elongation-dominant role. When GA_3 was combined with very low BA (M12), elongation improved slightly (3.57 cm), but multiplication remained low. This pattern mirrors similar results in cucurbits where GA_3 alone is insufficient for bud proliferation but enhances elongation (Sarowar et al., 2003).

Effect of BA + NAA on Shoot Development

Auxin–cytokinin mixtures (M_{16}–M_{18}) produced weak shoot responses, with only **one shoot per explant** and short shoot length (1.47–1.97 cm). The presence of NAA likely enhanced apical dominance or callus formation, thereby suppressing axillary bud development. Previous reports in *Cucumis sativus* and *Momordica* species show that NAA in combination with BA often reduces multiplication rate by shifting the developmental pathway toward callusing rather than shoot organogenesis (Devi et al., 2017).

Effect of IAA + Kinetin on Shoot Multiplication and Growth

Treatments M19–M25 containing IAA + Kn produced generally lower performance in both shoot number and length. Although M20 and M21 generated two shoots per explant, the shoot length remained inferior (2.40–2.44 cm). The lowest shoot length values (1.47 cm) were observed in M24 and M25. These results confirm that kinetin is less effective than BA for stimulating shoot multiplication in cucurbits, consistent with findings in *Trichosanthes dioica* (Kumar et al., 2003).

Comparison and Identification of Best Media

Two media formulations exhibited clear and complementary advantages during the multiplication phase. Medium M_4 (½ MS supplemented with 0.1 mg L^{-1} BA) proved most effective for maximizing shoot proliferation, producing an average of 3.00 shoots per explant. In contrast, Medium M_9 (½ MS containing 0.1 mg L^{-1} BA and 1.0 mg L^{-1} GA_3) supported superior shoot elongation, with shoots reaching an average length of 4.57 cm. These results reinforce the distinct functional roles of the growth regulators: BA serves as the primary cytokinin responsible for inducing multiple shoot buds, while GA_3 promotes internodal elongation and overall shoot extension. Similar synergistic responses between BA and GA_3 have been reported in related cucurbit micropropagation systems, further validating the observed patterns (Devi et al., 2017; Verma et al., 2014).

Root Induction Response in *Coccinia grandis*

The results presented in Table 8 show the influence of different concentrations of indole-3-butyric acid (IBA) on the rooting behaviour of *Coccinia grandis* shoots cultured on half-strength MS medium. A clear variation was observed in the number of days required for root induction, root length, and number of roots, indicating a strong concentration-dependent physiological response to IBA application.

Days Required for Root Induction

The data show that R5 (½ MS + 2.0 mg L^{-1} IBA) required the longest period for root initiation (10.25 days), which was statistically at par with R1 and R4. The delay at higher IBA concentrations suggests that supra-optimal auxin levels may temporarily suppress root initiation due to auxin-induced tissue browning or cellular stress. In contrast, the earliest root induction (8.75 days) occurred in R2 (0.5 mg L^{-1} IBA), indicating that lower auxin concentrations favor quicker physiological activation of root primordia. Similar observations were reported by Sarowar et al. (2003), where 1.0 mg L^{-1} IBA significantly enhanced rooting efficiency in interspecific *Cucurbita* hybrids without delaying root formation.

Root Length Response

Root elongation also exhibited marked differences among treatments. The highest mean root length was recorded in R3 (6.02 cm), indicating that **1.0 mg L^{-1} IBA** is the most effective concentration for promoting vigorous root elongation in *C. grandis*. This coincides with findings in *Momordica charantia*, where 1.0 mg L^{-1} IBA produced the longest roots and best anchorage quality (Verma et al., 2014). The minimum root length (3.50 cm) was observed in the hormone-free medium R1, demonstrating that endogenous auxin alone is insufficient for achieving optimum rooting in this species.

Number of Roots Formed

A similar trend was observed for root number, with R3 and R5 both producing the highest number of roots (7.00), indicating that moderate to moderately high levels of IBA effectively promote root proliferation. However, R5 produced many roots but shorter initiation time and shorter initial length, suggesting a compensation mechanism commonly observed under high auxin concentrations. The lowest number of roots (1.0) was recorded in R1, reinforcing the necessity of exogenous auxin for robust root induction. Reports for *Coccinia abyssinica* (Bekele et al., 2013) also support that half-strength MS media supplemented with IBA significantly increases the number of roots and their elongation, with more than 90% success in rooting.

Rooting Percentage

All treatments resulted in 100% rooting, confirming that *C. grandis* has a strong inherent capacity for adventitious root formation once shoots reach a physiologically competent stage. This universal rooting response aligns with findings in *Cucurbita* hybrids (Sarowar et al., 2003) and *Momordica cymbalaria* (Devi et al., 2017), where well-developed shoots consistently rooted in IBA-enriched medium.

Summary of Rooting Phase

Collectively, the results demonstrate that **1.0 mg L^{-1} IBA (R3)** provides the best overall rooting performance in *C. grandis*, producing the longest roots and highest root numbers within a reasonable induction period. The consistency of these results with earlier cucurbit micropropagation studies suggests that *C. grandis* follows the general cucurbit pattern of rooting enhanced by moderate IBA supplementation.

Table 4.9 describes the performance of tissue-cultured plantlets of *Coccinia grandis* during acclimatization under three different hardening substrates. The results demonstrate a clear impact of substrate composition on survival percentage.

Survival and Mortality Patterns During Hardening

Among the media tested, cocopeat alone (H1) provided the most favorable conditions for primary hardening, allowing two out of five plantlets to survive, corresponding to 40% survival (or 60% mortality). This higher survival rate may be attributed to the excellent water-holding capacity, aeration properties, and pathogen-free nature of cocopeat, which together create a gentle transition environment from in vitro to ex vitro conditions. In contrast, the addition of soil at either 20% (H2) or 50% (H3) resulted in complete mortality of plantlets during primary hardening. This can be attributed to the sudden exposure to heterogeneous microbial populations, higher compaction, and reduced moisture stability typically associated with soil-based substrates.

These results are consistent with those reported by Devi et al. (2017) in *Momordica cymbalaria*, where cocopeat alone or in combination with inert substrates such as perlite yielded higher survival rates compared to soil-containing mixtures. The physiological fragility of newly rooted micropropagated plants makes cocopeat advantageous because it provides a non-stressful environment with adequate moisture without hypoxia.

Secondary Hardening

The two surviving plants from the cocopeat treatment (H1) were successfully transferred to secondary hardening, where both survived completely, reducing the final mortality to 0%. This indicates that once the initial acclimatization stage is overcome, *C. grandis* plantlets possess strong adaptability to ex vitro conditions.

Table 1: Effect of different concentrations on fungal contamination

Treatments code	Chemical Concentration	Time period (Min.)			Hard node (%)	Soft node (%)	Shoot tip (%)
		Hard node	Soft node	Shoot tip			
T1	Bavistin (2000 ppm)	14	10	06	10 ± 1.2	9.0 ± 1.0	8.0 ± 0.0
	Cefotaxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptocycline (1000 ppm)	14	12	08			
	$HgCl_2$ 0.1%	08	05	02			
T2	Bavistin (2000 ppm)	14	10	06	8.0 ± 0.0	7.0 ± 1.0	5.0 ± 1.0
	Cefotaxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptocycline (1000 ppm)	16	12	10			
	$HgCl_2$ 0.1%	08	05	1.5			
T3	Bavistin (2000 ppm)	16	12	10	4.0 ± 0.0	3.0 ± 1.0	3.0 ± 1.0
	Cefotaxime (1000 ppm)	12	10	06			
	Kanamycine (1000 ppm)	12	10	06			
	Streptocycline (1000 ppm)	16	12	08			

	$HgCl_2$ 0.1%	08	05	02			
T4	Bavistin (2000 ppm)	18	16	10	3.0 ± 1.0	5.0 ± 1.0	2.0 ± 1.2
	Cefotaxime (1000 ppm)	14	12	06			
	Kanamycine (1000 ppm)	14	12	06			
	Streptocycline (1000 ppm)	18	16	08			
	$HgCl_2$ 0.1%	09	08	02			
T5	Bavistin (2000 ppm)	16	14	10	2.0 ± 1.2	2.0 ± 1.2	1.0 ± 1.0
	Cefotaxime (1000 ppm)	14	12	05			
	Kanamycine (1000 ppm)	14	12	05			
	Streptocycline (1000 ppm)	16	14	08			
	$HgCl_2$ 0.1%	08	06	02			
T6	Bavistin (2000 ppm)	18	16	10	0.0 ± 0.0	1.0 ± 1.0	1.0 ± 1.0
	Cefotaxime (1000 ppm)	14	12	06			
	Kanamycine (1000 ppm)	14	12	06			
	Streptocycline (1000 ppm)	18	16	08			
	$HgCl_2$ 0.1%	08	07	02			

Table 2: Effect of different chemical concentrations on bacterial contamination

Treatments code	Chemical Concentration	Time period (Min.)			Hard node (%)	Soft node (%)	Shoot tip (%)
		Hard node	Soft node	Shoot tip			
T_1	Bavistin (2000 ppm)	14	10	06	4.0 ± 0.0	6.0 ± 1.2	2.0 ± 1.2
	Cefotaxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptocycline (1000 ppm)	14	12	08			
	$HgCl_2$ 0.1%	08	05	02			
T_2	Bavistin (2000 ppm)	14	10	06	1.0 ± 1.0	3.0 ± 1.0	1.0 ± 1.0
	Cefotaxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptocycline (1000 ppm)	16	12	10			
	$HgCl_2$ 0.1%	08	05	1.5			
T_3	Bavistin (2000 ppm)	16	12	10	6.0 ± 1.2	3.0 ± 1.0	3.0 ± 1.0
	Cefotaxime (1000 ppm)	12	10	06			
	Kanamycine (1000 ppm)	12	10	06			
	Streptocycline (1000 ppm)	16	12	08			
	$HgCl_2$ 0.1%	08	05	02			
T_4	Bavistin (2000 ppm)	18	16	10	5.0 ± 1.0	3.0 ± 1.0	4.0 ± 0.0
	Cefotaxime (1000 ppm)	14	12	05			

	Kanamycine (1000 ppm)	14	12	05			
	Streptocycline (1000 ppm)	18	16	08			
	$HgCl_2$ 0.1%	09	08	02			
T_5	Bavistin (2000 ppm)	16	14	10	2.0 ± 1.2	1.0 ± 1.0	1.0 ± 1.0
	Cefotaxime (1000 ppm)	14	12	06			
	Kanamycine (1000 ppm)	14	12	06			
	Streptocycline (1000 ppm)	16	14	08			
	$HgCl_2$ 0.1%	08	06	02			
T_6	Bavistin (2000 ppm)	18	16	10	1.0 ± 1.0	1.0 ± 1.0	0.0 ± 0.0
	Cefotaxime (1000 ppm)	14	12	06			
	Kanamycine (1000 ppm)	14	12	06			
	Streptocycline (1000 ppm)	18	16	08			
	$HgCl_2$ 0.1%	08	07	02			

Table 3: Drying percentage of hard node, soft node and shoot tip explants (The values have been presented as a mean ± S.E.)

Treatments code	**Chemical Concentration**	**Time period (Min.)**			**Hard node (%)**	**Soft node (%)**	**Shoot tip (%)**
		Hard node	**Soft node**	**Shoot tip**			
T1	Bavistin (2000 ppm)	14	10	06	2.0 ± 1.2	3.0 ± 1.0	6.0 ± 1.2
	Cefotoxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptomcycline (1000 ppm)	14	12	08			
	$HgCl_2$ 0.1%	08	05	02			
T2	Bavistin (2000 ppm)	14	10	06	1.0 ± 1.0	1.0 ± 1.0	3.0 ± 1.0
	Cefotoxime (1000 ppm)	12	10	05			
	Kanamycine (1000 ppm)	12	10	05			
	Streptomcycline (1000 ppm)	16	12	10			
	$HgCl_2$ 0.1%	08	05	1.5			
T3	Bavistin (2000 ppm)	16	12	10	1.0 ± 1.0	1.0 ± 1.0	3.0 ± 1.0
	Cefotoxime (1000 ppm)	12	10	06			
	Kanamycine (1000 ppm)	12	10	06			
	Streptomcycline (1000 ppm)	16	12	08			
	$HgCl_2$ 0.1%	08	05	02			
T4	Bavistin (2000 ppm)	18	16	10	0.0 ± 0.0	1.0 ± 1.0	3.0 ± 1.0
	Cefotoxime (1000 ppm)	14	12	05			
	Kanamycine (1000 ppm)	14	12	05			
	Streptomcycline (1000 ppm)	18	16	08			
	$HgCl_2$ 0.1%	09	08	02			
T5	Bavistin (2000 ppm)	16	14	10	1.0 ±	0.0 ±	3.0 ±

	Cefotoxime (1000 ppm)	14	12	06	1.0	0.0	1.0
	Kanamycine (1000 ppm)	14	12	06			
	Streptomcycline (1000 ppm)	16	14	08			
	$HgCl_2$ 0.1%	08	06	02			
T6	Bavistin (2000 ppm)	18	16	10	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.0
	Cefotoxime (1000 ppm)	14	12	06			
	Kanamycine (1000 ppm)	14	12	06			
	Streptomcycline (1000 ppm)	18	16	08			
	$HgCl_2$ 0.1%	08	07	02			

Table 4: Sprouted plants and response (%) at 7, 14 and 21 days after initiation

Treatments	At 7 days		At 14 days		At 21 days		Number of days taken for sprouting
	Number of sprouted plants	Response %	Number of sprouted plants	Response %	Number of sprouted plants	Response %	
T1	5.00	20	10.00	40	12.50	50	20.75
T2	8.75	35	12.50	50	17.50	70	14.00
T3	8.00	32	15.00	60	17.00	68	11.75
T4	9.50	38	15.50	62	18.00	72	11.00
T5	10.75	43	16.25	65	21.25	85	9.00
T6	12.50	50	18.75	75	23.75	95	7.00
S.Em.	0.22		0.22		0.22		0.14
C.D. (p=0.05)	0.66		0.66		0.66		0.42
C.V.%	4.85		3.01		2.40		2.36

Table 5:

Media code	Media details	Number Of shoots	Length of Shoots(cm)	Shooting (%)
M1	½ MS	2.25	5.05	100
M2	½ MS + 0.01mgl^{-1} BA	2.00	4.70	100
M3	½ MS + 0.05 mgl^{-1}BA	1.00	4.88	100
M4	½ MS + 0.1 mgl^{-1} BA	3.00	5.13	100
M5	½ MS + 0.2 mgl^{-1} BA	2.00	4.53	100
M6	½ MS +1.0 mgl^{-1} GA_3	1.75	4.45	100
M7	½ MS + 0.01 mgl^{-1} BA+1.0 mgl^{-1} GA_3	1.00	3.98	100
M8	½ MS + 0.05 mgl^{-1} BA+1.0 mgl^{-1} GA_3	1.25	4.70	100
M9	½ MS + 0.1 mgl^{-1} BA+1.0 mgl^{-1} GA_3	1.00	5.45	100
M10	½ MS + 0.2 mgl^{-1} BA+1.0 mgl^{-1} GA_3	1.75	3.28	100
M11	½ MS +2.0 mgl^{-1} GA_3	1.00	3.25	100
M12	½ MS + 0.01 mgl^{-1} BA+2.0 mgl^{-1} GA_3	1.25	4.13	100

M13	½ MS + 0.05 mgl^{-1} BA+2.0 mgl^{-1} GA_3	1.00	4.35	100
M14	½ MS + 0.1 mgl^{-1} BA+2.0 mgl^{-1} GA_3	1.00	3.05	100
M15	½ MS + 0.2 mgl^{-1} BA+2.0 mgl^{-1} GA_3	2.00	3.25	100
M16	½ MS+0.02 mgl^{-1} BA+0.05 mgl^{-1} NAA	1.00	1.58	100
M17	½ MS+0.05 mgl^{-1} BA+0.05 mgl^{-1} NAA	1.00	1.63	100
M18	½ MS+0.1 mgl^{-1} BA+0.05 mgl^{-1} NAA	2.00	2.78	100
M19	½ MS+0.1 mgl^{-1} IAA+0.1 mgl^{-1}Kn	1.25	1.45	100
M20	½ MS+0.01 mgl^{-1} IAA+0.02 mgl^{-1}Kn	1.00	1.98	100
M21	½ MS+0.05 mgl^{-1} IAA+0.02 mgl^{-1}Kn	1.00	1.40	100
M22	½ MS+0.1 mgl^{-1} IAA+0.02 mgl^{-1}Kn	1.00	2.18	100
M23	½ MS+0.01 mgl^{-1} IAA+0.05 mgl^{-1}Kn	1.00	1.50	100
M24	½ MS+0.05 mgl^{-1} IAA+0.05 mgl^{-1}Kn	1.00	1.35	100
M25	½ MS+0.01 mgl^{-1} IAA+0.1 mgl^{-1}Kn	1.00	1.83	100
S.Em.		0.12	0.08	-
C.D. (p=0.05)		0.35	0.23	-
C.V.%		17.75	4.73	-

Table 6:

Media Code	Media details	Number of shoots	Length of Shoots(cm)	Shooting (%)
M1	½ MS	2.00	4.00	100
M2	½ MS + 0.01mgl^{-1} BA	2.00	4.70	100
M3	½ MS + 0.05 mgl^{-1}BA	1.00	3.25	100
M4	½ MS + 0.1 mgl^{-1} BA	2.75	5.00	100
M5	½ MS + 0.2 mgl^{-1} BA	1.25	4.32	100
M6	½ MS +1.0 mgl^{-1} GA_3	1.00	4.57	100
M7	½ MS + 0.01 mgl^{-1} BA+1.0 mgl^{-1} GA_3	1.00	4.70	100
M8	½ MS + 0.05 mgl^{-1} BA+1.0 mgl^{-1} GA_3	2.00	4.85	100
M9	½ MS + 0.1 mgl^{-1} BA+1.0 mgl^{-1} GA_3	1.75	5.35	100
M10	½ MS + 0.2 mgl^{-1} BA+1.0 mgl^{-1} GA_3	2.00	3.45	100
M11	½ MS +2.0 mgl^{-1} GA_3	1.00	3.37	100
M12	½ MS + 0.01 mgl^{-1} BA+2.0 mgl^{-1} GA_3	1.00	4.50	100
M13	½ MS + 0.05 mgl^{-1} BA+2.0 mgl^{-1} GA_3	2.00	3.50	100
M14	½ MS + 0.1 mgl^{-1} BA+2.0 mgl^{-1} GA_3	1.00	2.30	100
M15	½ MS + 0.2 mgl^{-1} BA+2.0 mgl^{-1} GA_3	1.00	3.09	100

M16	½ MS+0.02 mgl^{-1} BA+0.05 mgl^{-1} NAA	1.00	1.52	100
M17	½ MS+0.05 mgl^{-1} BA+0.05 mgl^{-1} NAA	1.00	1.65	100
M18	½ MS+0.1 mgl^{-1} BA+0.05 mgl^{-1} NAA	1.75	2.15	100
M19	½ MS+0.1 mgl^{-1} IAA+0.1 mgl^{-1}Kn	1.75	1.89	100
M20	½ MS+0.01 mgl^{-1} IAA+0.02 mgl^{-1}Kn	1.00	1.97	100
M21	½ MS+0.05 mgl^{-1} IAA+0.02 mgl^{-1}Kn	1.00	1.92	100
M22	½ MS+0.1 mgl^{-1} IAA+0.02 mgl^{-1}Kn	1.00	2.17	100
M23	½ MS+0.01 mgl^{-1} IAA+0.05 mgl^{-1}Kn	2.00	2.05	100
M24	½ MS+0.05 mgl^{-1} IAA+0.05 mgl^{-1}Kn	1.00	1.50	100
M25	½ MS+0.01 mgl^{-1} IAA+0.1 mgl^{-1}Kn	2.00	1.32	100
S.Em.		0.11	0.08	-
C.D. (p=0.05)		0.32	0.23	-
C.V.%		15.42	5.25	-

Table 7:

Media Code	Media details	Number of shoots	Length of shoots (cm)	Shooting (%)
M1	½ MS	1.00	3.62	100
M2	½ MS + 0.01mgl^{-1} BA	1.00	3.40	100
M3	½ MS + 0.05 mgl^{-1}BA	1.00	2.52	100
M4	½ MS + 0.1 mgl^{-1} BA	3.00	2.45	100
M5	½ MS + 0.2 mgl^{-1} BA	1.00	2.25	100
M6	½ MS +1.0 mgl^{-1} GA_3	1.00	3.76	100
M7	½ MS + 0.01 mgl^{-1} BA+1.0 mgl^{-1} GA_3	2.00	3.72	100
M8	½ MS + 0.05 mgl^{-1} BA+1.0 mgl^{-1} GA_3	1.00	3.47	100
M9	½ MS + 0.1 mgl^{-1} BA+1.0 mgl^{-1} GA_3	2.00	4.57	100
M10	½ MS + 0.2 mgl^{-1} BA+1.0 mgl^{-1} GA_3	2.00	3.55	100
M11	½ MS +2.0 mgl^{-1} GA_3	1.25	2.97	100
M12	½ MS + 0.01 mgl^{-1} BA+2.0 mgl^{-1} GA_3	1.00	3.57	100
M13	½ MS + 0.05 mgl^{-1} BA+2.0 mgl^{-1} GA_3	1.75	2.57	100
M14	½ MS + 0.1 mgl^{-1} BA+2.0 mgl^{-1} GA_3	2.00	4.25	100
M15	½ MS + 0.2 mgl^{-1} BA+2.0 mgl^{-1} GA_3	1.00	2.86	100
M16	½ MS+0.02 mgl^{-1} BA+0.05 mgl^{-1} NAA	1.00	1.47	100
M17	½ MS+0.05 mgl^{-1} BA+0.05 mgl^{-1} NAA	1.00	1.82	100
M18	½ MS+0.1 mgl^{-1} BA+0.05 mgl^{-1} NAA	1.00	1.97	100

M19	½ MS+0.1 mgl^{-1} IAA+0.1 mgl^{-1}Kn	1.75	1.90	100
M20	½ MS+0.01 mgl^{-1} IAA+0.02 mgl^{-1}Kn	2.00	2.40	100
M21	½ MS+0.05 mgl^{-1} IAA+0.02 mgl^{-1}Kn	2.00	2.44	100
M22	½ MS+0.1 mgl^{-1} IAA+0.02 mgl^{-1}Kn	1.25	2.06	100
M23	½ MS+0.01 mgl^{-1} IAA+0.05 mgl^{-1}Kn	1.25	1.49	100
M24	½ MS+0.05 mgl^{-1} IAA+0.05 mgl^{-1}Kn	1.25	1.47	100
M25	½ MS+0.01 mgl^{-1} IAA+0.1 mgl^{-1}Kn	1.00	1.47	100
S.Em.		0.12	0.05	-
C.D. (p=0.05)		0.35	0.14	-
C.V.%		17.25	3.53	-

Table 8: Number of roots, No. of days taken for rooting, root length and rooting percentage at 21 days

Media Code	**Media details**	**Days To root induction**	**Length of roots (cm)**	**Number of roots**	**Rooting (%)**
R1	½ MS	9.75	3.50	1.00	100
R2	½ MS+ 0.5 mgl^{-1} IBA	8.75	4.25	3.50	100
R3	½ MS+ 1.0 mgl^{-1} IBA	9.25	6.02	7.00	100
R4	½ MS+ 1.5 mgl^{-1} IBA	9.50	4.37	2.50	100
R5	½ MS+ 2.0 mgl^{-1} IBA	10.25	5.25	7.00	100
S.Em.		0.26	0.11	0.18	-
C.D. (p=0.05)		0.78	0.34	0.55	-
C.V.%		5.44	4.79	8.69	-

Table 9: Mortality percentage at hardening

Treatments	**Media details**	**Total number of plants taken for hardening**	**Number of plants survive**	**Mortality (%)**
H1	Coco peat (100%)	5	2	60
H2	Soil : Coco peat (20:80)	5	0	100
H3	Soil : Coco peat (50:50)	5	0	100


Sprouted explant


Multiple shoots


Rooted explant


Primary harden plant


Secondary harden plant

CONCLUSION

The overall study establishes a robust, efficient, and reproducible micropropagation protocol for *Coccinia grandis*, with each phase—sterilization, establishment, multiplication, rooting, and acclimatization—clearly optimized for maximum success. The sterilization results confirm that Treatment T_6, comprising Bavistin, broad-spectrum antibiotics, and $HgCl_2$ with adequate exposure time, provides the most effective microbial control while minimizing tissue drying, thereby ensuring high explant survival during establishment. Shoot multiplication across multiple subcultures consistently demonstrated that BA at 0.1 mg L^{-1}, either alone or in combination with GA_3, is the most reliable cytokinin treatment for inducing multiple shoots and achieving superior elongation, while kinetin- and NAA-based treatments remained less effective. The repeated success of BA-centered media across subcultures highlights its stability and suitability for large-scale propagation of this species. Rooting was maximized with IBA at 1–2 mg L^{-1}, producing vigorous and well-developed root systems. During acclimatization, cocopeat alone proved to be the best substrate for primary hardening, while soil-containing media caused high mortality due to early-stage stress—findings consistent with micropropagation behavior in other cucurbits. Collectively, the optimized protocol ensures high regeneration efficiency, genetic uniformity, and successful transfer of plantlets to ex vitro conditions, making it highly suitable for commercial-scale propagation and conservation of elite *Coccinia grandis* germplasm.

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