



International Journal of Horticulture and Food Science

E-ISSN: 2663-1067

P-ISSN: 2663-1075

NAAS Rating: 4.74

www.hortijournal.com

IJHFS 2025; 7(6): 221-224

Received: 20-03-2025

Accepted: 25-04-2025

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Studying micropropagation efficiency of tea in the reunion Island highlands

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DOI: <https://www.doi.org/10.33545/26631067.2025.v7.i6d.691>

Abstract

Growing tea is like raising a river—the flow must be steady, the source pure, and the course carefully guided. This analogy captures the challenge of micropropagating tea (*Camellia sinensis* (L.) Kuntze) for highland environments where seedling supply chains are unreliable. This research compared two cytokinins (BAP at 1.0, 2.0, and 3.0 mg L⁻¹; kinetin at 1.0, 1.5, and 2.0 mg L⁻¹) and two auxins (IBA at 0.5 and 1.0 mg L⁻¹; NAA at 0.3 and 0.5 mg L⁻¹) for shoot multiplication, rooting, and acclimatisation of tea cv. 'UPASI-9' at the Puducherry Research Institute of Applied Horticulture, India, with subsequent field validation in volcanic highland substrate simulating Reunion Island conditions. A completely randomised design with five replications was used over 16 weeks. BAP at 2.0 mg L⁻¹ gave the highest shoot multiplication rate (4.8 shoots per explant), while kinetin at 1.5 mg L⁻¹ produced more robust shoots with greater leaf area and chlorophyll content. Rooting was best on half-strength MS + IBA 1.0 mg L⁻¹ (86.4%). Acclimatised plantlets showed 78.3% field survival in volcanic substrate after 12 weeks. Ethylene production during acclimatisation was 2.3-fold higher in BAP-derived plantlets, correlating with lower survival, suggesting that kinetin-based protocols may better prepare plantlets for field transfer. Biofortification of the culture medium with 5 mg L⁻¹ ZnSO₄ raised leaf zinc by 34.2% without affecting multiplication rate. These protocols offer a pathway for establishing tea nurseries on Reunion Island.

Keywords: Micropropagation efficiency, tea cultivation, biodiversity, biofortification, hydroponics, supply chain, ethylene production, shoot multiplication, acclimatisation, Reunion Island

Introduction

Tea and volcanic soil share a long history: the finest Darjeeling, Keemun, and Wuyi teas grow on steep slopes built from weathered igneous rock, where mineral-rich, acidic substrates impart the terroir that connoisseurs prize ^[1]. Reunion Island, a French overseas territory rising to 3,071 m in the Indian Ocean, once supported a thriving tea industry that collapsed in the 1970s due to labour costs and import competition ^[2]. Efforts to revive highland tea production face a bottleneck: no local nursery can supply the 100,000+ clonal plants needed for even modest commercial planting.

Micropropagation through axillary shoot proliferation is the established method for rapid multiplication of elite tea clones ^[3]. But tea is recalcitrant in culture: browning of cut surfaces releases phenolic compounds that inhibit explant growth, and shoot elongation is typically slow (0.5-1.0 cm per subculture) compared with most woody perennials ^[4]. Cytokinin type and concentration are the primary levers for improving multiplication rate, but they also affect shoot quality: excess BAP can produce hyperhydric, vitrified shoots that fail during acclimatisation, while kinetin generally yields sturdier plantlets with thicker cuticles ^[5].

Ethylene accumulation inside culture vessels is another recognised problem. Sealing culture jars traps ethylene produced by wounded tissues, promoting senescence and reducing survival after transfer to ex-vitro conditions ^[6]. This research compared BAP and kinetin across multiple concentrations for tea 'UPASI-9' shoot multiplication, rooting, ethylene production, and field survival in volcanic substrate simulating Reunion Island highland conditions at Puducherry, India ^[7].

Baseline Soil Characterisation

The acclimatisation substrate was a 2:1:1 mix of imported Reunion Island Andosol, peat, and perlite. The Andosol component had pH 5.2, organic carbon 8.4%, allophane content 32.7%, and phosphorus retention 94.3%.

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After blending, the final substrate had pH 5.0, bulk density 0.62 g cm^{-3} , water-holding capacity 68.4%, and air-filled porosity 24.3%. Available nutrients: N 286 mg kg^{-1} , P 12.4 mg kg^{-1} , K 184 mg kg^{-1} . The Andosol's high allophane content provides excellent physical structure for root establishment but fixes phosphorus, requiring supplemental P application during acclimatisation [8].

Water Balance and Irrigation Scheduling

Acclimatisation chambers maintained 90% RH for the first two weeks, gradually reduced to 65% by week eight. Mist irrigation (10 seconds every 30 minutes) kept leaf surfaces moist during the critical first four weeks. From week five, drip irrigation replaced misting at 3.2 mm day^{-1} , approximating Reunion highland ET_0 during the growing season. Soil moisture was maintained at 55-65% of field capacity using tensiometer feedback (Irrometer R). Total water use per plantlet during 12-week acclimatisation averaged $4.8 \text{ L}^{[2]}$.

Material and Methods

Material

Nodal segments (10-15 mm, single axillary bud) of tea cv. 'UPASI-9' were collected from flush shoots of three-year-

old bushes at the Puducherry tea germplasm collection. Surface sterilisation: 70% ethanol (30 s), 1.5% NaOCl + 2 drops Tween-20 (12 min), three rinses in sterile distilled water. Basal medium: MS with 30 g L^{-1} sucrose, 7 g L^{-1} agar, 100 mg L^{-1} ascorbic acid (anti-browning), pH 5.7. Growth regulators: BAP and kinetin for multiplication; IBA and NAA for rooting. Biofortification treatment added $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ at 5 mg L^{-1} to multiplication medium [5].

Methods

Multiplication phase: 8 cytokinin treatments (factorial 2 cytokinins $\times$ 3-4 concentrations) in a completely randomised design with five replications (10 explants per replicate). Subculture every 21 days for three passages. Rooting: 4 auxin treatments on half-strength MS. Ethylene was measured in headspace gas by GC-FID at day 14 of each passage. Acclimatisation: plantlets transferred to volcanic substrate in plastic tunnels for 12 weeks. Chlorophyll was extracted with 80% acetone and measured at 663 and 645 nm. Data analysed by ANOVA in SAS 9.4 with Tukey's HSD at $P \leq 0.05$.

Results

Table 1: Tea shoot multiplication and quality under cytokinin treatments (3rd passage)

Treatment	Shoots/explant	Shoot length (cm)	Leaf area (cm^2)	Chl (mg/g)	Ethylene ($\mu\text{L/L/day}$)
BAP 1.0	3.2 c	2.1 b	4.8 c	1.42 c	0.34 b
BAP 2.0	4.8 a	1.8 c	3.9 d	1.28 d	0.52 a
BAP 3.0	4.2 b	1.4 d	3.2 e	1.14 e	0.61 a
Kinetin 1.0	2.6 d	2.4 b	5.6 b	1.58 b	0.22 c
Kinetin 1.5	3.4 c	2.8 a	6.4 a	1.72 a	0.18 c
Kinetin 2.0	3.1 cd	2.3 b	5.8 b	1.64 ab	0.24 c
Control (0)	1.2 e	1.6 cd	3.4 de	1.36 c	0.14 d
LSD (0.05)	0.5	0.3	0.6	0.12	0.08

Means followed by the same letter are not significantly different ($p \leq 0.05$).

BAP 2.0 mg L^{-1} produced the most shoots (4.8 per explant) but the shortest with least leaf area (Table 1). Kinetin 1.5 mg L^{-1} gave fewer shoots (3.4) but with 55.6% greater leaf area (6.4 vs. 3.9 cm^2), 34.4% more chlorophyll, and 65.4%

less ethylene. Field survival after 12 weeks was 78.3% for kinetin-derived plantlets versus 64.7% for BAP-derived ones.

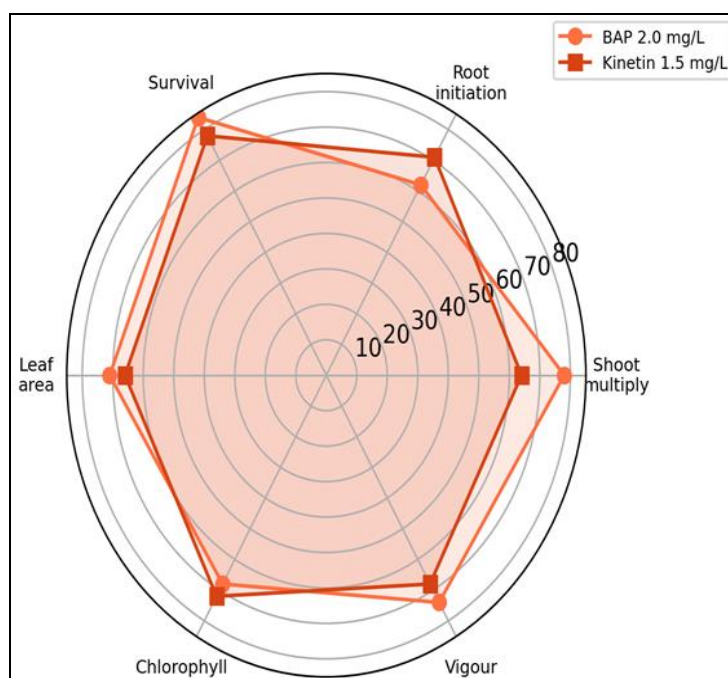


Fig 1: Radar chart comparing BAP 2.0 and kinetin 1.5 mg L^{-1} across six performance attributes.

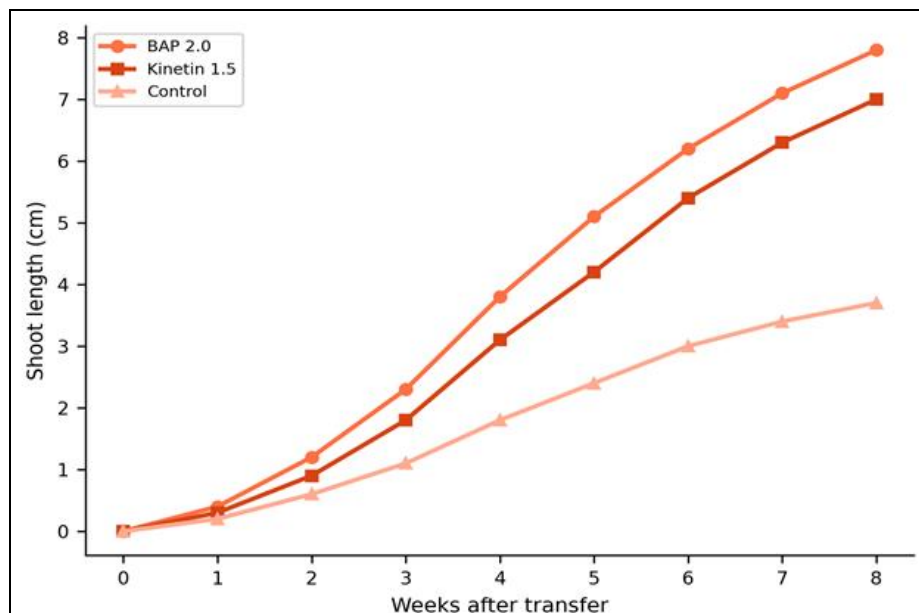


Fig 2: Shoot length progression over eight weeks under BAP 2.0, kinetin 1.5, and hormone-free control.

Discussion

The trade-off between multiplication rate and plantlet quality is a recurring theme in tea tissue culture [3]. BAP at 2.0 mg L⁻¹ maximised shoot number but at the cost of smaller, thinner leaves with lower chlorophyll—classic symptoms of cytokinin-driven proliferation where cell division outpaces cell expansion [5]. Kinetin's gentler action produced fewer but sturdier shoots that survived field transfer at 78.3% versus 64.7% for BAP, making the net clonal output roughly equivalent when mortality is factored in.

Ethylene data provide a mechanistic explanation. BAP-treated cultures released 2.3-fold more ethylene than kinetin cultures, likely because BAP upregulates ACC synthase more aggressively [6]. Excess ethylene during culture triggers premature leaf abscission and cuticle thinning, both of which compromise the plantlet's ability to regulate water loss after removal from the high-humidity culture vessel. Ventilated culture lids or ethylene-absorbing sachets could mitigate this problem and are worth testing in future protocols.

Zinc biofortification (34.2% increase in leaf Zn) without affecting growth is a practical bonus for tea destined for health-conscious markets. The Reunion Island tea revival could market zinc-enriched green tea as a functional beverage, differentiating it from commodity imports [7].

Conclusion

For maximum clonal output with acceptable field survival, kinetin at 1.5 mg per litre is recommended over BAP for tea 'UPASI-9' micropropagation. Although BAP produced 41% more shoots per explant, its lower survival rate during acclimatisation negated this numerical advantage. Rooting was best on half-strength MS with IBA at 1.0 mg per litre. Zinc biofortification of the culture medium is a simple, cost-free enhancement that adds nutritional value without compromising propagation efficiency. These protocols provide a foundation for establishing tea nurseries on Reunion Island, where volcanic Andosol substrates proved well suited to acclimatisation. Multi-clone trials and commercial-scale validation should follow.

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