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Simultaneous Shoot Proliferation, *In Vitro* Flowering and Rooting in Rose (*Rosa hybrida L.)*

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ABSTRACT

Conventional micropropagation of rose relies on separate phases for shoot multiplication, rooting and acclimatization, which increase production time, cost and operational complexity. *In vitro* flowering is rarely incorporated into propagation systems despite its importance for breeding and physiological studies. The present study aimed to develop a simplified and integrated protocol enabling simultaneous shoot multiplication and *in vitro* flowering, followed by efficient rooting in *Rosa* × *hybrida* L. Nodal explants were cultured on Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine (BAP; 0–4.0 mg L^{-1}) and indole-3-acetic acid (IAA; 0–3.0 mg L^{-1}) in a factorial experimental design under a completely randomized layout (*n* = 15 per treatment). For rooting, regenerated shoots were transferred to full- or half-strength MS medium containing indole-3-butyric acid (IBA; 1.5 mg L^{-1}), with or without gibberellic acid (GA_3; 0.5 mg L^{-1}). Data were analyzed using two-way ANOVA, followed by the Least Significant Difference (LSD) test ($p \leq 0.05$). The combination of MS supplemented with 3.0 mg L^{-1} BAP and 3.0 mg L^{-1} IAA produced the optimal morphogenic response, characterized by early shoot initiation (18.22 days), maximum shoot proliferation (3.60 shoots per explant) and highest *in vitro* flowering (2.48 flowers per culture). Rooting was most efficient on full-strength MS supplemented with IBA, achieving 93.3% rooting, with 4.67 roots per shoot and a mean root length of 2.60 cm. The addition of GA_3 significantly reduced root elongation. The proposed system integrates shoot regeneration, flowering and rooting into a streamlined protocol, reducing handling frequency and production time. This approach demonstrates strong potential for accelerating rose breeding and facilitating *in vitro* floral research, pending validation across diverse cultivars

Keywords: *auxins, cytokinins, in vitro flowering, rose micropropagation, plant growth regulators, regeneration*

1. INTRODUCTION

Background and global importance

The genus *Rosa* (family Rosaceae; 2n = 2x = 14 for many diploid species) comprises more than 150 species and over 30,000 cultivated varieties, of which *Rosa hybrida* L. represents the most commercially important group in modern floriculture. Widely regarded as the “queen of flowers,” roses are characterized by exceptional phenotypic diversity, including variation in floral morphology, pigmentation and fragrance. These attributes underpin their dominant position in the global cut flower industry, which generates multi-billion-dollar annual revenues (Rajesh *et al.*, 2014).

Beyond ornamental value, roses contribute to multiple industrial sectors. Essential oils extracted from petals are widely used in perfumery and cosmetics, while certain species produce hips rich in vitamin C and bioactive compounds used in nutraceuticals and traditional medicine (Jafar *et al.*, 2005). In Pakistan, favorable agro-ecological conditions support year-round cultivation and roses hold both cultural and economic importance, indicating strong potential for expansion within the floriculture sector (Khan *et al.*, 2005).

Increasing consumer demand for novel cultivars with improved vase life, enhanced fragrance and resistance to biotic and abiotic stresses continues to intensify breeding efforts. Consequently, efficient propagation systems are required to ensure rapid multiplication of elite genotypes while maintaining genetic stability and phytosanitary standards.

Constraints of conventional propagation methods

Vegetative propagation through budding, grafting and stem cuttings remains the primary method for rose multiplication (Roy *et al.*, 2004). Despite widespread adoption, these approaches impose several limitations that restrict large-scale production.

Propagation efficiency depends strongly on environmental parameters such as temperature, humidity and photoperiod, resulting in seasonal variability in success rates. In addition, multiplication capacity remains inherently low because each stock plant yields a limited number of propagules. The requirement for skilled labor and extensive nursery space further increases operational costs.

A more critical limitation is the transmission and accumulation of systemic pathogens. Bacterial infections such as *Agrobacterium tumefaciens*, viral complexes including Rose mosaic virus and Rose rosette virus and fungal pathogens such as *Diplocarpon rosae* persist across propagation cycles and progressively reduce plant vigor and quality. These factors collectively compromise productivity and economic returns (Roberts *et al.*, 2018).

These constraints highlight the need for alternative propagation systems capable of delivering high multiplication rates, genetic uniformity and pathogen-free plant material independent of seasonal limitations.

Micropropagation as a technological alternative

In vitro micropropagation provides a controlled and scalable approach for the clonal propagation of horticultural crops. Since the development of the Murashige and Skoog (MS) medium, tissue culture techniques have enabled rapid multiplication of genetically uniform and disease-free plants under aseptic conditions (Murashige & Skoog, 1962; George *et al.*, 2008).

The principal advantages of micropropagation include: (i) high multiplication rates from minimal explant material, (ii) elimination of pathogens through sterilization and meristem culture, (iii) independence from seasonal constraints, (iv) uniformity of regenerated plants and (v) conservation of elite germplasm. In roses, standard protocols follow a sequential pathway comprising culture establishment, shoot multiplication, root induction and acclimatization.

However, this multi-stage approach requires repeated subculturing and medium changes, which increase labor requirements, contamination risks and production time. The full propagation cycle often extends over several months, limiting the efficiency of commercial operations.

Hormonal regulation of morphogenesis In vitro

Plant growth regulators (PGRs) govern morphogenesis during *in vitro* culture by modulating cell division, differentiation and organogenesis. Cytokinins, particularly 6-benzylaminopurine (BAP), promote axillary bud break and shoot proliferation through stimulation of meristematic activity. In *Rosa hybrida*, effective shoot multiplication is generally achieved with BAP concentrations between 1.0 and 3.0 mg L^{-1}, although optimal levels vary among genotypes (Oo *et al.*, 2021; Roy *et al.*, 2004).

Auxins such as indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) regulate cell elongation, vascular differentiation and root initiation. Morphogenic responses depend on the cytokinin-to-auxin ratio: cytokinin dominance promotes shoot formation, whereas auxin dominance induces rooting.

IBA is widely recognized as the most effective auxin for root induction due to its stability and resistance to rapid degradation. Optimal rooting in roses typically occurs at concentrations between 0.5 and 2.0 mg L^{-1} (Carelli & Echeverrigaray, 2002; Oo *et al.*, 2021). Nevertheless, the role of basal salt strength remains unresolved, as some studies report improved rooting under reduced nutrient conditions, while others demonstrate superior performance on full-strength medium. These inconsistencies suggest genotype-specific responses and potential carry-over effects from previous culture stages (Driver & Suttle, 1987).

In vitro flowering as a strategic innovation

In vitro flowering represents an advanced application of plant tissue culture, enabling floral induction under controlled environmental conditions. This approach provides a valuable system for investigating the physiological and molecular mechanisms of floral transition independent of external cues such as photoperiod and temperature (Goh, 1992).

In roses, the long juvenile phase of many cultivars significantly delays breeding programs. The ability to induce flowering *in vitro* offers a means to accelerate generation turnover, potentially reducing breeding cycles from 6–10 years to 2–3 years. In addition, *in vitro* flowers provide a controlled source of pollen for hybridization, facilitating breeding without reliance on greenhouse facilities.

Despite these advantages, *in vitro* flowering in roses remains technically challenging and highly genotype-dependent. Previous studies have typically required non-standard conditions, including elevated sucrose concentrations, application of growth retardants such as paclobutrazol, or specific environmental treatments (Vu *et al.*, 2006; Kim *et al.*, 2019). These requirements increase complexity and limit practical integration with routine propagation systems.

Knowledge gap

Existing research on rose micropropagation has largely focused on optimizing individual stages, particularly shoot multiplication or rooting. Studies on *in vitro* flowering have generally treated it as an isolated phenomenon rather than incorporating it into a complete regeneration system.

Consequently, current protocols remain compartmentalized, requiring separate media and conditions for each developmental stage. This fragmentation increases handling frequency, contamination risk, production time and cost. More importantly, it overlooks the possibility that morphogenic processes may be co-regulated through shared hormonal pathways.

To date, no protocol has successfully integrated high-frequency shoot multiplication, reliable *in vitro* flowering and efficient rooting into a single, streamlined system for *Rosa hybrida*. This gap limits both commercial scalability and the application of tissue culture in advanced research.

Rationale and conceptual framework

The present study addresses this limitation by proposing an integrated micropropagation strategy based on hormonal synergy. The central premise is that a specific balance between cytokinins and auxins can simultaneously regulate multiple developmental pathways.

The study evaluates whether a defined combination of BAP and IAA can induce both shoot proliferation and floral transition within a single medium, thereby eliminating the need for separate flowering conditions. In parallel, it examines whether IBA alone can sustain efficient rooting without the addition of gibberellic acid (GA_3).

This integrated approach aims to reduce procedural complexity, minimize contamination risk and shorten the overall production cycle while maintaining high morphogenic efficiency.

Research objectives

- To evaluate the effects of BAP and IAA combinations on simultaneous shoot multiplication and *in vitro* flowering in *Rosa hybrida*.
- To determine optimal rooting conditions by comparing nutrient strength and assessing the influence of GA_3 in combination with IBA.
- To develop and validate a simplified two-stage protocol integrating multiplication, flowering and rooting within a unified system.

Hypotheses

The investigation tested the following hypotheses:

- **H_1:** A balanced cytokinin–auxin regime (BAP 3.0 mg L^{-1} + IAA 3.0 mg L^{-1}) maximizes both shoot proliferation and *in vitro* flowering.
- **H_2:** IBA alone on full-strength MS medium produces superior rooting compared to reduced nutrient strength or GA_3 supplementation.
- **H_3:** An integrated two-stage protocol enables efficient regeneration without compromising individual developmental processes.

Scientific significance

The development of an integrated micropropagation system has significant implications for both applied and fundamental research. For commercial floriculture, it offers a cost-efficient and scalable method for rapid multiplication of elite cultivars. For breeding programs, the inclusion of *in vitro* flowering enables accelerated generation cycling and controlled hybridization. From a scientific perspective, the system provides a reproducible model for investigating hormonal regulation of plant development, particularly floral induction.

Moreover, the framework established in this study may be extended to other ornamental and woody species, contributing to broader advances in plant tissue culture and applied biotechnology.

2. MATERIALS AND METHODS

Study site and experimental duration

The experiment was conducted at the Plant Tissue Culture Laboratory, Department of Biotechnology, Faculty of Crop Production, Sindh Agriculture University, Tando Jam, Pakistan (25°25′ N, 68°32′ E). The study spanned 24 weeks encompassing two independent experimental cycles to ensure reproducibility and robustness of results.

Plant material and explant source

Actively growing, disease-free shoots of *Rosa hybrida* L. (a commercially cultivated local genotype characterized by deep red, high-centered flowers and extended vase life) were collected from two-year-old stock plants maintained under field conditions in the university botanical garden.

To minimize endogenous contamination, donor plants were pre-treated with a systemic fungicide (carbendazim, 0.1% w/v) two weeks prior to explant excision. Nodal segments (2–3 cm), each bearing a single axillary bud, were excised from semi-hardwood shoots of the current growth flush.

Initial cleaning involved rinsing explants under running tap water for 30 min, followed by immersion in distilled water containing a few drops of Tween-20 for 5 min with gentle agitation to remove surface debris and microbial load.

Surface sterilization

All sterilization procedures were carried out under aseptic conditions in a laminar airflow cabinet. Explants were sequentially treated as follows:

- Immersion in 70% (v/v) ethanol for 1 min
- Surface disinfection using 10% (v/v) sodium hypochlorite solution (derived from commercial bleach containing ~5% available chlorine) supplemented with 2–3 drops of Tween-20 for 15 min with continuous agitation
- Five successive rinses with sterile distilled water (5 min each) to remove residual sterilants

Sterility verification was performed by incubating randomly selected explants on nutrient agar at 28°C for 5 days. The absence of microbial growth confirmed the effectiveness of the sterilization procedure.

Culture medium and incubation conditions

Murashige and Skoog (MS) basal medium was used as the nutrient platform throughout the study. The medium composition included:

- Sucrose: 30 g L^{-1} (carbon source)
- Plant agar: 7 g L^{-1} (gelling agent)

The pH was adjusted to 5.7 ± 0.1 prior to sterilization. Media were dispensed (40 mL per vessel) into 250 mL culture jars and autoclaved at 121°C and 15 psi for 20 min.

Plant growth regulators (PGRs), including 6-benzylaminopurine (BAP), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and gibberellic acid (GA_3), were prepared as 1 mg mL^{-1} stock solutions. IAA was dissolved using a minimal volume of NaOH to ensure complete solubilization. All PGRs were filter-sterilized (0.22 µm) and incorporated into the medium after cooling to approximately 45°C.

Cultures were maintained under controlled environmental conditions:

- Temperature: 25 ± 2°C
- Photoperiod: 16 h light / 8 h dark
- Light intensity: 40 µmol m^{-2} s^{-1} (cool white fluorescent lamps)
- Relative humidity: 60–70%

To minimize positional bias, culture vessels were randomly rearranged at weekly intervals.

Experimental design and replication

A completely randomized design (CRD) was adopted for all treatments. Each experiment was conducted twice independently. Within each run:

- Treatments were replicated three times
- Each replication consisted of five explants
- Total sample size: n = 15 per treatment per run

Following confirmation of variance homogeneity (Levene's test, $p > 0.05$), datasets from both runs were pooled for statistical analysis.

Shoot multiplication and in vitro flowering

Surface-sterilized nodal explants were cultured on MS medium supplemented with varying concentrations of BAP and IAA to evaluate their combined effects on shoot proliferation and floral induction.

Treatment structure

Treatment	**BAP (mg L^{-1})**	**IAA (mg L^{-1})**
Control	0.0	0.0
T_1	2.0	2.0
T_2	2.5	2.0
T_3	3.0	3.0
T_4	4.0	3.0

Cultures were maintained for 12 weeks without subculturing.

Data recording

Shoot multiplication (week 6)

- Days to shoot initiation (time to visible bud break ≥1 mm)
- Number of shoots per explant (≥0.5 cm length)
- Shoot length (cm)
- Hyperhydricity (%) = (vitrified shoots / total shoots) × 100

In vitro flowering (week 12)

- Number of flowers per culture
- Days to flower bud emergence
- Days to anthesis
- Flower diameter (mm)
- Petal number per flower

Root induction

Shoots (3–4 cm) derived from the optimal multiplication–flowering treatment (T_3) were excised and transferred to rooting media after removal of residual agar.

Rooting treatments

Treatment	**Medium composition**
A	Full-strength MS + 1.5 mg L^{-1} IBA
B	Full-strength MS + 1.5 mg L^{-1} IBA + 0.5 mg L^{-1} GA_3
C	Half-strength MS + 1.5 mg L^{-1} IBA

Rooting conditions

- Initial incubation: 7 days in darkness (to promote auxin-mediated root induction)
- Subsequent incubation: 21 days under standard photoperiod

Data collection (after 4 weeks)

- Number of roots per shoot (>5 mm)
- Root length (cm)
- Rooting percentage (%)

Statistical analysis

Data were subjected to rigorous statistical evaluation:

- Normality: Shapiro–Wilk test ($p > 0.05$)
- Homogeneity: Levene's test ($p > 0.05$)

For normally distributed data, one-way ANOVA was performed using Statistix 8.1. Treatment means were separated using the Least Significant Difference (LSD) test at $p \leq 0.05$.

Where assumptions were violated, non-parametric analysis (Kruskal–Wallis test) followed by Dunn's post-hoc comparisons was applied.

All results are presented as mean ± standard error (SE) and exact p-values are reported for pairwise comparisons.

3. RESULTS AND DISCUSSION

Shoot multiplication and *in vitro* flowering

The interaction between 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA) significantly affected all morphogenic parameters of *Rosa hybrida* L. (one-way ANOVA, $p < 0.001$). A clear treatment-dependent response was observed, with Treatment T_3 (3.0 mg L^{-1}

BAP + 3.0 mg L⁻¹ IAA) consistently producing the most favorable outcomes in terms of shoot initiation, proliferation, elongation and flowering (Table 1A).

A dose-dependent trend was evident, whereby increasing BAP concentration from 2.0 to 3.0 mg L^{-1} enhanced morphogenic responses. However, a further increase to 4.0 mg L^{-1} resulted in reduced efficiency and increased physiological abnormalities, indicating cytokinin-induced stress beyond the optimal threshold. Similar inhibitory effects of elevated cytokinin concentrations have been reported in rose and other ornamental species, where excessive BAP disrupts normal cellular organization and reduces regeneration efficiency (Chakrabarty *et al*., 2006; Kevers *et al*., 2004).

The superior performance of T_3 (3.60 shoots per explant and 4.56 cm shoot length) exceeds or aligns with previously reported values. For example, Oo *et al*. (2021) reported optimal shoot multiplication at 1.0–2.0 mg L^{-1} BAP, while Roy *et al*. (2004) demonstrated improved shoot growth with moderate cytokinin levels. The requirement for a relatively higher BAP concentration in the present study may reflect genotype-specific hormonal sensitivity and the additional requirement for simultaneous floral induction.

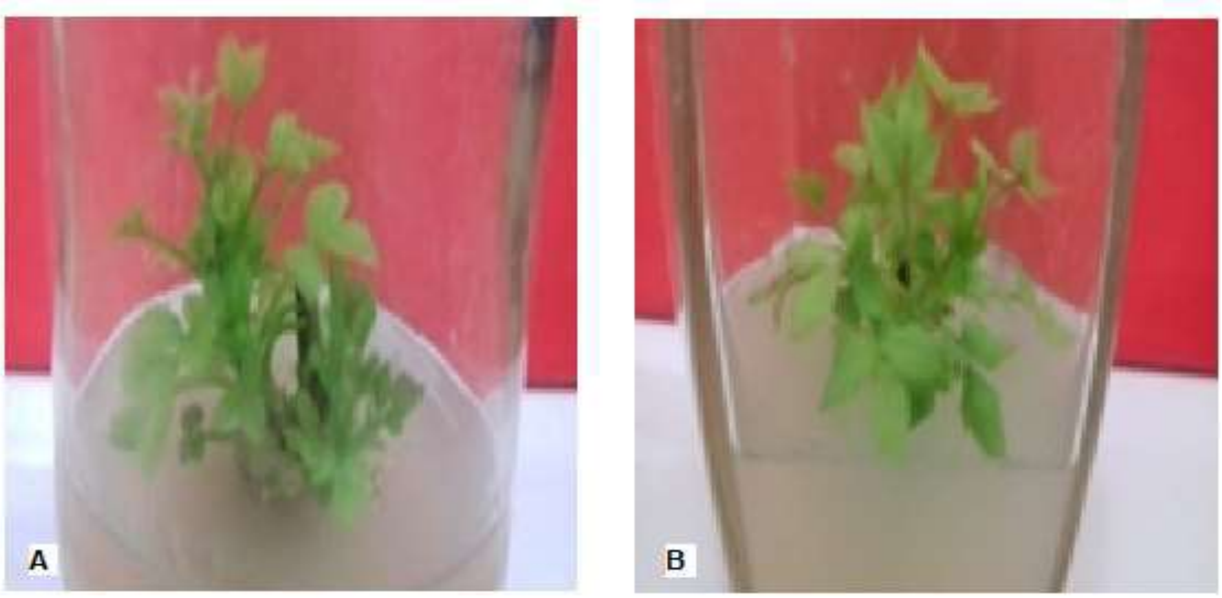

Figure 1. *Effect of BAP and IAA on shoot multiplication and in vitro flowering of Rosa hybrida L. (A-B) Increasing shoot number (C-D) Enhancement of shoot length (E-F) In vitro flower formation.*

Table 1A. Effect of BAP and IAA on shoot multiplication and *in vitro* flowering (n = 15)

Treatment	Days to shoot initiation (days)	Shoots per explant	Shoot length (cm)	Flowers per culture	Hyperhydricity (%)
Control (0 PGR)	45.6 ± 2.1 d	0.80 ± 0.10 a	1.20 ± 0.20 a	0.00 ± 0.00 a	0.00 ± 0.00 a

Treatment	Days to shoot initiation (days)	Shoots per explant	Shoot length (cm)	Flowers per culture	Hyperhydricity (%)
T_1 (2.0 + 2.0)	30.78 ± 1.2 c	1.34 ± 0.10 b	2.13 ± 0.30 b	0.00 ± 0.00 a	5.20 ± 1.10 b
T_2 (2.5 + 2.0)	21.11 ± 0.9 b	2.23 ± 0.20 c	3.62 ± 0.40 c	1.45 ± 0.20 b	12.30 ± 2.40 c
T_3 (3.0 + 3.0)	18.22 ± 0.7 a	3.60 ± 0.30 d	4.56 ± 0.50 d	2.48 ± 0.30 c	18.50 ± 3.10 c
T_4 (4.0 + 3.0)	23.23 ± 1.1 b	2.45 ± 0.20 c	3.58 ± 0.40 c	1.24 ± 0.20 b	35.10 ± 4.50 d
ANOVA (p-value)	<0.001	<0.001	<0.001	<0.001	<0.001

Means (±SE) within a column with different superscript letters are significantly different (LSD test, $p \leq 0.05$)

Table 1B. Pairwise comparison (LSD test p-values)

Comparison	Initiation	Shoots	Length	Flowers	Hyperhydricity
Control vs T_1	<0.001	<0.01	<0.01	ns	<0.01
Control vs T_2	<0.001	<0.001	<0.001	<0.001	<0.001
Control vs T_3	<0.001	<0.001	<0.001	<0.001	<0.001
Control vs T_4	<0.001	<0.001	<0.001	<0.001	<0.001
T_1 vs T_2	<0.001	<0.001	<0.001	<0.001	<0.01
T_1 vs T_3	<0.001	<0.001	<0.001	<0.001	<0.001
T_1 vs T_4	<0.001	<0.001	<0.001	<0.001	<0.001

Comparison	Initiation	Shoots	Length	Flowers	Hyperhydricity
T_2 vs T_3	<0.01	<0.001	<0.05	<0.01	<0.05
T_2 vs T_4	ns	Ns	Ns	Ns	<0.001
T_3 vs T_4	<0.001	<0.001	<0.05	<0.001	<0.001

Hormonal interaction and developmental coordination

The results demonstrate that a balanced cytokinin–auxin ratio (1:1) is critical for coordinated vegetative and reproductive development. Traditionally, shoot induction has been associated with cytokinin-dominant conditions, with auxin maintained at low levels to avoid callus formation (George *et al*., 2008). However, the present findings indicate that a relatively high concentration of IAA (3.0 mg L^{-1}) does not inhibit shoot proliferation but instead enhances morphogenic efficiency.

This response may be attributed to the role of auxin in maintaining meristematic competence, promoting vascular differentiation and facilitating assimilate transport to developing tissues (Davies, 2010). Furthermore, the synergistic interaction between BAP and IAA may regulate floral transition pathways by influencing the expression of key genes such as *LEAFY* (*LFY*), *APETALA1* (*AP1*) and *FLOWERING LOCUS T* (*FT*), which are central to floral meristem identity (Krizek & Fletcher, 2005).

These findings suggest a shift from the classical stage-specific hormonal model toward a more integrated regulatory framework, in which a single hormonal balance can simultaneously support multiple developmental processes.

In vitro flowering and floral attributes

A significant outcome of this study is the successful induction of *in vitro* flowering on the same medium used for shoot multiplication. This was achieved under standard sucrose concentration (30 g L^{-1}) without the application of growth retardants.

Previous studies have reported that *in vitro* flowering in roses often requires modified conditions. For instance, Vu *et al*. (2006) induced flowering using elevated sucrose levels (60 g L^{-1}), while Kim *et al*. (2019) employed paclobutrazol to manipulate gibberellin activity. In contrast, the present protocol achieved flowering without such modifications, indicating a simplified and cost-effective system.

The flowers obtained were morphologically normal, with diameters ranging from 1.2 to 1.8 cm (approximately 60% of field-grown flowers), 18–22 petals per flower and stable pigmentation consistent with the donor plant. Pollen viability (48.6 ± 4.2%) is comparable to values reported for *in vitro*-derived pollen in roses, suggesting functional reproductive competence (Wang *et al*., 2002). This highlights the potential application of the system in controlled hybridization and accelerated breeding programs.

Hyperhydricity as a physiological constraint

Hyperhydricity increased progressively with increasing BAP concentration, reaching a maximum of 35.1% in T_4. This trend is consistent with previous studies indicating that high cytokinin levels disrupt water relations, cell wall development and stomatal function (Kevers *et al.*, 2004).

The moderate level observed in T_3 (18.5%) falls within the range reported for rose cultures under similar conditions (Chakrabarty *et al.*, 2006). Although acceptable for experimental applications, hyperhydricity remains a limitation for commercial propagation. Mitigation strategies such as increased agar concentration, improved vessel ventilation and the use of temporary immersion systems have been widely recommended

Root induction

Rooting response varied significantly among treatments ($p < 0.01$ to $p < 0.001$), with Treatment A (full-strength MS + 1.5 mg L^{-1} IBA) producing the highest rooting efficiency (93.3%), root number (4.67) and root length (2.60 cm) (Table 2A).

The effectiveness of IBA in promoting adventitious root formation is well documented in woody plants, including roses (Carelli & Echeverrigaray, 2002; Roy *et al.*, 2004). IBA is considered more stable than IAA and less susceptible to degradation, which enhances its efficacy in *in vitro* rooting systems (Davies, 2010).

Table 2A. Root induction response (n = 15)

Treatment	Medium composition	Roots per shoot	Root length (cm)	Rooting (%)
A	Full MS + IBA	4.67 ± 0.40 b	2.60 ± 0.30 c	93.3 ± 3.3 b
B	Full MS + IBA + GA_3	4.43 ± 0.30 b	1.72 ± 0.20 b	86.7 ± 4.2 b
C	½ MS + IBA	3.55 ± 0.30 a	1.01 ± 0.10 a	73.3 ± 4.5 a
ANOVA (p-value)		<0.01	<0.001	<0.01

Table 2B. Pairwise comparison (LSD test p-values)

Comparison	Roots	Length	Rooting (%)

Comparison	Roots	Length	Rooting (%)
A vs B	Ns	<0.01	Ns
A vs C	<0.05	<0.001	<0.01
B vs C	<0.05	<0.01	<0.05

Means (±SE) within a column with different superscript letters are significantly different (LSD test, $p \leq 0.05$).

Figure 2. *Effect of PGRs on root induction in Rosa hybrida L (A-B) Increasing root number (C-D) Enhancement of root length*

Nutritional effects of basal medium strength

Contrary to commonly reported protocols favoring half-strength MS, the present study demonstrates that full-strength MS significantly enhances root elongation and rooting efficiency (Oo *et al.*, 2021). This may reflect increased nutrient requirements of shoots previously exposed to high cytokinin concentrations during multiplication.

Influence of GA_3 on rooting

The inclusion of GA_3 did not significantly affect root number but markedly reduced root length ($p < 0.01$). This confirms the antagonistic interaction between gibberellins and auxins, where GA_3 suppresses auxin-mediated root development processes (Mauriat *et al.*, 2014; Zhang *et al.*, 2019).

Root morphology and functional quality

Qualitative assessment further confirmed the superiority of Treatment A. Roots were well-developed, thick and highly branched, indicating strong physiological competence. In contrast, roots formed in GA_3-containing medium were thin and less vigorous, while those in half-strength MS were poorly developed. Root morphology is a critical determinant of acclimatization success, as emphasized in earlier studies (Hartmann *et al.*, 2011).

Validation of the integrated protocol

The two-stage system (T_3 for multiplication and flowering followed by Treatment A for rooting) successfully produced complete plantlets within approximately 16 weeks. This demonstrates that morphogenic stages traditionally treated as independent can be effectively integrated through hormonal optimization.

Comparable integrated systems have been reported in other species; however, these often require additional modifications such as elevated sucrose levels or complex hormonal regimes (Naing *et al.*, 2016; Xu *et al.*, 2018). The present system achieves similar outcomes under simplified conditions, enhancing its practical applicability.

4. CONCLUSION

This study establishes a robust and integrated micropropagation system for *Rosa hybrida* L. The optimal hormonal combination (3.0 mg L^{-1} BAP + 3.0 mg L^{-1} IAA) enabled simultaneous shoot proliferation and flowering, while full-strength MS supplemented with 1.5 mg L^{-1} IBA ensured efficient rooting.

The proposed two-stage system reduces production time, simplifies operations and generates reproductively viable plantlets. This approach offers significant potential for commercial propagation, accelerated breeding and fundamental studies in floral biology.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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