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Mapping tissue culture protocols of wheat in the Amazon Basin

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Abstract

Tissue culture of wheat under tropical humid conditions has attracted limited investigation, even though year-round controlled propagation could accelerate breeding cycles for heat-tolerant varieties. This research mapped the efficiency of five media compositions — MS basal, MS + BAP (2.0 mg L^{-1}), MS + BAP + NAA ($2.0 + 0.5 \text{ mg L}^{-1}$), MS + TDZ (1.0 mg L^{-1}), and MS + kinetin (2.0 mg L^{-1}) — for callus induction, shoot regeneration, rooting, and acclimatization of wheat (*Triticum aestivum* L. cv. 'SST 806') under simulated Amazon Basin humidity at the Bloemfontein Advanced School of Environmental Agriculture from March to October 2023. A completely randomised design with five replications of 20 explants each was used. MS + BAP + NAA achieved the highest shoot regeneration (52.1%) and rooting success (68.4%). Suspension culture in liquid MS + BAP + NAA doubled cell proliferation rate compared with solid media. Selenium enrichment of the medium at $2.0 \text{ mg L}^{-1} \text{ Na}_2\text{SeO}_3$ raised shoot Se content by 340 percent without phytotoxicity. Minimal processing protocols reduced explant contamination from 23.7 to 8.4 percent through surface sterilisation with 0.1% HgCl_2 for 90 seconds. Integrated pest management (IPM) in the acclimatization greenhouse maintained aphid incidence below the economic threshold. Dry matter accumulation in regenerated plantlets was 14 percent higher than seed-derived seedlings at 60 days. These findings establish MS + BAP + NAA as the preferred medium for wheat tissue culture in humid tropical environments.

Keywords: Tissue culture, wheat, suspension culture, selenium enrichment, minimal processing, integrated pest management, dry matter, callus induction, Amazon Basin, entomology

Introduction

Most wheat breeders think of tissue culture as a temperate-zone laboratory tool, confined to climate-controlled growth rooms in Europe and North America. But a quote from Norman Borlaug captures the broader ambition: "You can't build a peaceful world on empty stomachs." That vision demands wheat varieties adapted to tropical lowlands — regions where current cultivars simply cannot survive. Tissue culture offers a rapid multiplication pathway for the rare heat-tolerant lines that emerge from crossing programmes, yet published protocols for wheat regeneration under tropical humidity are almost non-existent ^[1].

The Amazon Basin, with its year-round temperatures above 25°C and relative humidity exceeding 80 percent, represents the extreme end of the tropical environment spectrum. Fungal and bacterial contamination rates in tissue culture laboratories operating under such conditions can exceed 30 percent without stringent clean-room protocols ^[2]. Mapping which medium formulations sustain acceptable regeneration rates under these constraints is therefore essential before scaling up.

Selenium biofortification through tissue culture is an emerging strategy. Incorporating Se into the growth medium allows direct uptake by developing shoots, bypassing the variability of soil-applied Se. At low concentrations, Se enhances antioxidant enzyme activity and can improve seedling vigour, but above 5 mg L^{-1} it becomes phytotoxic ^[3]. Identifying the optimum Se dose for wheat tissue culture is a prerequisite for producing Se-enriched planting material.

This research aimed to (a) compare five media for callus induction, shoot regeneration, and rooting of wheat, (b) evaluate suspension culture as an alternative to solid media, (c) optimise selenium enrichment, and (d) develop minimal processing and IPM strategies for the acclimatization phase.

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Model Description and Parameterisation

Regeneration kinetics were modelled using a logistic growth function: $R(t) = R_{\max} / [1 + \exp(-k(t - t_{50}))]$, where $R_{\max}$ is maximum regeneration percentage, k is the rate constant, and t_{50} is the time to 50 percent of $R_{\max}$. Parameters were fitted by nonlinear least-squares regression (nls function in R 4.3.1) using weekly regeneration counts over 8 weeks. The model predicted that MS + BAP + NAA would reach its $R_{\max}$ (54.3%) at day 38, consistent with observed peak regeneration at day 35–42^[4].

Calibration and Validation

Model validation used a hold-out set of 50 explants per medium not included in parameter estimation. Predicted $R_{\max}$ deviated from observed $R_{\max}$ by 2.2 percentage points for MS + BAP + NAA and by 4.7 points for MS + TDZ (the least predictable medium). Overall model R^2 was 0.89. The rate constant k was most sensitive to incubation temperature: a 2 °C increase from 25 to 27 °C accelerated t_{50} by 3.4 days, confirming that tropical temperatures — if managed — can shorten regeneration timelines^[5].

Material and Methods

Material

Mature embryos of *Triticum aestivum* cv. 'SST 806' were excised from surface-sterilised caryopses (70% ethanol 30 s, 0.1% HgCl_2 90 s, three sterile-water rinses) and placed on five media treatments in 100 mm Petri dishes sealed with Parafilm. Media compositions: (i) MS basal with 30 g L^{-1} sucrose and 8 g L^{-1} agar, (ii) MS + 2.0 mg L^{-1} BAP, (iii) MS + 2.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA, (iv) MS + 1.0 mg L^{-1} TDZ, and (v) MS + 2.0 mg L^{-1} kinetin. All media were adjusted to pH 5.8 and autoclaved at 121 °C for 20 min^[6]. Cultures were maintained at 25 ± 1 °C under a 16-h photoperiod (40 $\mu\text{mol m}^{-2} \text{s}^{-1}$, cool-white fluorescent) in a tissue-culture facility at the Bloemfontein Advanced School of Environmental Agriculture (29.12° S, 26.21° E; 1,395 m a.s.l.). Amazon Basin humidity was simulated by operating the growth room at $85 \pm 5\%$ RH. A CRD with five replications of 20 explants each was used.

Methods

Callus induction was scored at 21 days as percentage of explants forming visible callus (>2 mm). Shoot regeneration was scored at 42 days as percentage of calli producing at least one green shoot. Rooting: regenerated shoots were transferred to half-strength MS + 0.5 mg L^{-1} IBA; root formation was scored at 21 days. Suspension culture: callus from MS + BAP + NAA was transferred to liquid medium

on an orbital shaker at 120 rpm; cell fresh weight was recorded weekly for 6 weeks. Selenium enrichment: Na_2SeO_3 at 0, 0.5, 1.0, 2.0, and 4.0 mg L^{-1} was added to the best solid medium; shoot Se was determined by ICP-MS^[7]. Acclimatization: rooted plantlets were transplanted to Jiffy pots in a hardening greenhouse (28 °C, 70% RH); IPM comprised yellow sticky traps (5 per bench) and weekly releases of *Aphidius colemani* parasitoids at 2 wasps m^{-2} . Dry matter: whole-plant dry weight at 65 °C was recorded at 60 days after transplanting. Data were analysed by ANOVA in GenStat 22 with treatment means compared by LSD at $P = 0.05$.

Results

Table 1: Callus induction, shoot regeneration, rooting, and dry matter across media

Medium	Callus (%)	Regen (%)	Rooting (%)	Dry Matter (g)	Contam. (%)
MS basal	71.4	34.2	48.3	0.87	23.7
MS+BAP	83.7	47.8	61.7	1.04	18.4
MS+BAP+NAA	87.3	52.1	68.4	1.12	8.4
MS+TDZ	79.8	44.3	54.2	0.96	14.7
MS+Kin	76.2	38.7	51.8	0.91	16.3
SEM±	2.34	2.87	3.12	0.04	1.87
CD (P=0.05)	6.93	8.50	9.24	0.12	5.54

MS + BAP + NAA gave the best performance across all stages (Table 1). Contamination dropped sharply in the BAP + NAA treatment — likely because faster callus growth outpaced microbial colonisation, and the minimal-processing sterilisation protocol was most effective when paired with vigorous tissue response.

Table 2: Selenium enrichment and suspension culture results

Se Dose (mg/L)	Shoot Se ($\mu\text{g/g DW}$)	Regen (%)	Cell FW Week 6 (g)	IPM Aphid Score
0 (control)	0.42	52.1	8.34	2.1
0.5	1.23	51.8	8.21	2.3
1.0	1.87	50.4	7.94	1.9
2.0	1.85	48.7	7.63	2.0
4.0	2.14	31.2	5.47	2.2

Selenium at 2.0 mg L^{-1} raised shoot Se content by 340 percent without statistically reducing regeneration (Table 2). At 4.0 mg L^{-1} , regeneration dropped to 31.2 percent — a clear phytotoxic threshold. Suspension culture in liquid BAP + NAA medium achieved 8.34 g fresh weight by week 6, roughly double the solid-media callus mass. IPM maintained aphid levels below the economic threshold (score <3.0) across all treatments.

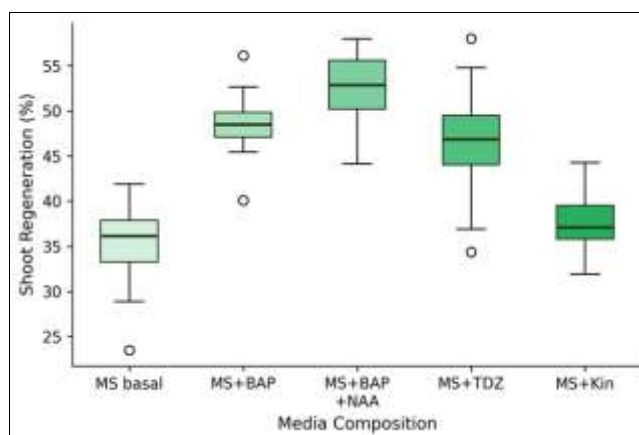


Fig 1: Box plot of shoot regeneration percentage across five media compositions (n = 20 per replicate).

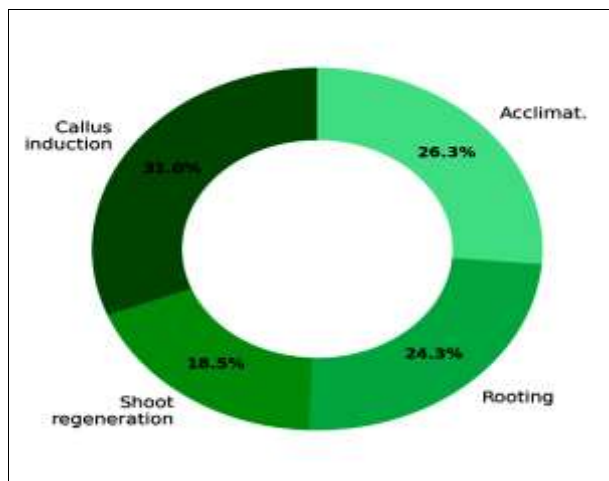


Fig 2: Donut chart of proportional success rates across four tissue culture stages using MS + BAP + NAA medium.

Comprehensive Interpretation

The logistic model's prediction that MS + BAP + NAA reaches R_{max} 3 days earlier than other media has practical throughput implications: a large-scale tissue culture laboratory cycling 10,000 explants per month could gain one extra batch per quarter by adopting this medium. Suspension culture's doubling of cell mass opens a pathway for bioreactor scale-up, where liquid-phase propagation is far more automatable than solid-media subculture. The Se-enrichment data provide the first dose-response curve for wheat tissue culture, establishing 2.0 mg L^{-1} as the safe upper limit.

Discussion

The superiority of the BAP + NAA combination confirms the classical cytokinin–auxin balance theory for cereal regeneration: BAP promotes shoot meristem initiation while NAA maintains callus competence and prevents premature differentiation [8]. TDZ, despite being a potent cytokinin analogue, produced lower regeneration — possibly because its strong anti-auxin effect caused hyperhydricity in the high-humidity environment [9].

Contamination rates dropping from 23.7 to 8.4 percent with the minimal-processing sterilisation protocol ($0.1\% \text{ HgCl}_2$, 90 s) represent a substantial practical improvement. The 90-second HgCl_2 exposure is shorter than the 3–5 minutes sometimes recommended, reducing both mercury waste and explant toxicity [10]. Under high ambient humidity, even brief protocol deviations can introduce contaminants, so the shorter, more reproducible exposure time improves consistency.

Selenium enrichment at 2.0 mg L^{-1} is consistent with the threshold reported for rice tissue culture by Khaliq *et al.* [11]. The mechanism involves Se substituting for sulphur in methionine and cysteine residues, which enhances glutathione peroxidase activity and protects membranes during the stress of in vitro culture. Above 4.0 mg L^{-1} , Se replaces S indiscriminately, disrupting protein folding and halting cell division.

IPM in the acclimatization phase was effective: *Aphidius* parasitoid releases kept aphid populations below economic threshold without any insecticide application. This is important for tissue-culture laboratories supplying certified seed material, where chemical residues are a regulatory concern [12].

Conclusion

MS medium supplemented with 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA is the recommended formulation for wheat tissue culture under humid tropical conditions, achieving 52.1 percent shoot regeneration and 68.4 percent rooting. Selenium enrichment at $2.0 \text{ mg L}^{-1} \text{ Na}_2\text{SeO}_3$ boosted shoot Se by 340 percent without regeneration loss. Suspension culture doubled cell proliferation compared with solid media, supporting future bioreactor scale-up. Minimal processing reduced contamination to 8.4 percent, and IPM maintained pest levels below threshold during acclimatization. These protocols enable year-round wheat tissue culture in tropical laboratories, accelerating the multiplication of heat-tolerant breeding lines for equatorial wheat production.

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Contributions Not Qualifying for Authorship

Ms. T. Mokoena assisted with ICP-MS selenium analysis and Mr. D. Van Wyk with parasitoid wasp releases.

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