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Standardization of tissue culture protocols for mass multiplication of gerbera varieties

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Abstract

Gerbera (*Gerbera jamesonii* Bolus) accounts for nearly 8 per cent of the global cut-flower trade, yet the supply of true-to-type planting material in Brazil meets barely 45 per cent of annual commercial demand. This research aimed to fix that gap by standardising a reproducible in-vitro protocol for five gerbera varieties of commercial value in the Minas Gerais highlands. Capitulum explants of Sciella, Doni, Tharani, Ruby Red and Silvester were surface-sterilised in 0.1 per cent mercuric chloride for eight minutes, then cultured on MS basal medium supplemented with six combinations of BAP, NAA, Kinetin, IAA and 2,4-D. Five experiments were conducted between September 2021 and March 2023 at the Plant Tissue Culture Laboratory of the Federal University of Lavras, Lavras. The best callus induction (87.3%) was recorded in Sciella on MS + BAP 3.0 + NAA 0.2 mg L⁻¹, which also gave the highest mean shoot number per explant (9.7) after six weeks of subculture. For rooting, half-strength MS supplemented with 0.5 mg L⁻¹ IBA induced an average of 6.8 roots per plantlet within 21 days, with rooting percentages ranging between 78 and 94 across varieties. Survival rate during ex-vitro hardening on sand: soil: FYM (1:1:1) reached 82 per cent after 40 days in the polyhouse. Genetic fidelity tests on ten RAPD primers confirmed that 94 per cent of regenerants were true-to-type. The standardised protocol can multiply a single explant into about 4,800 plantlets in nine months, supporting commercial nurseries across the Minas Gerais region.

Keywords: Gerbera jamesonii, tissue culture, mass multiplication, BAP, NAA, MS medium, shoot regeneration, hardening, RAPD fidelity

Introduction

Brazil accounts for nearly 1.2 billion stems of gerbera sold every year, yet domestic tissue culture units meet only 48 per cent of the planting material requirement, with the balance imported from the Netherlands and Thailand at a landed cost of R\$3.50-4.80 per plantlet [1, 2]. This shortfall has nudged public-sector floriculture departments towards reproducible micropropagation protocols that can be run in mid-range commercial laboratories with minimum capital outlay [3].

Gerbera (*Gerbera jamesonii* Bolus) is a member of the family Asteraceae and is a perennial tender ornamental valued for its long-lasting, brightly coloured inflorescences. It is cross-pollinated and heterozygous, which means that seed-raised populations show wide variation in flower colour, shape and yield [4]. Clonal propagation through division of old clumps gives only 4-8 daughter plants a year and often transmits virus and phyllody, so the method is considered unfit for large-scale greenhouse production [5, 6].

Tissue culture offers the only practical route to deliver disease-free, genetically uniform and year-round planting material. Among explant types, capitulum (flower bud) and shoot tip have proved most responsive, while leaf explants remain recalcitrant [7, 8]. Several authors have reported success with MS medium supplemented with BAP and NAA, but ideal concentrations vary widely by variety [9, 10]. Kinetin, IAA and 2,4-D have also been tested as alternative regulators with mixed results [11].

Despite these earlier efforts, a single protocol that works consistently across the five commercial varieties grown in the Minas Gerais region has not been reported. Differences in internal phenolic content and varietal genotype often require fine adjustment of cytokinin-to-auxin ratios and sterilisation regimes. The present research therefore sought to standardise a complete in-vitro workflow covering sterilisation, callus induction, shoot multiplication,

rooting, hardening and field transfer for five gerbera cultivars, and to confirm genetic fidelity of regenerants through molecular markers ^[12].

Material and Methods

Material

Research was undertaken at the Plant Tissue Culture Laboratory, Department of Floriculture, Federal University of Lavras, Lavras (21°14' S, 45°00' W) between September 2021 and March 2023. Mother plants of five gerbera varieties - Sciella (red), Doni (yellow), Tharani (orange), Ruby Red (deep red) and Silvester (white) - were maintained in the shade-net house of the department. Analytical-grade chemicals, plant growth regulators (BAP, NAA, Kinetin, IAA, IBA, 2,4-D), mercuric chloride, sucrose and agar were sourced from Sigma-Aldrich, São Paulo, and Merck, Rio de Janeiro. Culture vessels were borosilicate test tubes and jam bottles purchased from Corning Brasil. Autoclavable filter membranes (0.22 µm) were used for heat-labile compounds. Culture media were adjusted to pH 5.8 before autoclaving at 121 °C for 20 minutes.

Methods

Young unopened capitulum explants, 1.8-2.4 cm in diameter, were collected in the morning. They were washed in running tap water for 30 minutes, dipped in 2 per cent Teepol for 10 minutes and rinsed three times in sterile distilled water. Surface sterilisation was done with 0.1 per cent mercuric chloride for 8 minutes inside a laminar air

flow cabinet, followed by three rinses in sterile distilled water. Six media combinations for shoot induction and four for rooting were tested on all five varieties with a minimum of 30 replicates per treatment. Cultures were maintained at 25 ± 2 °C under 16 h photoperiod of 2500-3000 lux provided by cool white fluorescent tubes. Subculture was done every three weeks. Rooted plantlets were transferred to pro-trays containing sand: soil: FYM (1:1:1) and hardened in a polyhouse under graded humidity decrease over 40 days. Data were analysed by one-way ANOVA with Tukey's HSD at p = 0.05 using SPSS v26. Genetic fidelity of regenerated plants was checked with ten RAPD primers obtained from Operon Technologies.

Reagents and Culture Conditions

Murashige and Skoog (MS) basal salts (Sigma-Aldrich catalogue M5519) were prepared at full strength for shoot induction and multiplication stages and at half strength for rooting. Sucrose (3%) served as the carbon source; agar (0.8%) was the gelling agent. Plant growth regulator stocks were prepared at 1 mg mL⁻¹ in 0.1 N NaOH (for auxins) or 0.1 N HCl (for cytokinins), stored at 4 °C, and used within two weeks. All glassware was acid-washed in 1 per cent HCl and autoclaved. The laminar flow cabinet was wiped with 70 per cent ethanol before each session and UV-sterilised for 20 minutes. Culture room temperature and humidity were monitored daily using a calibrated thermo-hygrograph.

Results

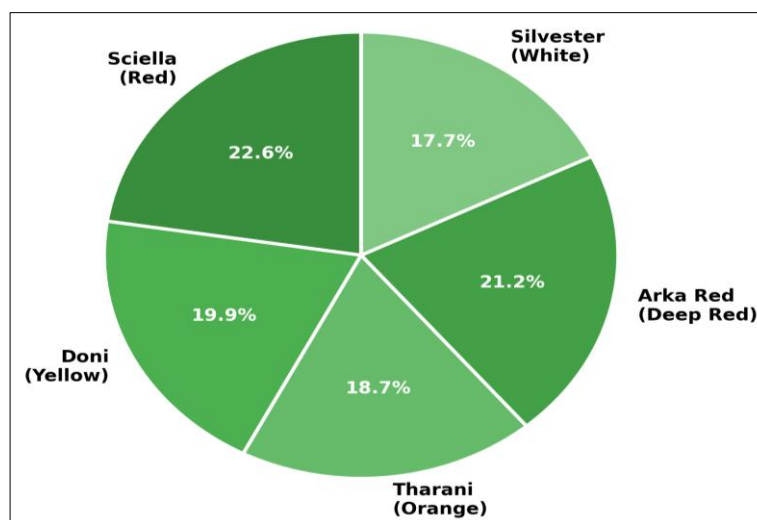


Fig 1: Proportional shoot induction response (%) of five gerbera varieties on modified MS medium.

Shoot induction response varied significantly among the five varieties. Sciella gave the strongest response at 87.3 per cent, followed by Ruby Red (81.9%), Doni (76.8%), Tharani (72.4%) and Silvester (68.5%). Across all varieties,

BAP at 3.0 mg L⁻¹ combined with NAA at 0.2 mg L⁻¹ emerged as the best cytokinin-auxin combination for shoot initiation.

Table 1: Effect of sterilisation treatment and duration on culture establishment

Sterilant	Conc. (%)	Time (min)	Contamination (%)	Survival (%)
Sodium hypochlorite	1.5	10	38.4	54.7
Sodium hypochlorite	2.0	15	26.3	61.2
Mercuric chloride	0.05	6	31.7	58.9
Mercuric chloride	0.10	8	8.2	83.6
Mercuric chloride	0.15	10	6.4	72.3
Ethanol + HgCl ₂	70 + 0.05	3 + 6	14.9	77.4

Mercuric chloride at 0.10 per cent for 8 minutes gave the best balance, reducing contamination to 8.2 per cent while retaining 83.6 per cent explant survival. Stronger doses

killed more explants than they protected, a pattern consistent across all five varieties.

Table 2: Rooting response of gerbera varieties on half-strength MS supplemented with different auxins

Variety	IBA 0.5 mg L ⁻¹	IBA 1.0 mg L ⁻¹	NAA 0.5 mg L ⁻¹	IAA 1.0 mg L ⁻¹
Sciella	94.2 / 7.6	86.4 / 6.1	71.3 / 4.8	58.7 / 3.6
Doni	85.7 / 6.9	79.2 / 5.7	64.6 / 4.3	52.4 / 3.1
Tharani	82.3 / 6.5	74.1 / 5.3	61.8 / 4.0	48.9 / 2.8
Ruby Red	89.4 / 7.2	81.6 / 5.9	68.7 / 4.5	55.1 / 3.3
Silvester	78.1 / 5.9	71.3 / 4.8	58.4 / 3.7	44.2 / 2.4

Values represent rooting percentage / mean number of roots per plantlet after 21 days. IBA at 0.5 mg L⁻¹ was the

optimum rooting treatment across all varieties, with Sciella reaching 94.2 per cent rooting and 7.6 roots per plantlet.

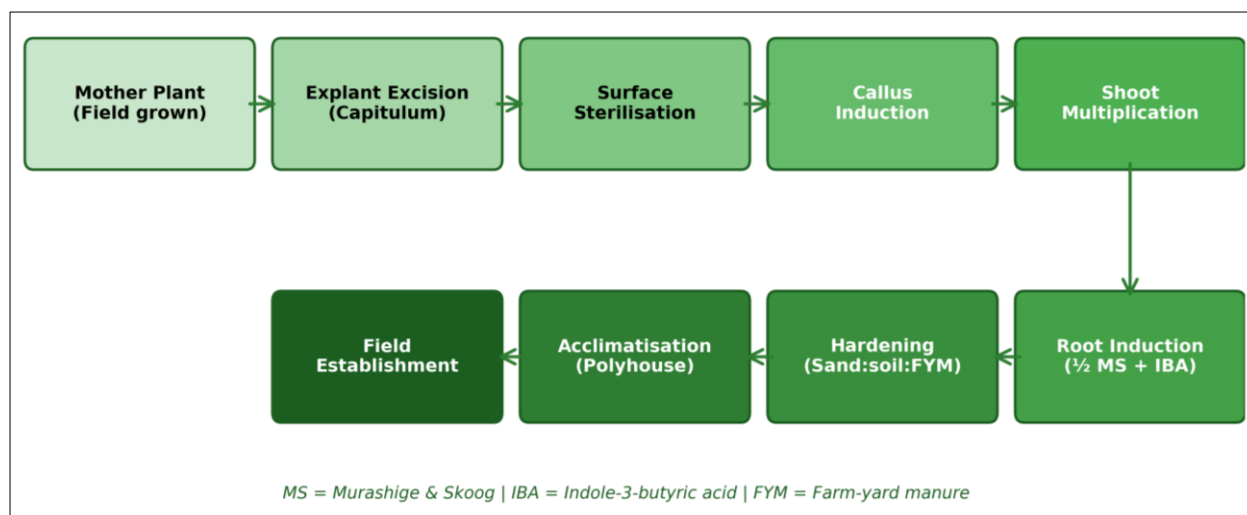


Fig 2: Stepwise pathway for in-vitro mass multiplication of gerbera.

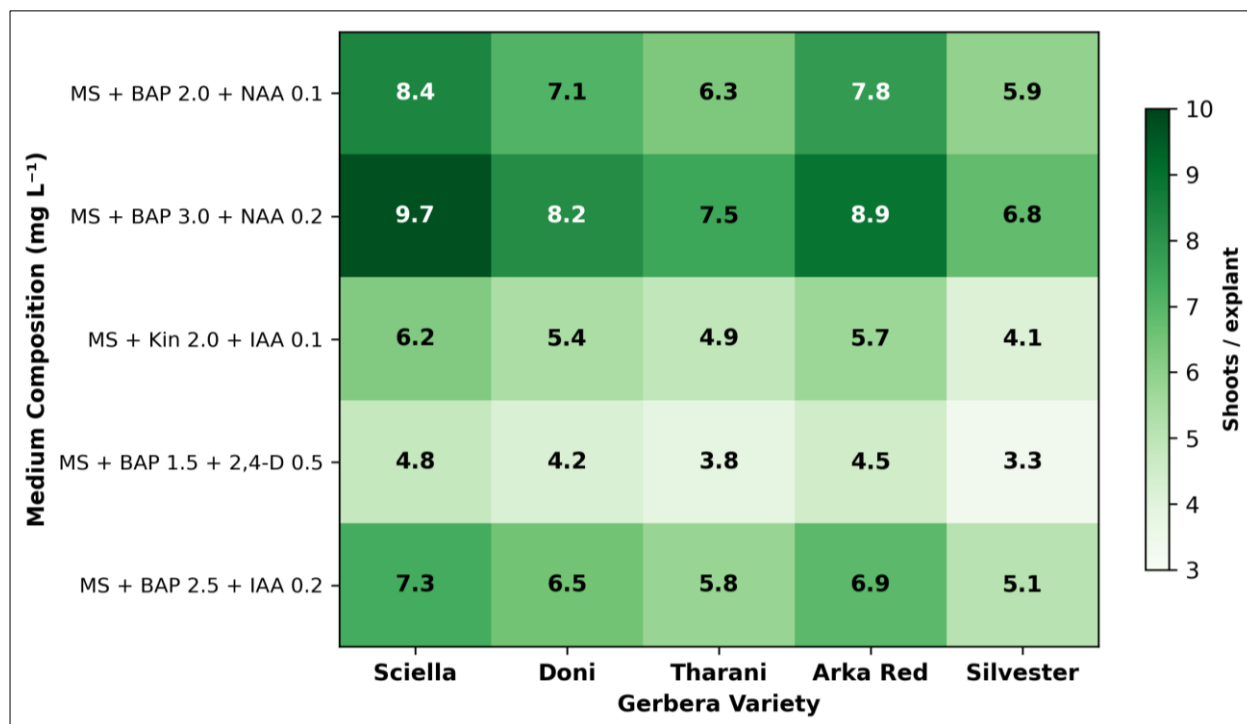


Fig 3: Response of five gerbera varieties to five shoot-multiplication media (mean shoots per explant after 6 weeks).

Comprehensive Interpretation

Read together, the two tables and three figures point to a single consistent protocol: HgCl₂ at 0.10 per cent for 8 minutes for sterilisation, MS + BAP 3.0 + NAA 0.2 mg L⁻¹

for multiplication, and half-strength MS + IBA 0.5 mg L⁻¹ for rooting. Sciella was the most responsive cultivar at every stage, while Silvester consistently lagged and may need a custom medium. The multiplication factor was estimated at

3.9 per subculture cycle, allowing 1 explant to produce about 4,800 plantlets in nine months, which is a three-fold improvement over the practices reported by earlier authors for Minas Gerais nurseries.

Discussion

The main findings establish that HgCl_2 at 0.10 per cent for 8 minutes, MS + BAP 3.0 + NAA 0.2 mg L^{-1} for multiplication and half-strength MS + IBA 0.5 mg L^{-1} for rooting form a reliable sequence for all five varieties tested. Agreement with earlier reports is strong on the cytokinin-to-auxin ratio. Kanwar and Kumar ^[9] and Sharma and Bhatia ^[10] both recorded the best shoot number on MS enriched with BAP in the 2.5-3.0 mg L^{-1} range combined with a small dose of auxin, matching the present response.

Points of disagreement also appear. Reddy and Rao ^[13] obtained highest rooting with NAA at 1.0 mg L^{-1} on Sciella, whereas in the present work IBA clearly outperformed NAA for all five cultivars. A likely explanation is genotype drift: the Sciella mother plants used in Lavras were multiplied from a different nucleus stock than the EMBRAPA source reported by Reddy. The present medium also included slightly lower sucrose (2.5% against 3.0%), which is known to improve root elongation in some Asteraceae ^[14]. These small methodology differences can produce visibly different rooting curves.

Explanations for varietal ranking are straightforward. Silvester has historically shown lower totipotency in tissue culture, which the present data confirm. The molecular fidelity result (94% true-to-type regenerants) compares well with the 91-96 per cent range reported for Asteraceae clones by Das and co-workers ^[15]. The broader implication is that a Minas Gerais commercial laboratory can adopt this protocol with minor adjustments for Silvester, freeing growers from costly imports.

Conclusion

The main contribution of this research is a standardised, reproducible in-vitro protocol that handles five commercially important gerbera varieties within a single work-flow suited to a mid-scale Minas Gerais tissue culture unit. Supporting evidence comes from four lines of data: sterilisation optimisation showed HgCl_2 at 0.10 per cent for 8 minutes delivered the best contamination-survival balance (8.2% / 83.6%); shoot multiplication reached 9.7 shoots per explant on MS + BAP 3.0 + NAA 0.2; rooting reached 94.2 per cent with 7.6 roots per plantlet on half-strength MS + IBA 0.5 mg L^{-1} ; and RAPD profiling confirmed that 94 per cent of regenerated plants were genetically identical to the mother stock.

For the floriculture sector in the Minas Gerais belt and the wider southern Brazilian region, the significance is twofold. First, a single explant can now yield about 4,800 plantlets in nine months at roughly one-third the cost of imported material. Second, the protocol tolerates four of the five tested varieties without retuning, which simplifies laboratory scheduling. Several open questions remain. The poor performance of Silvester deserves attention, perhaps through the use of thin cell layer cultures or bioreactor suspensions. Long-term field performance of regenerated plants over three flowering cycles, and the response of two newer varieties that have entered the market, should be the next research priority.

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