Research Journal of Biotechnology and Life Science
Volume 6, Issue 1, 2026 (pp. 19-33)
www.abjournals.org

IN VITRO MULTIPLICATION OF TWO LOCAL TARO VARIETIES (*COLOCASIA ESCULENTA* (L.) SCHOTT) USING SHOOT TIP EXPLANTS IN TOGO

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Cite this article:

Kola, K. E., Bammite, D., Bodjona, B. P. I T., Akan, L., Toundou, O., Odah, K., Tozo, K. (2026), In Vitro Multiplication of Two Local Taro Varieties (Colocasia Esculenta (L.) Schott) Using Shoot Tip Explants in Togo. Research Journal of Biotechnology and Life Science 6(1), 19-33. DOI: 10.52589/RJBLS-PQQK4CMS

Manuscript History

Received: 18 Mar 2026

Accepted: 20 Apr 2026

Published: 21 May 2026

ABSTRACT: *Taro (Colocasia esculenta (L.) Schott) is an important crop for global food security. In Togo, its production remains low due to limited high-quality planting material. This study developed an efficient in vitro micropropagation protocol for local taro varieties 'Kpèkpèou rouge' (TGUL Ce4) and 'Kpèkpèou vert' (TGUL Ce3), using shoot tip explants. Following initiation on modified MS medium, plantlets were evaluated on ten culture media (M0–M9). Quantitative data were analysed using GenStat (12th edition). Treatment effects were assessed by one-way ANOVA, and mean separation was performed using Duncan's Multiple Range test. For shoot multiplication, the highest number of shoots was obtained on MS+1 mg L^{-1} BAP+0.75 mg L^{-1} IAA for 'TGUL Ce4' (7.33 ± 2.64) and on MS+2.5 mg L^{-1} BAP+1 mg L^{-1} kinetin for 'TGUL Ce3' (10.2 ± 2.39). For rooting, optimal root formation was observed on the control medium, with 14.88 ± 3.44 and 16.8 ± 3.52 roots per explant for 'TGUL Ce4' and 'TGUL Ce3', respectively. Plant acclimatisation success exceeded 96% for both varieties. These findings establish variety-specific and efficient micropropagation protocols that can support the rapid and sustainable production of improved taro planting material in Togo.*

KEYWORDS: Acclimatisation; *Colocasia esculenta*; *in vitro*; multiplication; Togo.

Article DOI: 10.52589/RJBLS-PQQK4CMS
DOI URL: https://doi.org/10.52589/RJBLS-PQQK4CMS

INTRODUCTION

Taro (*Colocasia esculenta* (L.) Schott) is a perennial herbaceous species of the family Araceae (Okonkwo, 1993). Native to Southeast Asia (Matthews, 1991), it is widely distributed across tropical and subtropical regions (Onwueme, 1999), with Africa accounting for 76% of global production (FAOSTAT, 2023). Often termed a "poor man's crop" (Onyeka, 2014), taro possesses significant nutritional, medicinal, and economic value (Tamadaho et al., 2024). In addition, its tolerance to diverse agroecological conditions and capacity to enhance food systems under climate change make it a strategic crop for addressing food insecurity (Bamidele et al*.,* 2014).

Despite these attributes, taro remains under-researched and underutilised due to limited investment in research and development (Onyeka 2014). Production is constrained by post-harvest storage limitations, irregular rainfall (Houngbo et al., 2015), and diseases like leaf blight (Singh et al., 2006). Additionally, vegetative propagation facilitates pathogen transmission, notably Dasheen mosaic virus, causing severe yield losses (Nassar et al., 2009).

In Togo, taro is classified among neglected and underutilised crops and is generally cultivated on a small scale (Beyene et al., 2010), resulting in lower production levels compared with neighboring Ghana (Houngbo et al., 2015). Farmers face multiple constraints, including low soil fertility, poorly structured marketing systems, and a limited supply of improved planting material (Bammite et al., 2018a). Soil fertility represents a critical limitation, as less than 10% of Togolese soils are considered moderately fertile (Toundou et al., 2020), with documented deficiencies in nitrogen and phosphorus in several agricultural zones of southern Togo (Kola et al., 2023). In addition, inadequate post-harvest management leads to significant losses of planting material between growing seasons, further restricting production (Bammite et al., 2018a).

The limited availability of high-quality planting material, coupled with increasing demand, constitutes a major bottleneck to taro production. In this context, *in vitro* micropropagation offers a viable alternative to conventional propagation methods by enabling the rapid multiplication of disease-free and genetically uniform planting material. Previous studies have demonstrated that the response of taro to *in vitro* culture is highly genotype-dependent and influenced by the composition of growth regulators in the culture medium (Kebede and Abera, 2015). For instance, optimal shoot proliferation has been reported on MS medium supplemented with 0.50 mg L^{-1} BAP and 0.50 mg L^{-1} NAA in Egypt (ElDajin et al., 2023), while a concentration of 2 mg L^{-1} BAP proved more effective in Kenya (Kepue et al., 2021). However, to date, no specific study has optimized these protocols for a large-scale seed production in Togo. This limit of healthy planting materials.

Therefore, the present study aims to optimise *in vitro* multiplication protocols for two local taro varieties (*C. esculenta*), locally known as ‘Kpèkpèou rouge’ and ‘Kpèkpèou vert’ (Bammite, 2018), to enhance the availability of improved planting material and support sustainable taro production in Togo.

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MATERIALS AND METHODS

Plant material

The plant material used in this study consisted of two local varieties of taro (*Colocasia esculenta* (L.) Schott), locally known as " Kpèkpèou vert" (reference code: TGUL Ce3) and " Kpèkpèou rouge " (reference code: TGUL Ce4). The samples were obtained from the taro and cocoyam germplasm collection established and maintained in the field at the Togolese Institute of Agronomic Research (ITRA) (Bammite, 2018). Both varieties belong to the *eddoe* type (*Colocasia esculenta* (L.) Schott var. *antiquorum*). The TGUL Ce 3 variety comprises accessions characterized by a low number of suckers, green petioles, a yellow petiole junction pattern, and drooping leaves. The TGUL Ce 4 other variety consists of accessions exhibiting a high number of suckers, a small purple petiole junction pattern, and petioles that are in the upper third while being brown in the middle and basal thirds (Bammite et al., 2018a). To produce the initial explant material, plants were cultivated in a greenhouse under natural photoperiod conditions and irrigated with tap water as required. Shoots were harvested after eight weeks of growth and used as explants for *in vitro* culture initiation.

Culture media and growth conditions

In vitro initiation was carried out on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with growth regulators, specifically 1.127 mg L^{-1} 6-benzylaminopurine (BAP) and 0.0931 mg L^{-1} α-naphthaleneacetic acid (NAA). The basal medium was prepared using MS powder (4.54 g L^{-1}; Plant Cell Technology), supplemented with sucrose (30 g L^{-1}) and solidified with agar (8 g L^{-1}). The pH was adjusted to 5.7 ± 0.1 prior to autoclaving at 120°C for 20 min under 1 bar pressure. Cultures were maintained in a growth room at 25 ± 2°C under a 16 h photoperiod. Illumination was provided by cool white fluorescent lamps installed on each shelf.

Explant preparation and surface sterilisation

Shoots collected from the field collection were initially trimmed to approximately 4 cm in length and 1.5 cm in basal width, bearing three to four petioles. Upon transfer to the Crop Protection and Biosecurity Laboratory at ITRA, roots and leaves were removed, and the tubers were washed under running tap water, followed by immersion in liquid soap for 5 min. Explants measuring 2–3 cm and containing the apical bud with two to three petioles were then transferred to a horizontal laminar airflow cabinet for surface sterilisation. Two sterilisation protocols were evaluated using different disinfecting agents: sodium hypochlorite and calcium hypochlorite. In the first protocol, explants were immersed in 70% (v/v) ethanol for 5 min and rinsed three times with sterile distilled water, followed by immersion in 20% (v/v) sodium hypochlorite for 20 min, rinsed three times with sterile distilled water, and finally treated with 10% (v/v) sodium hypochlorite for 10 min. In the second protocol, explants were similarly pre-treated with 70% ethanol, then immersed in 20% (w/v) calcium hypochlorite for 20 min, followed by three rinses with sterile distilled water, and subsequently treated with 10% (w/v) calcium hypochlorite for 30 min, with intermediate rinsing between steps. For both protocols, sterilisation was completed with three final rinses using sterile distilled water. Under aseptic conditions, the explants were dissected to obtain segments approximately 5 mm in diameter, each containing one or two petioles. Individual explants were then cultured in test tubes containing the initiation medium.

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Shoot multiplication and rooting

Following successful initiation, explants were transferred to specific culture media containing different types and concentrations of plant growth regulators to assess their effects on shoot proliferation and rhizogenesis (Table 1). Transfers were conducted under a horizontal laminar airflow cabinet. Explants were removed from culture tubes using sterile forceps, placed on a sterile Petri dish, and dissected with a sterile scalpel to remove roots and leaves. Individual shoots were then isolated and transferred to fresh media. Nine different hormonal combinations were tested, in addition to a control medium without growth regulators, resulting in a total of ten media.

Table 1: Concentration of each growth regulator in each medium.

Medium	Growth Regulators (mg L^{-1})			
	BAP	NAA	Kinetin	IAA
M0	0	0	0	0
M1	0.5	0.0625	0	0
M2	2.5	0.5	0	0
M3	0.25	0	0.125	0
M4	2.5	0	1	0
M5	0.25	0	0	0.125
M6	1	0	0	0.75
M7	2.5	0	0	1
M8	2	0.25	0.75	0.75
M9	2.5	0.5	1	1

Rooting and acclimatisation

Rooting of *in vitro*-derived plantlets of both varieties was evaluated using MS media supplemented with different concentrations of α-naphthaleneacetic acid (NAA; 0.5, 1.0, and 2.0 mg L^{-1}), as well as a control medium without growth regulators. During the rooting phase, the number of roots, together with shoot and root lengths, was recorded over eight weeks following culture initiation.

Well-rooted plantlets were gently removed from the culture vessels and rinsed with tap water to eliminate residual culture medium. Individual plantlets were then transferred to disposable plastic containers filled with forest soil. Each plantlet was labelled according to variety and maintained in a greenhouse for three weeks under ambient light conditions. Subsequently, the plantlets were gradually exposed to ambient temperature conditions. Throughout the acclimatisation period, the plantlets were irrigated daily with tap water. Plantlet survival was assessed after four weeks, and the overall survival rate was calculated after eight weeks according to the following formula: Survival rate (%) = (number of surviving plantlets / total number of plantlets transplanted) × 100.

Research design and data collection

The experiments were conducted using a Completely Randomized Design. For the initiation phase, a total of 40 explants were established per variety to account for potential losses; at this

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stage, each individual explant was cultured in a single test tube. For the micropropagation phase, the experimental unit was defined as one culture tube containing a single explant. A total of 100 explants were used per variety, distributed across 10 different media (including the control) with 10 replicates (tubes) per medium. During the initiation phase, contamination rate, necrosis rate, and budburst rate were recorded. During the micropropagation phase, the number of shoots, roots, and leaves, as well as plant height, were recorded weekly for eight weeks following subculture.

Statistical analysis

Quantitative data were subjected to statistical analysis by one-way analysis of variance (ANOVA) using GenStat software (version 12). When significant differences were detected, means were separated using Duncan's Multiple Range Test (DMRT) at the 5% probability level to compare the effects of the types and concentrations of growth regulators on the measured parameters**.**

RESULTS

Shoot initiation stage

Observations after four weeks showed that surface sterilisation with sodium hypochlorite (NaClO) resulted in lower contamination rates compared to calcium hypochlorite ($Ca(ClO)_2$) for both taro varieties (**Table 2**). With NaClO, contamination rates were 40% for 'TGUL Ce4' and 30% for 'TGUL Ce3'. Conversely, calcium hypochlorite resulted in higher contamination, reaching up to 65% (**Table 2**). Explant viability was 75% (30/40) for 'TGUL Ce4' and 77.5% (31/40) for 'TGUL Ce3'. The budburst rate was 52.5% for 'TGUL Ce3'and 45% for 'TGUL Ce4'.

Table 2: Effectiveness of the sterilization method and bud break of cultured explants

Sterilizing Agents	Cultivars	Number of Explants in Culture	Number of Necrotic explants	Contamination (%)	Reactive Explants
Sodium hypochlorite (NaClO)	TGUL Ce4	40	6	40	18
	TGUL Ce3	40	7	30	21
Calcium hypochlorite ($Ca(ClO)_2$)	TGUL Ce4	40	9	52.50	10
	TGUL Ce3	40	5	65	9

Shoot Proliferation Stage

Results in Table 3 show that all measured growth parameters were significantly affected by the culture media ($p < 0.001$). M4 and M6 (MS + 1 mg/L BAP + 0,75 mg/L AIA) were most effective for multiplication, yielding 10.2 ± 2.39 shoots for 'TGUL Ce3' and 7.33 ± 2.64 shoots

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for 'TGUL Ce4'. Conversely, the control (M0), which produced the highest number of roots in both varieties, with 14.88 ± 3.44 roots per explant for 'TGUL Ce4' and 16.8 ± 3.52 roots per explant for 'TGUL Ce3'. Furthermore, the greatest shoot height was also recorded on the M0 medium for both varieties (Table 3).

Table 3: Influence of growth regulator combinations on growth parameters in TGUL Ce4 and TGUL Ce3

Media	Mean Number of shoots		Mean Shoot Height		Mean Number of Leaves		Mean number of Roots	
	TGUL Ce4	TGUL Ce3	TGUL Ce4	TGUL Ce3	TGUL Ce4	TGUL Ce3	TGUL Ce4	TGUL Ce3
	(p<0.001)	(p<0.001)	(p<0.001)	(p<0.001)	(p<0.001)	(p<0.001)	(p<0.001)	(p<0.001)
M0	1.33±0.5 ^{e}	1.3±0.48 ^{d}	10.77±4.20 ^{a}	9.23 ± 1.42 ^{a}	5.77 ± 1.39 ^{e}	11.7±2.75 ^{cd}	14.88±3.44 ^{a}	16.8±3.52 ^{a}
M1	3.33 ± 1.5 ^{bc}	6.2 ± 2.69 ^{bc}	3.2 ± 2.08 ^{b}	1.14 ± 0.47 ^{c}	7±3 ^{de}	15.4±6.18 ^{bc}	9.77 ± 4.20 ^{ab}	3.1 ± 2.46 ^{de}
M2	5±2.34 ^{abc}	5.7 ± 4.78 ^{bc}	1.38 ± 0.58 ^{b}	2.5±0.72 ^{b}	7.55 ± 4.15 ^{cde}	11.1 ± 4.43 ^{cd}	2±2.39 ^{cd}	0±0 ^{de}
M3	4.33 ± 3.27 ^{abc}	3±1.05 ^{cd}	2.04 ± 0.89 ^{b}	1.78 ± 0.24 ^{bc}	8±5.74 ^{cde}	6.6±2.59 ^{d}	9.88 ± 6.90 ^{ab}	3.6 ± 2.11 ^{de}
M4	5.44 ± 3.84 ^{ab}	10.2±2.39 ^{a}	1.92 ± 0.59 ^{b}	2.23 ± 0.58 ^{b}	5.11±4.59 ^{e}	22.8±7.52 ^{a}	0.11 ± 0.33 ^{d}	5.1 ± 1.79 ^{bcd}
M5	5.33 ± 2.73 ^{ab}	6±2 ^{bc}	1.84 ± 0.20 ^{b}	2.09 ± 0.44 ^{b}	20.33 ± 8.21 ^{ab}	22.6±5.33^{a}	7±3.77 ^{bc}	8±4.21 ^{bc}
M6	7.33 ± 2.64 ^{a}	8.3±2.93 ^{ab}	2.01 ± 0.46 ^{b}	2.15 ± 0.40 ^{b}	24.88±6.09 ^{a}	20.6 ± 4.59 ^{ab}	6.22 ± 3.80 ^{bc}	9±4.42 ^{b}
M7	4.22 ± 2.27 ^{abc}	5±1.33 ^{bc}	1.47 ± 0.22 ^{b}	1.93 ± 0.35 ^{bc}	15.33 ± 7.17 ^{bc}	16.8±3.70 ^{abc}	0.55 ± 0.88 ^{d}	4.4 ± 4.55 ^{cd}
M8	4.66 ± 2.17 ^{abc}	8.1±2.18 ^{ab}	2.6 ± 0.83 ^{b}	1.99 ± 0.26 ^{bc}	14.66 ± 5.09 ^{bcd}	17.3±3.91 ^{abc}	4.88 ± 3.55 ^{bcd}	3.5±2.06 ^{de}
M9	2.55±1.33 ^{bc}	2.9 ± 0.99 ^{cd}	1.5±0.50 ^{b}	2.03 ± 0.63 ^{bc}	6.66 ± 2.23 ^{e}	5.9 ± 0.73 ^{d}	0±0 ^{d}	0±0 ^{e}

In a column, means followed by the same letter are statistically identical at the 5% threshold (least significant difference).

Shoot induction

A significant difference in the mean number of shoots were observed between the two varieties. For 'TGUL Ce4', medium M6 (MS +1 mg L^{-1} BAP + 0.75 mg L^{-1} IAA) was most effective (7.33 ± 2.64 shoots). In contrast, 'TGUL Ce3' exhibited its highest response on medium M4 (MS + 2.5 mg L^{-1} BAP +1 mg L^{-1} kinetin), yielding 10.2 ± 2.39 shoots. In both varieties, the

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control medium (M0) resulted in the lowest shoot production (approximately 1.3 shoots per explant).

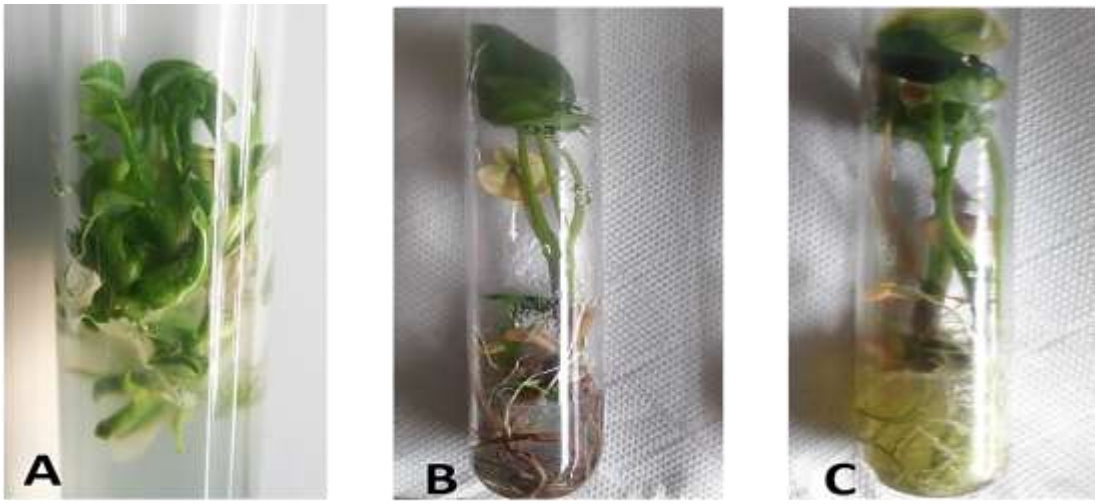

Figure 1: Effect of M6 (MS + 1 mg/L BAP + 0.75 mg/L IAA) on shoot proliferation (A); effect of M0 on TGUL Ce4 and TGUL Ce3 respectively (B, C)

Rhizogenesis

Statistical analysis revealed a highly significant effect of culture medium on root induction ($p < 0.001$; **Table 3**). The control medium (M0) resulted in the highest root formation in both varieties, with 16.8 ± 3.52 roots for 'TGUL Ce3' and 14.88 ± 3.44 roots for 'TGUL Ce4'. In contrast, no root development was recorded on multiplication media (M1 to M9), particularly medium M9.

Shoot height

Shoot height differed significantly among culture media in both varieties ($p < 0.001$). Among the nine tested hormonal combinations and the control, the highest shoot lengths were recorded on the control medium (M0), with mean lengths of 10.77 ± 4.20 cm for 'TGUL Ce4' and 9.23 ± 1.42 cm for 'TGUL Ce3'. All media supplemented with growth regulators resulted in reduced in lower shoot height compared to the control.

Number of leaves

The analysis of leaf number showed that media with high shoot proliferation also recorded the highest leaf numbers. For TGUL Ce4, medium M6 induced the highest number of leaves (24.88 ± 6.09), while for TGUL Ce3, the highest values occurred on media M4 (22.8 ± 7.52) and M5 (22.6 ± 5.33). While these media increased the total number of leaves, leaf size was lower compared to those recorded on the control medium (**Figure 1**).

Rooting Stage

Statistical analysis revealed a highly significant effect of the culture medium on root induction after eight weeks ($p < 0.001$; **Table 4**). The control medium (M0) recorded the highest

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rhizogenesis, yielding 14.00 ± 1.92 roots in TGUL Ce3 and 13.75 ± 3.73 roots in TGUL Ce4. These values were significantly higher than those obtained on MS medium supplemented with 1 mg L^{-1} NAA. Additionally, medium M0 induced the greatest root elongation (8.2 ± 0.4 cm, **Table 4**) and produced more robust roots (**Figure 2**).

Table 4: Effect of NAA and the control medium on rhizogenesis of the 2 varieties of taro

Varieties	NAA (mg/L)		Mean Number of Shoots	Mean Shoot Height	Mean Number of Leaves	Mean Number of Roots	Mean Root Length
TGUL Ce4				(p<0.001)	(p<0.001)	(p<0.001)	(p<0.001)
	Control		1.5 ± 0.53 ^{ns}	12.62 ± 4.24 ^{a}	9.12 ± 1.95 ^{a}	13.75±3.73 ^{a}	8.2 ± 0.4 ^{a}
		0.5	1.12 ± 0.35 ^{ns}	5.36 ± 1.48 ^{b}	5.62 ± 1.40 ^{b}	6.5±1.77 ^{b}	1.3 ± 0.72 ^{b}
		1	1.37 ± 0.51 ^{ns}	2.85±1.06 ^{c}	5.62 ± 1.40 ^{b}	2±0.75 ^{c}	0.36±0.16 ^{c}
		2	1±0 ^{ns}	2.36±0.46 ^{c}	3.87 ± 0.83 ^{c}	2.25 ± 1.03 ^{c}	0.31 ± 0.09^{c}
TGUL Ce3				(p<0.001)	(p<0.001)	(p<0.001)	(p<0.001)
	Control		1.75 ± 1.03 ^{ns}	7.8±2.73 ^{a}	11.25±3.32^{a}	14±1.92 ^{a}	8.9 ± 0.6 ^{a}
		0.5	1.62 ± 0.91 ^{ns}	4.87 ± 1.63 ^{b}	6.75 ± 4.77^{b}	8.37 ± 2.97^{b}	0.98±1.23^{b}
		1	1.75 ± 0.70 ^{ns}	3.5±1.19^{bc}	7.125 ± 2.53^{b}	5.75±2.05 ^{c}	0.3 ± 0.10^{c}
		2	1.5 ± 0.53 ^{ns}	2.85±0.75 ^{c}	6±1.51 ^{b}	7.75±2.96^{bc}	0.4 ±0.07^{bc}

In a column, means followed by the same letter are statistically identical at the 5% threshold (least significant difference).

Figure 2: Taro shoots after rooting on M0 (A: TGUL Ce3; B: TGUL Ce4); (C: effect of 2 mg/L of NAA on rhizogenesis in both varieties); (D: TGUL Ce4 explant removed from culture medium)

Acclimation stage

The acclimation protocol resulted in a survival rate exceeding 96 % for both varieties (**Table 5 and Figure 3**).

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Table 5: Taro plant survival rate

Varieties	Number of acclimated plants	Number of surviving plants	Acclimation rate (%)
TGUL Ce4	30	29	96.66
TGUL Ce3	30	30	100

Figure 3: Acclimation and field transfer: (A), taro shoots undergoing acclimation (hardening) in plastic bags after 21 days in the greenhouse; (B), acclimated shoot adapted to natural conditions; (C), plants in the field 75 days after planting

DISCUSSION

In vitro establishment and sterilisation

Effective sterilisation is a critical prerequisite, as microbial contamination is a major constraint limiting explant establishment **(**Rai et al., 2010). The contamination rate obtained with NaClO for 'TGUL Ce4' (40%) was comparable to the 43.3% reported by Some et al. (2024). Higher contamination with calcium hypochlorite was potentially due to preparation difficulties leading to uneven disinfection. Necrosis was likely associated with contamination **(**Mohammède et al., 2022**)**. The variation in reactivity highlights the influence of genotypic factors on in vitro establishment. The relatively lower shoot emergence observed in 'TGUL Ce4' may be related to tuber characteristics that hinder complete surface sterilisation of the explants, thereby affecting meristem viability **(**Rai et al., 2010).

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Shoot multiplication and hormonal influence

A significant difference in mean number of shoots was observed between varieties, a response attributed to genotypic variation (Toledo et al., 1998). Overall, " TGUL Ce3" displayed a higher regeneration capacity, further highlighting the influence of genotype on *in vitro* responsiveness (Kebede and Abera, 2015). The results clearly demonstrate that combinations of cytokinin and auxins are essential for optimising shoot multiplication.

For 'TGUL Ce4', the superior performance of medium M6 (MS + 1 mg/L BAP + 0,75 mg/L AIA) supports the principle that a relatively low auxin concentration combined with a higher cytokinin level shifts the hormonal balance towards cytokinin dominance, thereby promoting shoot proliferation (Chung & Goh, 1994). BAP is known to release apical dominance and stimulate lateral bud outgrowth (Hailu et al., 2013), and its effectiveness in axillary bud induction in taro is well documented (Chung and Goh, 1994). Results for TGUL Ce4 are consistent with studies reporting optimal shoot multiplication on media combining cytokinins with auxins, such as Droh (2010). These findings confirm that a synergistic interaction between cytokinins and auxins is required to optimise caulogenesis in taro. However, this synergy is essential the specific optimal concentrations remain strongly genotype-dependent, as evidenced by the varying success of different BAP/IAA ratios across the literature (ChienYing et al., 2008). In contrast, for 'TGUL Ce3', the most effective treatment consisted exclusively of cytokinins. Medium M4 (MS + 2,5 mg/L BAP + 1 mg/L Kinétine) induced the highest shoot number (10.2 ± 2.39 shoots per tube), suggesting a synergistic interaction between the two cytokinins. Such combinations are known to enhance cell division and promote axillary shoot emergence by effectively breaking apical dominance (Gutiérrez-Mora et al., 2012). The importance of cytokinins, particularly BAP, during the multiplication stage has also been emphasised by Manju et al. (2017) and Ramakrishan et al. (2017). The contrasting optimal media requirements observed between the two varieties further confirm the critical role of genotypic variation in *in vitro* regeneration response (George et al., 2008; Alam et al., 2022).

Regarding growth parameters, the reduction in shoot height is likely associated with supra-optimal cytokinin concentrations, particularly BAP, which have been reported to inhibit elongation growth beyond a certain threshold (Beyene et al., 2010). Similar inhibitory effects of increasing BAP concentrations on *in vitro* shoot growth have been documented by Bekele et al. (2013) and Beyene et al. (2010). For leaf number, findings align with reports indicating that maximum leaf and shoot production in taro is achieved with cytokinin such as BAP (Képu et al., 2021). However, excessively high BAP concentrations (e.g., 6 mg L^{-1}) can cause a decline in numbers (El-Sayed et al., 2016).

Rhizogenesis and acclimation

The superior performance of M0 is attributed to the unaltered endogenous hormonal balance, where apical auxin naturally stimulates root initiation (Hayward et al., 2009). The fact that M0 was more effective for rooting than NAA-supplemented media suggests exogenous auxin concentrations exceeded the optimal threshold, exerting an inhibitory effect (El-Sayed et al., 2016). These discrepancies with previous findings (Behera and Sahoo 2008) highlight the strong influence of varietal differences. Collectively, these results confirm that *in vitro* rooting responses are genotype-specific, necessitating tailored protocols for efficient taro micropropagation systems. Similar observations were reported for a Malaysian taro variety cultured on hormone-free MS medium (Alam et al., 2022) and by Some et al. (2024) in Burkina

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Faso, who identified the control medium as the most favourable for taro root system development. However, contrasting results have been reported in other taro studies. Hala et al. (2023), for example, observed the lowest root number on hormone-free medium in an Egyptian taro variety, while Akpoglan et al. (2021) reported an absence of rooting on control medium in certain Beninese varieties. Nevertheless, in the present study, the strong rhizogenic response obtained o, the medium without growth regulators represents a major advantage for subsequent acclimatisation, as plantlets developed without exogenous growth regulators generally exhibit improved physiological stability and adapt more rapidly to ex vitro conditions (ElDajin et al., 2023). The survival rate exceeding 96 % indicated that the regenerated plantlets were of good physiological quality and that the weaning conditions, including humidity control, substrate composition, and irrigation regime, were appropriately optimised (George et al., 2008b). This demonstrates that the critical transition phase from *in vitro* conditions to the greenhouse environment, often associated with substantial losses in micropropagation systems, was successfully achieved for both local taro varieties.

CONCLUSION

This study demonstrated that the supplementation of cytokinin-type growth regulators was essential for maximising the multiplication rate per explant. In particular, MS medium supplemented with 1 mg/L BAP and 0.75 mg/L IAA (M6) was optimal for the TGUL Ce4 variety (7.33 ± 2.64 shoots), while MS medium containing 2.5 mg/L BAP and 1 mg/L kinetin (M4) proved most effective for TGUL Ce3 (10.2 ± 2.39 shoots). Conversely, the hormone-free control medium (M0) was the most suitable for root induction in both varieties (14.88 ± 3.44 and 16.8 ± 3.52 roots per explant for 'TGUL Ce4' and 'TGUL Ce3', respectively). Overall, this optimization of culture media enabled the establishment of a complete and efficient *in vitro* regeneration protocol, encompassing shoot induction, multiplication, and rooting. The protocol provides a reliable basis for the large-scale and rapid production of TGUL Ce4 and TGUL Ce3 plantlets, representing a valuable tool for the dissemination of high-quality planting material and for improving the productivity of this crop in Togo. Regarding the technical process, the initiation phase remained challenging and requires further optimization to improve the overall efficiency of micropropagation.

Disclosure statement

The authors report there are no competing interests to declare

Data availability statement

The data sets generated during and/or analysed during the current study are available from the corresponding author on request.

Acknowledgments

Authors are grateful to the International Foundation for Science (Research Grant No. C-5866-2 (2020-2023) and C-5866-2-Supplementary support 2025) for providing research funds and Institut Togolais de Recherche Agronomique (ITRA) for providing tissue culture facilities for initiation.

Article DOI: 10.52589/RJBLS-PQQK4CMS
DOI URL: https://doi.org/10.52589/RJBLS-PQQK4CMS

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