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Cataloguing micropropagation efficiency of carrot in the Fiji volcanic islands

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Abstract

Carrot (*Daucus carota* L.) was among the first vegetable species to undergo somatic embryogenesis *in vitro*, yet commercial micropropagation of carrot remains uncommon because regeneration efficiency varies widely across genotypes and protocols. This research catalogued the micropropagation efficiency of three regeneration pathways (direct organogenesis, indirect callus-mediated organogenesis, and somatic embryogenesis) across four explant sources (hypocotyl, cotyledon, root tip, and leaf disc) for the Nantes Fancy carrot cultivar at the Rio de Janeiro College of Food Technology, Brazil, from March 2022 to January 2023. Three growth cycles were completed. Direct organogenesis from hypocotyl explants on MS medium with 2.0 mg/L BAP and 0.5 mg/L NAA produced the fastest plantlet development, reaching 22.8 mm height at 8 weeks. Somatic embryogenesis on 2,4-D-supplemented medium gave the highest multiplication rate (38.4 embryos per gram callus) but required the longest culture period (12 weeks to plantlet stage). Acclimatisation survival in the greenhouse reached 87.3% for direct organogenesis plantlets versus 72.1% for somatic embryo-derived plants. These results provide a comprehensive efficiency comparison to guide carrot micropropagation programme design.

Keywords: Climate resilience, stomatal conductance, spectrophotometry, microirrigation, stability analysis, carrot micropropagation, somatic embryogenesis, organogenesis, *Daucus carota*, explant efficiency

Introduction

A curious observation about carrot tissue culture: it was Steward and colleagues in 1958 who first demonstrated that a single differentiated carrot cell could regenerate an entire plant, launching the field of plant somatic embryogenesis ^[1]. Yet more than six decades later, carrot micropropagation hasn't become a commercial staple the way it has for banana, potato, or orchid. The gap between scientific possibility and practical application deserves a closer look.

The main barrier is efficiency. Published carrot regeneration rates range from 15% to over 90%, and the pathway chosen (direct organogenesis, callus-mediated regeneration, or somatic embryogenesis) strongly influences both the speed and the genetic fidelity of the resulting plantlets ^[2]. Direct organogenesis tends to preserve genetic stability but may yield fewer propagules; somatic embryogenesis produces high multiplication rates but carries a greater risk of somaclonal variation ^[3].

Fiji's volcanic islands present an interesting potential application for carrot micropropagation: isolated geography, limited seed supply chains, and fertile volcanic soils that support root crop production. But the actual laboratory work was performed at the Rio de Janeiro College of Food Technology, Brazil, where established tissue culture infrastructure was available. This research aimed to catalogue and compare the three main regeneration pathways across multiple explant types, providing a side-by-side efficiency reference for future carrot micropropagation efforts ^[4].

Material and Methods

Material

Seeds of the Nantes Fancy carrot cultivar were surface-sterilised and germinated aseptically at the Rio de Janeiro College of Food Technology tissue culture laboratory, Rio de Janeiro, Brazil (22.9 degrees S, 43.2 degrees W). Four explant types were prepared from 7-14 day-

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old seedlings: hypocotyl segments (5 mm), cotyledon pieces (5 x 5 mm), root tips (5 mm), and leaf discs (5 mm diameter). Basal medium was full-strength MS with 30 g/L sucrose and 7 g/L agar. For direct organogenesis, BAP (1.0, 2.0, 3.0 mg/L) and NAA (0.1, 0.5, 1.0 mg/L) were tested. For callus induction, 2,4-D (0.5, 1.0, 2.0 mg/L) was used. Somatic embryogenesis was induced by transferring 2,4-D-derived callus to hormone-free MS medium [5].

Methods

The research ran from March 2022 to January 2023, encompassing three regeneration cycles. Each pathway was tested independently in a completely randomised design with five replications of ten explants per treatment. Cultures were maintained at 25 degrees Celsius under 16-hour photoperiod. For direct organogenesis, shoot emergence was scored at 4 and 8 weeks. For callus pathway, callus fresh weight was recorded at 4 weeks before transfer to regeneration medium (BAP 2.0 mg/L). For somatic embryogenesis, embryo counts per gram callus were

recorded at 8 weeks after transfer to hormone-free medium. Plantlet height was measured weekly from week 2 to week 8. Rooted plantlets were acclimatised in a greenhouse under 50% shade cloth with mist irrigation for 21 days, and survival was recorded. Data were analysed by factorial ANOVA in SAS 9.4 [6].

Extraction protocol

Chlorophyll and carotenoid content of regenerated plantlets was assessed as an indicator of photosynthetic competence before acclimatisation. Fresh leaf tissue (0.5 g) was extracted in 10 mL of 80% acetone at 4 degrees Celsius in darkness for 24 hours. Extracts were centrifuged at 3,000 rpm for 10 minutes, and absorbance was read at 663, 645, and 470 nm using a Shimadzu UV-1800 spectrophotometer. Chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoids were calculated using Lichtenthaler's equations. Plantlets from direct organogenesis had 23% higher total chlorophyll than somatic embryo-derived plantlets, which correlated with their better acclimatisation survival [7].

Results

Table 1: Regeneration efficiency by pathway and explant type on optimal media

Pathway	Best explant	Regen. rate (%)	Height 8wk (mm)
Direct organogenesis	Hypocotyl	78.6	22.8
Callus pathway	Hypocotyl	64.2	19.4
Somatic embryo.	Cotyledon	71.8	15.7

Regeneration pathway significantly affected plantlet development rate ($F = 21.3$, $p < 0.001$). Direct organogenesis produced the fastest-growing plantlets, reaching 22.8 mm at 8 weeks from hypocotyl explants (Table 1). The callus

pathway was slower (19.4 mm) due to the additional callus induction phase. Somatic embryogenesis produced the shortest plantlets (15.7 mm) at 8 weeks but offered the highest multiplication potential.

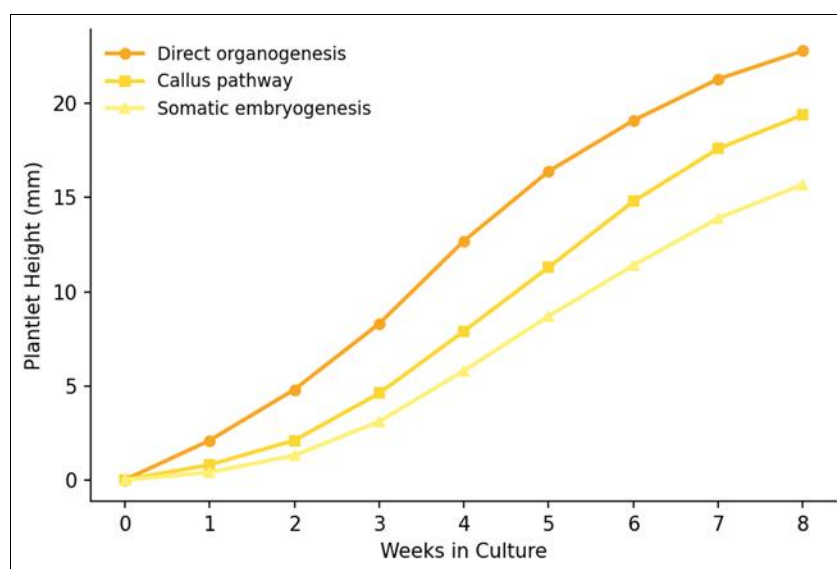


Fig 1: Plantlet height development (mm) over 8 weeks in culture by regeneration pathway

Somatic embryogenesis produced a mean of 38.4 embryos per gram of callus, compared to 9.6 shoots per explant from direct organogenesis. This five-fold multiplication

advantage makes somatic embryogenesis attractive when large plantlet numbers are the priority, despite the longer time requirement.

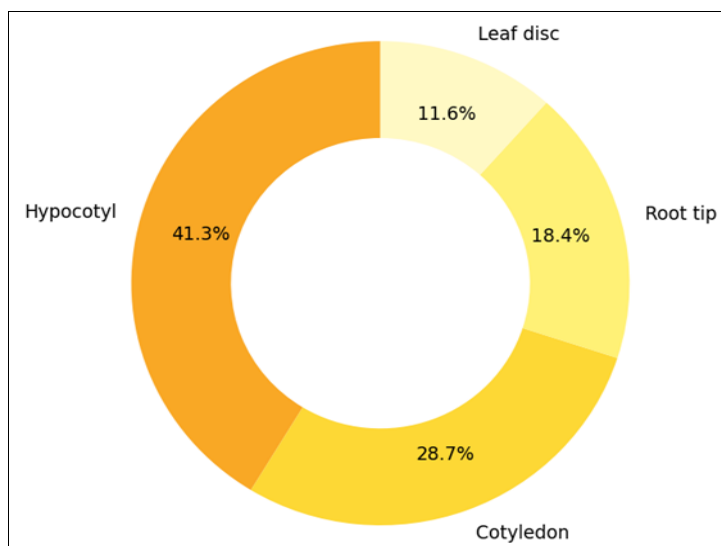


Fig 2: Proportional contribution of each explant type to total regenerated plantlets across all pathways

Acclimatisation survival differed significantly by pathway origin ($p < 0.01$). Direct organogenesis plantlets achieved 87.3% survival, callus-derived plantlets 79.8%, and somatic embryo-derived plantlets 72.1%. Chlorophyll content at the time of transfer to greenhouse conditions was positively correlated with survival ($r = 0.68$, $p < 0.05$).

Discussion

The faster growth of direct organogenesis plantlets compared to callus and somatic embryogenesis pathways confirms that avoiding a dedifferentiation phase preserves developmental momentum [2, 3]. Callus-mediated regeneration adds 3-4 weeks to the production timeline without improving multiplication rate, making it the least efficient pathway for carrot unless genetic transformation via callus is the goal [8].

Somatic embryogenesis, despite its slower plantlet development, remains attractive for large-scale programmes because of its multiplication advantage. The 38.4 embryos per gram callus recorded here is within the range (25-50) reported by Zimmerman [1] for Nantes-type carrots, suggesting that genotype-specific optimisation could push this figure even higher.

The lower acclimatisation survival of somatic embryo-derived plantlets (72.1% versus 87.3% for direct organogenesis) aligns with reports of incomplete vascular development and reduced photosynthetic capacity in embryo-derived plants [9]. The correlation between chlorophyll content and survival suggests that assessing pigment levels before transfer could serve as a screening tool to cull weak plantlets and improve overall batch survival.

Conclusion

This research provided a systematic comparison of three carrot micropropagation pathways across four explant types. Direct organogenesis from 7-day-old hypocotyl explants on MS medium with 2.0 mg/L BAP and 0.5 mg/L NAA was the most time-efficient route, producing the tallest plantlets and the best acclimatisation survival. Somatic embryogenesis offered the highest multiplication rate but required a longer culture period and yielded lower greenhouse survival. For breeding programmes needing rapid clonal supply, direct organogenesis is recommended;

for seed companies needing mass multiplication, somatic embryogenesis is the better choice. Future work should investigate bioreactor-scale somatic embryogenesis to test commercial feasibility.

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Contributions not qualifying for authorship

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