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Cost-Effective Tissue Culture Protocols for Neglected Indigenous *Dioscorea* Varieties: Optimization of Disinfection, Browning Control, and Growth Enhancement

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Abstract

Indigenous *Dioscorea* species are important neglected and underused species (NUS) that represent rich genetic resources for food security and medicinal applications, offering exceptional climate resilience and nutritional diversity crucial for sustainable agriculture. However, their propagation is hampered by expensive tissue culture techniques and high equipment expenses. The study optimized disinfection techniques, browning control, and growth promotion to develop comprehensive low-cost tissue culture procedures for purple- and white-fleshed indigenous *Dioscorea* varieties. Nodal explants were examined with commercial bleach (5% - 20%) for surface disinfection, polyvinylpyrrolidone, PVP (0 - 1,500 mg/L) for browning management, hormone combinations (BA and NAA at 0.5 - 2.0 mg/L) for morphogenesis, and alternative medium disinfection methods (bleach 0.5 - 4.0 mL/L versus autoclaving). Optimal explant disinfection was achieved using 10% commercial bleach, resulting in 67% - 73% survival at a cost of 0.117 THB (Thai Baht) per surviving plant. PVP at 1,000 mg/L substantially suppressed browning while increasing survival to 80% in white-fleshed and 67% in purple-fleshed varieties, with white-fleshed varieties regularly outperforming, purple-fleshed variants. BA at 2.0 mg/L increased shoot growth (5.87 shoots per explant), while NAA at 1.0 mg/L increased root formation (5.89 roots per explant). Bleach-based medium disinfection at 1 - 2 mL/L achieved autoclaving performance while substantially lowering equipment investment from 60,000 to 2,500 THB. These integrated protocols establish complete low-cost tissue culture systems utilizing commercial bleach for all disinfection steps, eliminating expensive laboratory equipment requirements and enabling community-based propagation systems for indigenous *Dioscorea* conservation in resource-constrained environments.

Keywords: *Dioscorea* spp., Low-cost tissue culture, Bleach disinfection, Browning control

Introduction

Contemporary agricultural systems encounter unprecedented vulnerabilities as climatic disruption compromises global food networks [1,2]. Increasing temperature stress, unpredictable rainfall patterns, and more severe extreme weather events all damage the yield of staple crops in different areas [3]. Alarmingly, agricultural biodiversity continues its precipitous decline, with fewer than 150 commercially cultivated species now supplying 90% of human caloric requirements [4]. Such genetic narrowing establishes perilous dependencies that severely compromise agricultural resilience against environmental stresses and emerging pest pressures. Therefore, agricultural diversity becomes basically necessary to make ecosystems better able to respond to changes in climate [3,4].

In this situation, neglected and underused species (NUS) are very important genetic resources for the growth of sustainable food systems [5-7]. These crops are naturally adapted to marginal areas and exhibit extraordinary resilience while requiring fewer inputs than conventional cultivars, typically sustaining consistent output under harsh circumstances [7]. In addition to their agronomic benefits, NUS offer exceptional nutritional properties, including concentrated proteins, essential minerals, and bioactive compounds that are critical in the fight against hunger [5,8]. When integrated into existing farming systems, they amplify dietary diversity while simultaneously supporting agrobiodiversity conservation and strengthening smallholder farmer livelihoods through expanded market opportunities [6,9], thereby establishing themselves as pivotal assets in food system diversification strategies.

Distinguished among these promising NUS, indigenous *Dioscorea* species present an exceptional convergence of climate adaptability with nutritional and pharmaceutical significance. Over 600 species are found across the world, and several varieties have long been used for medical purposes [10]. Within their tubers, bioactive compounds, including steroidal glycosides, diosgenin, and phenolic substances, demonstrate associations with anti-cancer, anti-diabetic, anti-inflammatory, and cardioprotective properties. Additionally, *Dioscorea* is also a major natural source of diosgenin, which is required for steroid hormone production. Their high antioxidant capacity, together with their diverse nutritional profiles, significantly supports functional food applications [10-12], while many species are being threatened by habitat destruction and unsustainable harvesting practices.

Conventional *Dioscorea* propagation encounters substantial technical constraints, notably including reduced multiplication rates, extended growth cycles, and heightened susceptibility to viral infections. Wild species experience these challenges most acutely, lacking standardized protocols while maintaining limited and expensive access to pathogen-free planting materials [13,14]. As a promising solution, plant tissue culture (PTC) offers several benefits, including faster multiplication, continuous year-round production, and reliable development of genetically homogeneous, pathogen-free plantlets. Beyond propagation benefits, it actively supports germplasm conservation initiatives and breeding program development [15,16]. However, PTC application in *Dioscorea* propagation faces some significant challenges. Surface disinfection is particularly challenging, as microbial contamination often affects explant viability and contributes to overall culture failure [13,17]. Moreover, *Dioscorea* has a lot of phenolic browning, which is caused by natural chemical oxidation in reaction to tissue damage and makes the processes of both shoot and root regeneration much slower [18-20]. The high cost of tissue culture—driven primarily by skilled labor, culture media components, and disinfection processes—has been repeatedly highlighted as a major barrier [21]. Reported unit costs vary depending on crop and method, ranging from approximately US$0.13 per sugarcane plantlet in high-throughput systems [22] to more than US$1.00 per banana plantlet under low-tech protocols [23], with intermediate costs such as US$0.73 per cacao plantlet (somatic embryogenesis) [24] and Rs.20.02 for dragon fruit micropropagation [25]. These expenses, together with specialized labor requirements and energy inputs for controlled environments [21], further limit accessibility, particularly in low-resource settings, underscoring the need for cost-effective, efficient protocols for *Dioscorea* species [26-28]. Such technical and economic barriers emphasize the urgent necessity for developing cost-effective, efficient tissue culture protocols specifically optimized for *Dioscorea* species.

Although the potential gains of NUS are gaining increasing recognition, comprehensive low-cost protocols for indigenous *Dioscorea* propagation remain remarkably limited. This investigation addressed these gaps by developing economically viable tissue culture protocols specific to white- and purple-fleshed indigenous varieties of *Dioscorea*. We optimized commercial bleach-based disinfection, tested PVP supplementation for browning prevention, optimized growth regulator formulations to enhance morphogenesis, and validated affordable medium disinfection alternatives. These integrated methods enhance indigenous *Dioscorea* genetic resources preservation and utilization and also promote sustainable agricultural development in resource-limited areas.

Materials and methods

Plant preparation

Fresh tubers of indigenous *Dioscorea* species with purple and white flesh were obtained from the Khok Kruat plant market, Khok Kruat Subdistrict, Mueang District, Nakhon Ratchasima Province, Thailand, and subsequently cultured in a greenhouse under controlled conditions. The cultivation employed the use of pot-based systems composed of a 1:1:1 proportion of loam soil, rice husk ash, and coconut coir substrate. The greenhouse microclimate was characterized by an air temperature of approximately 30 - 35 °C, relative humidity of 60% - 70%, and natural daylight averaging 11 - 12 h per day. Mulching was applied to stabilize soil moisture and temperature, while staking of vines facilitated optimal light interception for foliage development. These cultivation conditions were adapted with reference to previously reported protocols for *Dioscorea* greenhouse husbandry and photoperiod management [29-30]. Water was applied daily to maintain the right level of moisture until the vines emerged from planted tubers. After establishment, the nodal segments with axillary buds were harvested selectively to be used as experimental explants. Morphological characterization revealed distinct varietal differences between the two genotypes. Purple-fleshed varieties were noted to have a square stem with four longitudinal wings of brownish-red to dark purple. Cordate leaves positioned opposite each other defined this variety, and the shoot together with the young leaves had reddish-purple to dark red pigments. Tuber flesh appeared dark purple with brown outer bark. In contrast, white-fleshed varieties possessed similar square stems with four longitudinal wings but featured green stems with light pink to pinkish wing coloration. Although the leaves were arranged in a cordate and opposite manner, the shoots and young leaves were yellowish-green in color and sometimes had pink to light red cataphylls. This variety was characterized by white tuber flesh and brown outer bark (**Figure 1**).

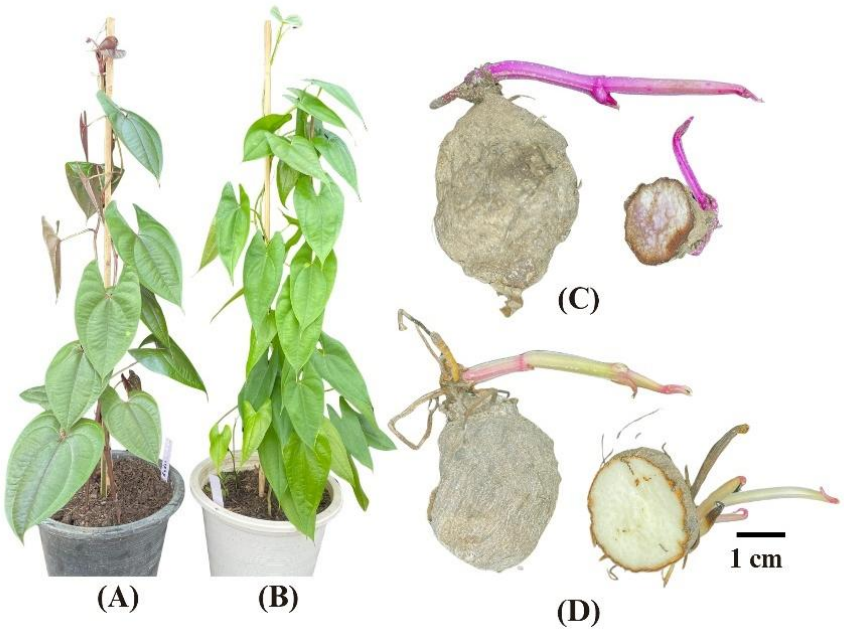

Figure 1 Morphological characteristics of indigenous *Dioscorea* spp. (A) Purple-fleshed and (B) white-fleshed varieties after 45 days of cultivation. (C) Purple-fleshed tubers with rough skin, violet flesh, and purple sprouts. (D) White-fleshed tubers with smoother skin, white flesh, and pale sprouts. Scale bar = 1 cm (C-D).

Bleach disinfection protocol optimization

Following successful plant establishment, optimization of surface disinfection was undertaken as the critical step to achieve contamination-free culture initiation. Disinfection efficiency was evaluated using commercial bleach (Haiter®; 6% w/w sodium hypochlorite) at four concentrations (5%, 10%, 15% and 20%) applied to explants of two *Dioscorea* varieties

(purple- and white-fleshed). Tender vine stems (approximately 0.5 - 1.0 cm each with one axillary bud) of the 2 varieties of plants were washed with tap water containing a few drops of detergent, rinsed under running tap water for 10 min, immersed in 0.2 % (w/v) Captan for 30 min, and finally rinsed three times with sterile distilled water (5 min each). These explants were then isolated for subsequent treatments, following protocols modified from Sookruksawong [27] and Homhual *et al.* [31]. The experimental design followed a completely randomized design (CRD) with two factors: Bleach concentration and plant variety. Each treatment consisted of three replicates with five explants per replicate. Individual explants were disinfected in 20 mL of the respective commercial bleach solution (contained in a 4-oz glass vessel) for 10 min with a drop of detergent to reduce surface tension. After disinfection, explants were rinsed three times with sterile distilled water (5 min each) before culture. Explant culture was performed on 20 mL of modified MS free hormone medium, which was prepared with 0.7% (w/v) agar, adjusted to pH 5.8 and autoclaved at 121 °C for 15 min [32], supplemented with the corresponding bleach treatment. Explants were maintained under *in vitro* conditions, and experimental results were recorded after 4 weeks of culture. Evaluation parameters included contamination rate, survival rate, and cost per surviving explant, calculated from the volume of bleach used in each treatment.

PVP supplementation for browning control

After finding the appropriate disinfection conditions, the focus switched to tackling the phenolic browning issues that frequently compromise *Dioscorea* tissue culture success. The effectiveness of polyvinylpyrrolidone (PVP, K-30, Himedia) supplementation was examined for its property of reducing tissue browning and promoting growth in *Dioscorea* varieties. The experimental design employed the CRD scheme with a total of 2 factors, which were PVP concentration (0, 500, 1,000 and 1,500 mg/L) and plant variety (purple-fleshed and white-fleshed), and 3 replications per treatment and 5 explants per replica. The concentration range was selected based on earlier reports in *Dioscorea* and other phenolic-rich species, where moderate supplementation (0.2 - 1.0 g/L) was found to effectively reduce browning, while concentrations above 1.0 g/L were often associated with decreased explant survival and growth [18,19,33,34]. Disinfected single-node segments (0.5 - 1.0 cm, each containing one axillary bud) obtained from the prior bleach concentration trial that provided the most suitable disinfection outcome were selected based on healthy morphology and subsequently cultured on modified MS medium supplemented with the respective PVP concentrations, to ensure consistency and reliability of the experimental results. Culture vessels contained 20 mL of MS medium each, with data collection conducted after 4-week incubation periods. Evaluation variables involved Browning Index (BI) testing on a 0 - 4 scale (0 = no browning, 4 = severe browning involving both explant and medium), survival rate, average number of shoots, and average number of roots per explant. Cost calculations involved dividing total PVP expenses per treatment by surviving plant numbers, excluding basal medium costs that remained constant across treatments.

Growth regulator formulation and culture protocols

To optimize shoot and root growth of *Dioscorea* varieties, we tested various cytokinin and auxin combinations in culture media. The CRD experimental structure encompassed two primary variables: Hormone formulation (six distinct levels) and plant variety (2 genotypes). Hormonal treatments comprised 0.5, 1.0 and 2.0 mg/L of 6-benzylaminopurine (BA) and the equivalent dosages of 1-naphthaleneacetic acid (NAA). These treatments were applied to both purple-fleshed and white-fleshed varieties with three replicates per treatment, each containing five explants. Disinfected yam shoots from the optimized bleach trials were used, and single-node segments (0.5 - 1.0 cm with one axillary bud) were cultured on MS medium containing the assigned growth regulators. An 8-week evaluation period was conducted with cultures in 20 mL culture vessels. Growth parameters from surviving explants were measured, including shoot height, leaf number, shoot number, root length, and root number.

Low-cost medium disinfection methods and economic evaluation

Medium sterilization options were tested to obtain a systematic assessment of medium selection for reducing overall propagation costs while maintaining

equivalent culture performance. Various economical medium disinfection approaches underwent comparative analysis for their effects on contamination, survival, and growth parameters of *Dioscorea* spp. *in vitro* cultures. In the CRD experimental design, two factors were considered: Disinfection method (autoclaving, non-autoclaving, and commercial bleach, Haiter® at 0.5, 1.0, 2.0, and 4.0 mL/L) and plant variety (purple-fleshed and white-fleshed), with three replicates per treatment and five explants per replicate. Aseptic yam shoots obtained from previous disinfection experiments underwent trimming into nodal segments bearing single axillary buds (0.5 - 1.0 cm with one axillary bud) before culture establishment in MS medium treated according to respective disinfection protocols. Bleach treatment procedures included adding disinfectants to the culture medium after it had cooled to about 60 °C before pipetting into culture vessels. Each culture vessel contained 20 mL of medium, and the cultures were evaluated after 8 weeks. Analytical variables included contamination rate, survival rate, shoot number, shoot height, root length, and cost per surviving plant. Economic analyses incorporated comprehensive cost comparisons encompassing medium components, energy expenditure, and equipment requirements across all disinfection methods.

Culture environment and maintenance

All experimentation was done in a laboratory environment with controlled temperature of 25 ± 2 °C and lighting conditions of about 37 μmol $m^{-2}s^{-1}$ under 16/8-hour light/dark photoperiods. Throughout the culture period, aseptic conditions were maintained to preserve experimental integrity and prevent contamination.

Statistical analysis

All experimental observations were statistically analyzed according to appropriate models. Analysis of variance (ANOVA) (two-way and one-way) was employed to evaluate main factors and their interactions. When significant effects were detected ($p \leq 0.05$), mean separation was performed using Duncan's New Multiple Range Test (DMRT) at the 95% confidence level. DMRT letters were assigned within rows to indicate differences among treatments with more than 3 levels (e.g., bleach concentrations, PVP concentrations, growth regulator formulations, low-cost medium disinfection methods). Pairwise comparisons between varieties (two levels only) were interpreted directly from ANOVA results rather than DMRT. Percentage data were arcsine-transformed prior to analysis to satisfy variance assumptions. Results were presented as mean ± standard deviation (SD) throughout all experimental reporting.

Results and discussion

Bleach concentration optimization for explant disinfection

Varying bleach concentrations significantly affected both contamination and survival parameters ($p \leq 0.05$) across *Dioscorea* spp. explants, whereas plant variety showed minimal influence ($p = 0.150$ for contamination; $p = 0.151$ for survival). No statistically significant interaction was detected ($p = 0.118$ for contamination; $p = 0.639$ for survival) (**Table 1**). Bleach treatment at 20% achieved a better disinfection rate, with contamination levels of 0.00 ± 0.00% in purple-fleshed varieties and 13.33 ± 11.55% in white-fleshed varieties. Nevertheless, such elevated concentrations severely compromised tissue viability, with survival rates of 26.67 ± 11.55% in purple-fleshed and 26.67 ± 23.09% in white-fleshed varieties, thereby indicating pronounced phytotoxic responses. Intermediate concentrations (10% - 15%) established optimal equilibrium between contamination suppression and cellular viability. While 20% bleach eliminated contamination, the associated survival rates of only ~26% in both varieties clearly demonstrate severe phytotoxicity, rendering this concentration unsuitable for propagation purposes. This result illustrates the critical balance between sterilization efficiency and tissue viability, as higher bleach concentrations ensure maximum microbial control but simultaneously impose detrimental effects on explant survival. In contrast, 10% bleach achieved a more balanced response by sufficiently suppressing microbial growth while maintaining relatively high survival (67% - 73%). This reduction in tissue viability can be explained by the fact that high concentrations of disinfectants also induce oxidative stress and membrane disruption in plant cells, similar to their effects on microorganisms. Consequently, plant tissues may experience ion leakage,

protein denaturation, and the release of phenolic compounds. Upon oxidation, these compounds lead to tissue browning and necrosis. Although some cells may remain alive, their ability to divide and regenerate into new organs is severely impaired. In summary, this study confirms that moderate bleach levels represent the most practical and sustainable option for *Dioscorea* propagation, as they provide an acceptable compromise between microbial control and explant viability. Thus, treatment with 10% bleach produced moderate contamination (33.33 ± 11.55% in purple-fleshed and 46.67 ± 11.55% in white-fleshed varieties), while simultaneously maintaining relatively high survival rates (66.67 ± 11.55% and 73.33 ± 11.55%, respectively), thereby demonstrating an optimal balance between contamination control and explant viability. In addition, this concentration had the lowest cost in terms of surviving plants (0.117 ± 0.02 THB) when compared to concentrations that had good biological responses. Although 5% bleach application resulted in survival rates of 66.67 ± 11.55% in purple-fleshed and 86.67 ± 11.55% in white-fleshed varieties (the maximum observed), contamination levels persisted at unacceptable thresholds (86.67 ± 11.55% in purple-fleshed and 73.33 ± 11.55% in white-fleshed varieties). Economic evaluation demonstrated that cost per surviving plant escalated substantially with increasing bleach concentrations. A lower concentration of between 5% - 10% was most economical (0.054 ± 0.01 - 0.117 ± 0.02 THB), with costs increasing massively at higher concentrations of 15% - 20% (0.250 ± 0.06-0.560 ± 0.22 THB; $p \leq 0.05$) as a result of low survival rates, not necessarily reagent cost.

Mechanically, sodium hypochlorite can be used to disinfect using the generation of hypochlorous acid (HOCl), which specifically targets the peptide bonds, amino groups, and thiol groups of microbial cells [35]. Our findings highlight that 10% commercial bleach represents the optimal balance between contamination suppression, tissue viability, and economic efficiency, making it the most appropriate and sustainable disinfection strategy for *Dioscorea* explants. Similarly, Eliwa *et al.* [36] demonstrated that 20% sodium hypochlorite treatment for 15 min achieved the highest responsiveness (82.81%) and survival (96.61%) with minimal contamination (0.24%) in peach rootstock explants, highlighting the critical importance of optimizing sterilization protocols for different plant species. These observations align with prior *Dioscorea* sterilization investigations emphasizing the critical importance of balancing disinfection effectiveness against tissue viability [37]. The absence of significant varietal effects indicates that our optimized 10% bleach protocol possesses universal applicability across diverse *Dioscorea* genotypes, thereby simplifying standardization procedures for commercial propagation enterprises. Economically, this cost-effective disinfection technology allows community-based propagation systems that improve food security in resource-constrained regions where autoclave equipment remains prohibitively expensive [27].

Table 1 Effects of bleach concentration and plant variety on contamination rate, survival rate, and cost per surviving plant of *Dioscorea* spp.

Variety	Bleach concentration (%)			
	5	10	15	20
	Contamination rate (%) ± SD			
Purple	86.67 ± 11.55^{a}	33.33 ± 11.55^{b}	13.33 ± 11.55^{c}	0.00 ± 0.00^{c}
White	73.33 ± 11.55^{a}	46.67 ± 11.55^{b}	26.67 ± 11.55^{bc}	13.33 ± 11.55^{c}
	Survival rate (%) ± SD			
Purple	66.67 ± 11.55^{a}	66.67 ± 11.55^{a}	46.67 ± 11.55^{ab}	26.67 ± 11.55^{b}
White	86.67 ± 11.55^{a}	73.33 ± 11.55^{ab}	53.33 ± 11.55^{bc}	26.67 ± 23.09^{c}

Variety	Bleach concentration (%)			
	5	**10**	**15**	**20**
	Cost per survival plant (THB) ± SD			
	0.054 ± 0.01[c]	0.117 ± 0.02[c]	0.250 ± 0.06[b]	0.560 ± 0.22[a]
Source of variation	**Contamination**		**Survival**	**Cost**
Varieties	0.150		0.151	N/A
Bleach concentration (%)	0.000*		0.000*	0.000*
Interaction	0.118		0.639	N/A

Note Distinct letters denote significant differences at $p \leq 0.05$ using DMRT analysis within individual rows (horizontal comparisons across bleach concentrations). N/A represents single-factor analyses. Asterisks (*) signify statistical significance ($p \leq 0.05$). Cost calculations utilized Haiter® pricing at 25 THB per 600 mL bottle.

PVP-mediated browning reduction and growth enhancement

After the optimal disinfection protocol with 10% bleach was established, the disinfected single-node explants were subsequently used as uniform starting material for the PVP browning reduction experiment. This ensured that the observed responses were attributable to PVP supplementation rather than differences in explant health. The level of PVP supplementation and plant varieties significantly affected all the studied parameters ($p \leq 0.05$), while no significant interaction between factors was detected (p = 0.480 for browning index; p = 0.107 for survival; p = 0.435 for shoot number; p = 0.371 for root number) (**Table 2**). PVP treatment at 1,000 mg/L suppressed browning, lowering BI values to 1.93 ± 0.50 in purple-fleshed and 1.00 ± 0.20 in white-fleshed types. However, maximum browning suppression was achieved at 1,500 mg/L in white-fleshed varieties (BI = 0.87 ± 0.16), though this came at the cost of reduced survival rates and growth performance compared to the 1,000 mg/L treatment. This was significantly lower than the severe browning observed in the untreated controls, which consisted of explants cultured on MS medium without PVP supplementation (0 mg/L PVP), with mean browning indices of 3.73 ± 0.23 in the purple-fleshed variety and 3.07 ± 0.23 in the white-fleshed variety (**Figure 2**). PVP absence (0 mg/L) resulted in significant tissue browning and reduced explant viability, especially for purple-fleshed types (26.67 ± 11.55 % survival). Intermediate PVP supplementation (500 - 1,000 mg/L) created an ideal balance between browning suppression and morphogenic stimulation. Treatment with 1,000 mg/L elevated survival rates to 66.67 ± 11.55% in purple-fleshed and 80.00 ± 0.00% in white-fleshed varieties while exhibiting superior shoot and root development responses. Shoot proliferation increased from control baselines (0.27 ± 0.12 and 0.87 ± 0.12) to 1.93 ± 0.31 and 2.87 ± 0.23 at 1,000 mg/L PVP concentrations, respectively. Root growth increased considerably at 500 - 1,000 mg/L compared to controls, reaching 1.53 ± 0.12 in the purple-fleshed variety and 2.33 ± 0.12 in the white-fleshed variety at optimal concentrations. The morphological responses of the explants are shown in **Figure 3**. Economic analysis demonstrated that when PVP supplementation levels increased, the cost per surviving plant increased significantly. Lower concentrations of 0 - 500 mg/L were the most cost-effective (0.00 ± 0.00 - 0.14 ± 0.03 THB), but expenses increased significantly at 1,000 - 1,500 mg/L (0.29 ± 0.04 - 0.61 ± 0.13 THB; $p \leq 0.05$), primarily due to higher reagent costs rather than poor biological performance.

Mechanically, the reason behind the prevention of browning is that the oxidation of the phenolic compounds by the polyphenol oxidase (PPO) and peroxidase (POD) enzymes are blocked, preventing the formation of quinones and melanin pigment. Additionally, PAL (phenylalanine ammonia-lyase) activity is regulated, reducing the production of phenolic

precursors in the phenylpropanoid pathway. PVP acts as a valuable antioxidant aid when it is used in tissue culture preparations, where it prevents quinone buildup and attendant tissue necrosis. The protective mechanism involves PVP forming hydrogen bonds with phenolic compounds while also functioning as a free radical scavenger and chelating agent [19,20]. This multi-modal action makes PVP particularly effective for high-phenolic plant species, though concentration optimization remains crucial for balancing browning control with optimal growth conditions. Furthermore, this suggests that optimization protocols should consider both plant species characteristics and the specific stage of tissue culture development to achieve maximum browning control while maintaining optimal growth conditions. This mechanism elucidates the substantial survival improvements observed throughout our investigations. Our findings substantiate that 1,000 mg/L PVP achieves the optimal balance between browning suppression and tissue development in *Dioscorea*, consistent with prior studies in high-phenolic yam species. In *Dioscorea alata* var. purpurae, [38] supplementation with PVP reduced phenolic leaching and improved callus induction and organogenesis, particularly in combination with BAP and 2,4-D. Similarly, *Dioscorea hispida* [39] was reported to suffer from severe phenolic exudation, and the use of antioxidant supplementation and optimized culture conditions was essential to promote shoot regeneration and reduce browning. Complementary biochemical evidence from *Dioscorea esculenta* [40] linked tissue browning directly to ionic peroxidase isozyme activity, highlighting the mechanistic basis for PVP-mediated suppression of enzymatic browning. Collectively, these studies support that moderate supplementation with PVP, such as 1,000 mg/L, is effective in mitigating phenolic oxidation and enhancing morphogenic responses in phenolic-rich *Dioscorea* species. The findings can be compared with previous *Dioscorea* browning suppression studies based on the ability of antioxidants to promote successful culturing *in vitro* [20]. Significant varietal effects indicate that genotype-specific phenolic composition influences antioxidant requirements, as purple-fleshed varieties consistently exhibited elevated browning indices and demanded more intensive interventions. This finding supports that of Graham-Acquaah *et al*. [41], who observed that pigmented *Dioscorea rotundata* tissues had a higher rate of polyphenol oxidase activity and higher phenolic content. Even though 1,500 mg/L maintained the reduction of browning, with decreased survival and growth compared to 1,000 mg/L, this would indicate that there may be inhibitory actions at high concentrations, and therefore the moderate methods of supplementation with PVP should be used. The concentration-specific responses noted are consistent with the previous reports that the concentrations of PVP between 0.2 and 0.5 g/L are effective in the control of browning in the plant tissue culture [19,20].

Economically, this streamlined PVP approach can result in the economical control of browning, thereby improving the efficiency of propagation of indigenous *Dioscorea* conservation. The concentration-dependent responses observed provide valuable knowledge for developing antioxidant strategies specific to varietal phenolic differences, particularly in resource-limited environments.

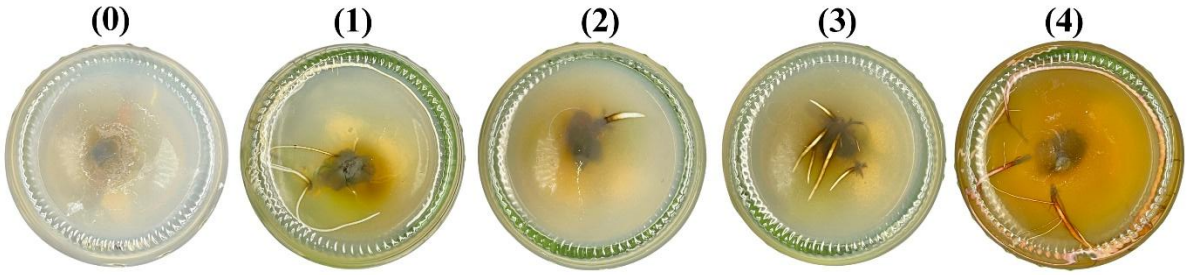

Figure 2 Visual scale of browning index (BI) used for assessment in *Dioscorea* spp. explants. The scale ranges from 0 (no browning) to 4 (severe browning observed in both explant and medium).

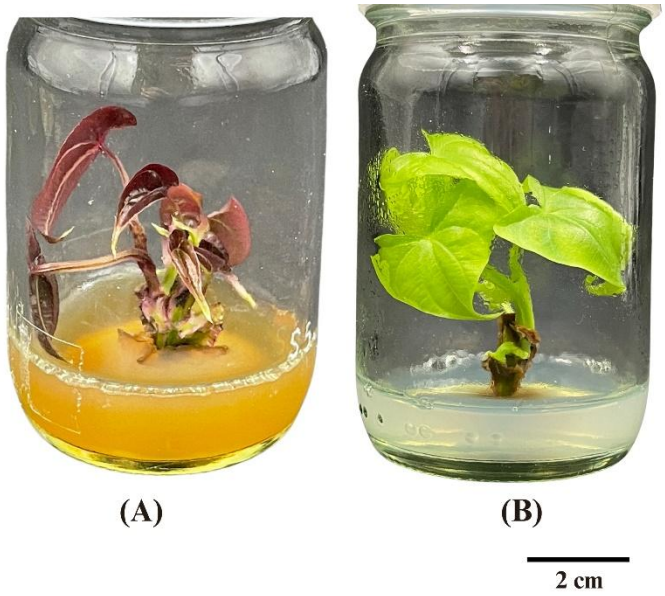

Figure 3 Morphological response of *Dioscorea* spp. explants cultured in MS medium supplemented with 1,000 mg/L PVP. (A) Purple-fleshed variety with visible browning and limited shoot development. (B) White-fleshed variety with minimal browning and vigorous shoot growth. Scale bar = 2 cm.

Table 2 Effect of PVP concentration and plant variety on browning index, survival, growth, and cost per survival of *Dioscorea* spp.

Variety	PVP concentration (mg/L)			
	0	**500**	**1,000**	**1,500**
		BI ± SD		
Purple	3.73 ± 0.23^{a}	3.07 ± 0.42^{b}	1.93 ± 0.50^{c}	2.13 ± 0.16^{c}
White	3.07 ± 0.23^{a}	2.00 ± 0.53^{b}	1.00 ± 0.20^{c}	0.87 ± 0.16^{c}
		Survival rate (%) ± SD		
Purple	26.67 ± 11.55^{b}	66.67 ± 11.55^{a}	66.67 ± 11.55^{a}	46.67 ± 11.55^{ab}
White	66.67 ± 11.55^{bc}	86.67 ± 11.55^{a}	80.00 ± 0.00^{ab}	60.00 ± 0.00^{c}
		Shoot No. ± SD		
Purple	0.27 ± 0.12^{d}	1.33 ± 0.23^{b}	1.93 ± 0.31^{a}	0.73 ± 0.12^{c}
White	0.87 ± 0.12^{d}	2.67 ± 0.31^{b}	2.87 ± 0.23^{a}	1.67 ± 0.12^{c}
		Root No. ± SD		
Purple	0.20 ± 0.00^{b}	1.33 ± 0.58^{a}	1.53 ± 0.12^{a}	0.93 ± 0.23^{a}
White	1.40 ± 0.40^{c}	1.93 ± 0.31^{ab}	2.33 ± 0.12^{a}	1.73 ± 0.12^{bc}
		Cost per survival plant (THB) ± SD		
	0.00 ± 0.00^{d}	0.14 ± 0.03^{c}	0.29 ± 0.04^{b}	0.61 ± 0.13^{a}

Source of variation	BI	Survival	Shoot No.	Root No.	Cost
Varieties	0.000*	0.000*	0.000*	0.000*	N/A
PVP concentration (mg/L)	0.000*	0.000*	0.000*	0.000*	0.000*
Interaction	0.480	0.107	0.435	0.371	N/A

Note Distinct letters denote significant differences at $p \leq 0.05$ using DMRT analysis within individual rows (horizontal comparisons across PVP concentrations). N/A represents single-factor analyses. Asterisks (*) signify statistical significance ($p \leq 0.05$). Cost calculations utilized PVP K-30 (Himedia) pricing at 1,045 THB per 100 g.

Comparative growth analysis under different media formulations

Hormonal formulations and plant varieties significantly affected all growth parameters of indigenous *Dioscorea* genotypes ($p \leq 0.05$), with statistically significant interactions observed for shoot height, leaf number, shoot number, root length, and root number (p = 0.000, 0.000, 0.000, 0.003 and 0.020, respectively) (**Table 3**). BA treatments consistently surpassed control and NAA formulations for shoot development, whereas NAA demonstrated superiority for root formation. White-fleshed varieties exhibited superior responses across all parameters, substantiating genotype-specific differences in hormone responsiveness with important implications for commercial propagation protocols.

BA treatment at a concentration of 2.0 mg/L provided the best shoot growth in both varieties and produced the highest shoot heights of 24.88 ± 0.87 mm in purple-fleshed and 31.64 ± 0.72 mm in white-fleshed variety compared to the controls of 21.94 ± 0.20 mm and 26.77 ± 0.87 mm, respectively. Comparable patterns of leaf development were observed, with BA at 2.0 mg/L yielding 3.93 ± 0.12 and 5.13 ± 0.12 leaves in purple- and white-fleshed varieties, respectively, compared to control values of 3.00 ± 0.00 and 3.53 ± 0.31 leaves. Shoot multiplication was most significantly improved, reaching 3.07 ± 0.31 (purple-fleshed) and 5.87 ± 0.12 (white-fleshed) shoots compared to 2.20 ± 0.00 and 4.07 ± 0.76 shoots in the control group.

NAA applications consistently reduced all the shoot parameters to below the control values, with the strongest effect being noticeable at 2.0 mg/L, which reveals definite antagonism between auxins and cytokinins in shoot organogenesis. Morphological differences between varieties seemed to be quite apparent, with white-fleshed varieties characterized by vigorous shoot growth as well as abundant leaf system and white rooting system, whereas purple-fleshed varieties had moderate shoot growth with characteristic pigmentation of stems and dense purple rooting (**Figure 4**). Root development exhibited contrasting hormone responses, with NAA applications proving superior to both BA and control formulations. NAA at 1.0 mg/L generated optimal root numbers of 4.67 ± 0.23 and 5.89 ± 0.12 roots in purple-fleshed and white-fleshed varieties, respectively, compared to control baselines of 2.07 ± 0.31 and 2.47 ± 0.12 roots. Root length development increased substantially with NAA treatments, with 1.0 mg/L producing optimal root lengths of 33.22 ± 0.51 mm in purple-fleshed and 40.53 ± 0.71 mm in white-fleshed varieties, compared to control baselines of 22.06 ± 1.41 mm and 25.04 ± 1.04 mm, respectively. While 2.0 mg/L achieved maximum length in purple-fleshed varieties (35.47 ± 3.81 mm), 1.0 mg/L provided the best overall root development considering both number and length parameters. BA applications maintained root parameters near control levels across all concentrations, indicating that cytokinin supplementation does not inhibit root formation while promoting shoot development.

High BA effectiveness corresponds with the established functions of cytokinins in promoting axillary bud break and overcoming apical dominance. Previous studies agree with our results as supplementation with BAP was found to be the most appropriate for the multiplication of *Dioscorea* species and it supports the main functions of cytokinins in the shoot organogenesis [42]. Comparable optimal responses were documented by Shah and Lele [38], who attained maximum callus and shoot formation utilizing BAP combinations in *Dioscorea alata* var. purpurae. Our experimental results clearly substantiate that BA promotes shoot formation. The dose-dependent responses observed reflect the critical importance of hormone balance, as concentrations exceeding optimal thresholds can paradoxically suppress growth and morphogenesis [43]. Contrasting root development responses emphasize the complementary roles of auxins and cytokinins in plant morphogenesis. NAA effectiveness for root growth verifies recognized auxin roles in rhizogenesis, as evidenced by similar IBA responses in *Dioscorea* species indicating optimum root formation at certain auxin concentrations [44]. This finding remains consistent with the study of Dang *et al.* [14], which reported that the NAA concentration at 0.5 mg/L was appropriate in *Dioscorea nipponica* in relation to rooting frequency, confirming the importance of special consideration of the concentration in auxin in order to have healthy root systems to ensure the processes of *Dioscorea* propagation become efficient. Similarly, Sanchez-Lopez *et al*. [45] found that NAA effectively

induces roots in *Dioscorea composita*, achieving 95% root formation with 1.0 mg/L IBA. This supports the fact that auxin supplementation significantly improves rhizogenesis in various *Dioscorea* species, promoting adventitious root development in large-scale micropropagation systems.

Stable excellent performance of white-fleshed varieties in all parameters is a manifestation of genotype-specific tissue culture responses reported in *Dioscorea* species. These varietal differences are probably due to the differences in levels of endogenous hormones and phenolic compound content, particularly anthocyanins in purple-fleshed varieties that may inhibit hormone responsiveness, and other cellular features that promote morphogenic actions [46]. Control treatments revealed important insights into endogenous hormone capabilities, demonstrating that both varieties possess sufficient natural growth regulators for basic development. This finding supports earlier reports that certain varieties of yams can grow well on hormone-free medium as this is evidence that they have adequate endogenous plant growth regulators [43]. Nevertheless, significant gains due to hormone supplementation demonstrate that exogenous applications are required to commercially propagate them optimally. Such variety-specific reactions underline the necessity to devise novel and more specific procedures that will be both highly efficient and cost-effective in a commercial and conservation setting.

Table 3 Effect of culture media formulations and plant variety on growth of *Dioscorea* spp.

Variety	Media formulation (mg/L)						
	Control	**0.5 BA**	**1.0 BA**	**2.0 BA**	**0.5 NAA**	**1.0 NAA**	**2.0 NAA**
				Shoot height (mm.) ± SD			
Purple	21.94 ± 0.20^{b}	24.21 ± 0.20^{a}	24.75 ± 0.99^{a}	24.88 ± 0.87^{a}	19.65 ± 0.15^{c}	19.79 ± 0.56^{c}	19.43 ± 0.57^{c}
White	26.77 ± 0.87^{c}	29.52 ± 0.35^{b}	30.36 ± 0.50^{b}	31.64 ± 0.72^{a}	23.35 ± 0.28^{d}	23.26 ± 0.12^{d}	22.29 ± 0.08^{e}
				Leaf No. ± SD			
Purple	3.00 ± 0.00^{cd}	3.27 ± 0.12^{c}	3.60 ± 0.20^{b}	3.93 ± 0.12^{a}	2.87 ± 0.12^{d}	2.93 ± 0.12^{d}	2.53 ± 0.23^{e}
White	3.53 ± 0.31^{d}	4.27 ± 0.12^{c}	4.73 ± 0.31^{b}	5.13 ± 0.12^{a}	3.33 ± 0.12^{de}	3.13 ± 0.12^{ef}	2.80 ± 0.20^{f}
				Shoot No. ± SD			
Purple	2.20 ± 0.00^{c}	2.40 ± 0.20^{bc}	2.53 ± 0.12^{bc}	3.07 ± 0.31^{a}	2.40 ± 0.20^{ab}	2.73 ± 0.46^{bc}	2.07 ± 0.12^{c}
White	4.07 ± 0.76^{c}	5.07 ± 0.12^{b}	5.47 ± 0.31^{ab}	5.87 ± 0.12^{a}	3.13 ± 0.12^{d}	3.20 ± 0.20^{d}	2.87 ± 0.31^{d}
				Root length (mm.) ± SD			
Purple	22.06 ± 1.41^{c}	22.26 ± 0.29^{c}	22.12 ± 0.30^{c}	22.39 ± 1.35^{c}	30.94 ± 0.59^{b}	33.22 ± 0.51^{ab}	35.47 ± 3.81^{a}
White	25.04 ± 1.04^{c}	26.98 ± 0.41^{c}	26.55 ± 0.40^{c}	27.11 ± 0.66^{c}	38.69 ± 0.62^{a}	40.53 ± 0.71^{a}	35.67 ± 3.18^{b}
				Root No. ± SD			
Purple	2.07 ± 0.31^{c}	1.80 ± 0.35^{cd}	1.47 ± 0.23^{d}	2.27 ± 0.31^{c}	3.47 ± 0.31^{b}	4.67 ± 0.23^{a}	4.67 ± 0.42^{a}
White	2.47 ± 0.12^{c}	2.73 ± 0.23^{c}	2.60 ± 0.20^{c}	2.87 ± 0.99^{c}	5.07 ± 0.12^{ab}	5.89 ± 0.12^{a}	4.60 ± 0.60^{b}

Source of variation	**Shoot height**	**Leaf No.**	**Shoot No.**	**Root length**	**Root No.**
Varieties	0.000*	0.000*	0.000*	0.000*	0.000*
Media formulations	0.000*	0.000*	0.000*	0.000*	0.000*
Interaction	0.000*	0.000*	0.000*	0.003*	0.020*

Note Distinct letters denote significant differences at $p \leq 0.05$ using DMRT analysis within individual rows (horizontal comparisons across hormone treatments). N/A represents single-factor analyses. Asterisks (*) signify statistical significance ($p \leq 0.05$).

Figure 4 Morphological response of *Dioscorea* spp. explants cultured with 2 mg/L BA. (A) Purple-fleshed variety with moderate shoot growth and dense purple roots. (B) White-fleshed variety with vigorous shoot proliferation and long white roots. Scale bar = 2 cm.

Cost-effectiveness and growth outcomes of low-cost disinfection approaches

Following the optimization of hormonal combinations for shoot and root development, subsequent experiments addressed the cost and technical limitations associated with medium sterilization. Low-cost disinfection approaches employing sodium hypochlorite (NaOCl) in combination with induction heating were systematically compared with conventional autoclaving, including an assessment of their economic feasibility. Statistical analysis indicated that both disinfection methods and plant varieties significantly influenced contamination (p = 0.024), survival (p = 0.006), shoot number (p = 0.001), shoot height (p = 0.000), and root length (p = 0.000). Significant interactions between factors were also detected for shoot number (p = 0.000), shoot height (p = 0.006), and root length (p = 0.005) (**Table 4**). These findings demonstrate that medium sterilization strategy and genotype exert combined effects on the overall culture performance, particularly in parameters related to shoot and root development. Bleach-based disinfection approaches exhibited promising biological performance while substantially reducing equipment and operational expenditures compared to conventional autoclaving.

Autoclaving achieved optimal performance, especially on the white-fleshed varieties (100% survival rate compared to 80% on purple-fleshed), highest shoot number (5.73 ± 0.12), shoot height (34.53 ± 0.42 mm), and root length (34.06 ± 0.69 mm). However, this methodology required significant equipment investment (60,000 THB) and incurred high operational costs (24.80 THB per vessel) (**Table 5**). Bleach concentrations of 1.0 - 2.0 mL/L established optimal equilibrium between contamination control and economic viability. Treatment with 2.0 mL/L concentration accomplished excellent disinfection (0.00 ± 0.00% in purple-fleshed and 13.33 ± 11.55% in white-fleshed varieties) while preserving robust survival rates (73.33 ± 11.55% and 86.67 ± 11.55%, respectively). Most surprisingly, 1.0 mL/L produced the best results in

shoot proliferation in purple-fleshed types (4.20 ± 1.04 shoots), which was better than autoclaved treatments (2.80 ± 0.20 shoots) (**Figure 5**). Non-autoclaving controls proved inadequate due to excessive contamination (73.33 ± 11.55% in purple-fleshed and 66.67 ± 30.55% in white-fleshed varieties) and poor survival rates.

Economic evaluation demonstrated compelling advantages for bleach-based disinfection methodologies across multiple cost parameters. Medium preparation expenses remained consistently low across bleach concentrations (20.804 - 20.832 THB per vessel) compared to autoclaving (24.80 THB per vessel) (**Table 5**). Equipment investment requirements varied enormously, whereas bleach methods require only 2,500 THB (induction cooker and stainless steel pot), whereas autoclave equipment requires an investment of 60,000 THB. Analysis of energy consumption showed that significant operational savings were achieved, with bleach preparation methods utilizing 4 THB per liter of preparation compared to 24 THB for autoclaving. Cost per surviving plant analysis also confirmed the economic feasibility, with bleach treatments coming in at 20.81 ± 10.04 - 36.75 ± 13.01 THB in comparison to 27.90 ± 3.40 THB for autoclaving. Bleach methodologies produced more attractive cost-effectiveness ratios when taken in the context of dramatically reduced equipment and operational costs necessary to achieve large-scale propagation systems, especially relevant in resource-limited settings where autoclave equipment is prohibitively expensive.

Mechanistically, the mode of action of bleach is that sodium hypochlorite is converted to hypochlorous acid (HOCl) in aqueous solutions that then diffuse across the cell membranes of microorganisms, destroying vital cellular functions, in addition to the formation of protective antimicrobial films that form and protect continuously over the cultures. Our findings align with the CSUP (Chemical Sterilization Using Powder) technique demonstrated by Peiris *et al.* [26], which achieved contamination-free cultures using 5% sodium hypochlorite while reducing equipment costs by 97%. Their investigation showed that sodium hypochlorite forms thin chemical films on culture vessel surfaces, providing antimicrobial protection even in non-aseptic environments. Comparable cost-effective approaches have been validated by Sookruksawong [27] in *Dioscorea bulbifera* tissue culture, where 2.0 mL/L bleach accomplished 100% disinfection and survival rates at 20.81 THB per vessel compared to 24.80 THB for autoclaving.

Chemical disinfection versatility extends beyond *Dioscorea* species, as demonstrated by Weber *et al.* [47] in potato micropropagation, where 5% NaOCl household bleach at 9 ppm active chlorine-controlled microorganism growth while maintaining plantlet performance. These results are consistent with Gavilan *et al.* [48], who successfully applied 0.001-0.005% active chlorine for chemical sterilization in *Cochlospermum regium* micropropagation, achieving effective contamination control and maintaining tissue viability without adverse morphogenic effects. Moreover, Datta *et al.* [49], who found that low-cost tissue culture technologies can reduce production costs by 50% - 90% while maintaining quality, making biotechnological tools accessible to resource-constrained businesses. Sookruksawong [50] confirmed cost-effectiveness in Kratom (*Mitragyna speciosa*) micropropagation. Commercial bleach at 2 mL/L proved to be the most cost-effective medium disinfection option, balancing efficacy, survival and economics.

Purple-fleshed varieties performed efficiently at 1.0 mL/L, but white-fleshed varieties responded better at 2.0 mL/L, indicating genotype-specific variations in phenolic content and disinfection tolerance. This variance complements prior findings of increased polyphenol oxidase activity in pigmented *Dioscorea* tissues. Our findings demonstrate that bleach-based medium disinfection is a feasible and cost-effective alternative to traditional autoclaving for *Dioscorea* tissue culture, with significant implications for conservation initiatives and commercial replication facilities in resource-limited environments.

Table 4 Effect of medium disinfection methods on contamination, survival, growth, and cost of *Dioscorea* spp.

Variety	Medium disinfection method					
	Autoclave (Positive)	Bleach concentration (mL/L)				Non-autoclave (Negative)
		0.5	1	2	4	
Contamination rate (%) ± SD						
Purple	13.33 ± 11.55^{c}	40.00 ± 20.00^{b}	20.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	73.33 ± 11.55^{a}
White	0.00 ± 0.00^{b}	6.67 ± 11.55^{b}	0.00 ± 0.00^{b}	13.33 ± 11.55^{b}	0.00 ± 0.00^{b}	66.67 ± 30.55^{a}
Survival rate (%) ± SD						
Purple	80.00 ± 0.00^{a}	86.67 ± 11.55^{a}	80.00 ± 0.00^{a}	73.33 ± 11.55^{ab}	53.33 ± 11.55^{bc}	40.00 ± 20.00^{c}
White	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	93.33 ± 11.55^{a}	86.67 ± 11.55^{a}	73.33 ± 30.55^{ab}	46.67 ± 23.09^{b}
Shoot No. ± SD						
Purple	2.80 ± 0.20^{b}	3.20 ± 0.53^{b}	4.20 ± 1.04^{a}	2.67 ± 0.50^{b}	1.27 ± 0.12^{c}	0.93 ± 0.42^{c}
White	5.73 ± 0.12^{a}	3.93 ± 0.23^{b}	2.53 ± 0.90^{c}	4.06 ± 0.83^{b}	2.33 ± 1.02^{cd}	1.23 ± 0.12^{d}
Shoot height (mm.) ± SD						
Purple	22.07 ± 0.61^{a}	21.80 ± 2.69^{a}	27.13 ± 6.71^{a}	19.60 ± 4.06^{a}	11.20 ± 2.78^{b}	9.27 ± 4.31^{b}
White	34.53 ± 0.42^{a}	27.13 ± 2.12^{ab}	20.13 ± 3.89^{bc}	28.40 ± 3.99^{a}	19.93 ± 7.38^{bc}	13.47 ± 2.34^{c}
Root length (mm.) ± SD						
Purple	20.97 ± 0.15^{ab}	20.39 ± 1.95^{b}	27.37 ± 6.13^{a}	18.71 ± 2.98^{b}	11.78 ± 2.57^{c}	9.16 ± 4.77^{c}
White	34.06 ± 0.69^{a}	27.50 ± 2.08^{ab}	20.20 ± 3.25^{bc}	28.47 ± 3.55^{a}	18.63 ± 8.01^{c}	13.89 ± 4.45^{c}
Cost per survival plant (THB) ± SD						
	27.90 ± 3.40^{b}	22.54 ± 2.69^{b}	20.81 ± 10.04^{b}	26.60 ± 4.48^{b}	36.75 ± 13.01^{a}	0.00 ± 0.00^{c}

Source of Variation	Contamination	Survival	Shoot No.	Shoot height	Root length	Cost
Varieties	0.024*	0.006*	0.001*	0.000*	0.000*	N/A
Disinfection methods	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
Interaction	0.056	0.968	0.000*	0.006*	0.005*	N/A

Note Distinct letters denote significant differences at $p \leq 0.05$ using DMRT analysis within individual rows (horizontal comparisons across disinfection methods). N/A represents single-factor analyses. Asterisks (*) signify statistical significance ($p \leq 0.05$). Cost evaluations utilized Haiter® pricing at 25 THB per 600 mL bottle.

Table 5 Comparative cost analysis of different medium disinfection methods showing medium components, energy expenditure, and equipment requirements (THB)

Component	Bleach (mL/L)				Positive control	Negative control
	0.5	1	2	4		
Culture medium per 100 mL						
Medium	100	100	100	100	100	100
Bleach	0.02	0.04	0.08	0.16	-	-
Electricity cost of Autoclave	-	-	-	-	24	-
Electricity cost Induction cooker	4	4	4	4	-	4
Culture medium cost per 100 mL	104.02	104.04	104.08	104.16	124	104
Culture medium cost per 20 mL (vessel)	**20.804**	**20.808**	**20.816**	**20.832**	**24.8**	**20.8**

Component	Bleach (mL/L)				Positive control	Negative control
	0.5	1	2	4		
Equipment						
Autoclave	-	-	-	-	60,000	-
Pot	500	500	500	500	-	500
Induction cooker	2,000	2,000	2,000	2,000	-	2,000
Total equipment cost (THB)	**2,500.00**	**2,500.00**	**2,500.00**	**2,500.00**	**60,000.00**	**2,500.00**

Note: Disinfectant: Haiter® bleach (600 mL, 25 THB). Equipment: 50 L autoclave (3,000 W; LS-50L Ditoplanding™), 2.5 L pots (ZEBRA®), induction cooker (2,000 W; Electrolux®). Electricity cost = (watts×h×4 THB/unit) / 1,000 (rate as of year 2024). Sterilization: 2 h (autoclave), 0.5 h (boiling). One experiment = 1 L MS medium.

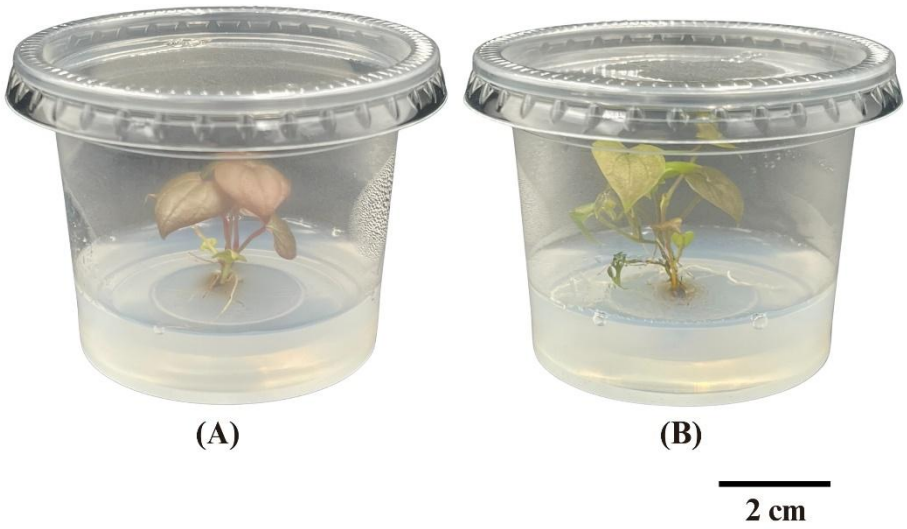

Figure 5 Morphological response of *Dioscorea* spp. explants cultured in MS medium disinfected with low-cost bleach treatment. (A) Purple-fleshed variety grown in 1 mL/L bleach showing enhanced shoot proliferation. (B) White-fleshed variety grown in 2 mL/L bleach displaying vigorous shoot and root development. Scale bar = 2 cm.

Conclusions

Our study effectively created complete, commercially viable tissue culture techniques for purple and white-fleshed indigenous *Dioscorea* species, overcoming significant hurdles that have previously hampered their propagation and conservation efforts. These integrated methodologies demonstrate that effective tissue culture can be accomplished using readily available household materials while maintaining biological performance comparable to conventional expensive approaches.

Commercial bleach treatment at 10% concentration provided optimal explant disinfection that led to 67% - 73% survival with each resulting plant costing 0.117 THB. The supplementation with 1,000 mg/L of PVP controlled browning, and also increased the survival rates to 80% of the white- and 67% of the purple-fleshed varieties. Across all parameters analyzed, white-fleshed varieties offered significantly better performance overall than purple-fleshed varieties and hence offer excellent advice in variety selection strategies. When BA (cytokinin) at 2.0 mg/L and NAA (auxin) at 1.0 mg/L were applied, shoot development was maximized (5.87 shoots per explant) and root formation was optimized (5.89 roots per explant). Remarkably, bleach-based medium disinfection at 1 - 2 mL/L achieved performance comparable to autoclaving while substantially lowering equipment costs from 60,000 to 2,500 THB. This breakthrough eliminates the primary economic obstacle to tissue culture adoption across developing regions.

These cost-effective methodologies enable complete tissue culture systems utilizing household bleach for all sterilization processes, representing a paradigm shift toward democratized biotechnology accessibility.

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Declaration of generative AI in scientific writing

The authors acknowledge utilizing generative AI tools—including ChatGPT by OpenAI, and Claude—exclusively for language editing and grammar correction during manuscript preparation. All content generation and data interpretation were performed solely by the authors. The authors retain full responsibility for the research content, analyses, and conclusions presented in this work.

CRediT author statement

Suchonma Sookruksawong: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - Original Draft, Visualization, and Funding acquisition; **Warisa Pilahome:** Formal analysis and Writing - Review & Editing.

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