

Cost-Effective Micropropagation of ABACA (*Musa textilis* NEE cv. Agbayanon) Using Locally Sourced Starches as Gelling Agents

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Abstract

This study evaluated the suitability of cassava and sago starch as alternative gelling agents to agar in the in vitro propagation of *Musa textilis* (cv. Agbayanon). Culture media were prepared using cassava starch, sago starch, their combination, and standard agar, and their effects on media properties, explant survivability, morphogenesis, and cost efficiency were assessed. Explants cultured on starch-based media exhibited significantly higher survival rates, with sago starch yielding the highest (87.5%) compared to agar (65.6%). Callus formation occurred in all treatments (100%). The earliest callus initiation was observed in the cassava starch treatment (55.25 days), while the slowest was recorded in the cassava + sago starch combination (58.25 days). Shoot emergence was fastest in agar (32.50 days), while shoot count was highest in cassava starch and agar (2.00 per explant). Leaf number and shoot height did not significantly differ across treatments, and no hyperhydricity was observed. The media pH remained stable after autoclaving in all treatments, indicating chemical compatibility. Starch-based gels appeared less transparent but sufficiently firm to support plant growth. Cost analysis revealed cassava starch as the most economical gelling agent (₱397.41 per liter), offering potential cost savings for large-scale propagation. Overall, cassava starch demonstrated performance comparable to agar, highlighting its promise as a sustainable, low-cost alternative in abaca micro propagation. Further optimization of starch blends may enhance their applicability in commercial tissue culture systems.

Keywords: Cassava Starch, Micropropagation, *Musa Textilis*, Plant Tissue Culture, Sago Starch, Starch-Based Medium.

Introduction

Abaca (*Musa textilis* Nee), commonly referred to as Manila hemp, is one of the most economically important fiber crops endemic to the Philippines (1). Globally renowned for its exceptional tensile strength, saltwater resistance, and biodegradability, abaca fiber is widely used in various industries, including cordage, textiles, currency paper, and increasingly, in sustainable composites for automotive and packaging applications (2-4). The Philippines remains the world's leading producer and exporter of abaca fiber, contributing over 85% of the global supply (5,6). Among the recognized cultivars, 'Agbayanon' is a notable variety due to its high-quality fiber and adaptability to local agro climatic conditions (7). Despite its global demand and national importance, abaca production continues to face significant constraints. Conventional propagation through suckers is slow and inefficient, resulting in limited

availability of high-quality planting materials. Moreover, the susceptibility of abaca to viral diseases such as abaca bunchy top virus and abaca mosaic virus further reduces productivity and poses a threat to genetic resources (8). These challenges highlight the urgent need for a reliable and scalable propagation method that ensures disease-free and uniform plantlets. Plant tissue culture, specifically micropropagation, offers a powerful solution to the limitations of traditional propagation methods. It enables the rapid multiplication of elite genotypes under aseptic and controlled conditions, ensuring clonal fidelity and pathogen-free planting stock (9). However, the practical application of tissue culture in developing countries like the Philippines is often hindered by the high cost of inputs—particularly agar, which is widely used as a gelling agent in culture media. Agar is one of the inputs that can account for more

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than 70% of the total cost of culture medium preparation, primarily due to its dependence on imports and fluctuating market prices (10). To address these economic constraints, researchers have explored the use of locally available and inexpensive starches as alternatives to agar. Starches derived from sago (*Metroxylon sagu*) and cassavas (*Manihot esculenta*) are promising candidates due to their wide availability in the Philippines, renewable nature, and favorable gelling properties when processed appropriately (11, 12). These starches have demonstrated utility as gelling agents in the micropropagation of several crops, such as banana, pineapple, and taro, with varying degrees of success depending on concentration, purity, and media compatibility (12-14). For example, cassava starch has successfully supported shoot proliferation in banana and pineapple, while sago starch has been shown to sustain callus formation in taro. However, challenges such as reduced medium clarity, inconsistent gel firmness, and, in some cases, slower morphogenic responses compared to agar have also been reported. These mixed outcomes demonstrate that while starch-based gelling agents hold promise, they also have apparent limitations, making it essential to test and validate their performance specifically for abaca. Despite these advances, very few studies have systematically evaluated the properties and biological performance of sago and cassava starches as gelling agents for abaca tissue culture, particularly for commercially important cultivars such as 'Agbayanon'. Moreover, the combination of both starches has not been thoroughly investigated, suggesting a potential synergy that could enhance gel consistency and improve plant growth response. Previous work on abaca micropropagation has relied almost exclusively on agar as the standard gelling agent, focusing primarily on hormonal optimization and explant source. The present study, therefore, differs by introducing and assessing starch-based alternatives, thereby addressing both cost and supply limitations associated with agar.

This study seeks to develop a cost-effective micropropagation protocol for abaca cv. Agbayanon by substituting agar with locally sourced starches, such as sago, cassava, and a combination of both, as gelling agents in the culture medium. Specifically, it aims to evaluate the

medium properties, like clarity and pH stability, and compare their effects on explant survival, shoot formation, and overall development. By directly contrasting starch-based gelling agents with the conventional agar system, our study provides new insights into how alternative substrates influence abaca micropropagation performance. By integrating low-cost, indigenous resources into the tissue culture system, this research supports a more sustainable and accessible biotechnology platform for abaca propagation—one that is adaptable for use by smallholders and rural tissue culture laboratories. Furthermore, this study contributes to the national agenda of enhancing abaca productivity and resilience while aligning with the global movement toward sustainable and locally driven agricultural innovations. The findings could significantly reduce the cost of micropropagation, increase the availability of planting materials, and ultimately strengthen the competitiveness of the Philippine abaca industry in the global fiber market.

Methodology

This study employed a Randomized Complete Block Design (RCBD) to assess the effectiveness of locally sourced cassava starch, sago starch, and their combination as alternatives to tissue culture-grade agar in the micropropagation of *M. textilis* nee cv. Agbayanon. Healthy and vigorously growing sword-leaf suckers were collected from abaca plants in Aklan, Philippines, and served as the source of explants. Shoot tips and axillary buds were excised from the collected suckers. After removing roots and leaf sheaths, the explants were thoroughly washed under running tap water to eliminate soil and debris. Basal portions were trimmed to approximately 2.5 cm³. Surface sterilization began with immersion in a detergent solution, followed by rinsing and draining. Explants were then treated with 50% (v/v) commercial sodium hypochlorite for 15 minutes and rinsed three times with distilled water. Under aseptic conditions in a laminar flow cabinet, further disinfection was performed using 25% (v/v) sodium hypochlorite for 10 minutes. To eliminate any residual disinfectant, explants were rinsed multiple times with sterile distilled water prior to inoculation.

A basal culture medium previously developed for plant tissue culture was used and supplemented

with 20 g/L sucrose (15). The pH was adjusted to 5.8 using 1 N NaOH or HCl before autoclaving. To induce shoot formation, the medium was enriched with 5 ppm 6-Benzylaminopurine (BAP). Four gelling treatments were tested: 17 g/L tissue culture-grade agar (control), 80 g/L cassava starch, 130 g/L sago starch, and a 50:50 (w/w) combination of cassava and sago starches at 40 g/L and 65 g/L, respectively, totaling 105 g/L. Concentrations were selected based on preliminary tests to ensure suitable gel strength. The media were dispensed into autoclavable glass jars at 30 mL per jar, sealed with either polypropylene plastic or aluminum foil, and sterilized at 121°C for 15 minutes.

Prepared media were visually assessed for clarity, and pH values were recorded both before and after autoclaving to evaluate pH stability. Explants were inoculated onto the culture media under aseptic conditions inside a laminar flow cabinet. Cultures were maintained at $25 \pm 2^\circ\text{C}$ under a 16:8-hour light-to-dark photoperiod. Each treatment was replicated four times with 20 explants per replicate, for a total of 80 explants per treatment. One explant was cultured per jar to ensure uniform contact with the medium. Cultures were maintained for duration of 12 weeks, with observations recorded at regular intervals. Subculturing was performed every four weeks to replenish nutrients and maintain aseptic conditions.

Observations were made weekly for three months. The recorded parameters included explant survival, the number of shoots per explant, shoot length, the number of leaves per explant and shoot, and the days to callus and shoot initiation.

Quantitative data were analyzed using analysis of variance (ANOVA) through the Statistical Tool for Agricultural Research (STAR) software (16). When significant differences among treatments were detected, mean separation was carried out using Tukey's Honest Significant Difference (HSD) test at a 5% significance level.

Results

The physical and chemical properties of the culture media varied according to the gelling agent used. Media prepared with cassava and sago starches were consistently cloudy and opaque, contrasting with the clear, transparent appearance of agar-based media. This reduction in clarity, although visually apparent, did not hinder gelation, as all media formed sufficiently firm gels capable of supporting explant growth. All culture media maintained a near-neutral pH of 5.8 before and after autoclaving, with minimal fluctuation and no significant differences, indicating adequate buffering capacity during sterilization.

Explant performance parameters varied significantly among treatments (Table 1). Survivability differed according to the gelling agent used (ANOVA, $p < 0.05$). Post-hoc analysis revealed that survival was highest in sago starch (87.50%), followed by cassava starch and the cassava + sago blend (both 81.25%), while agar resulted in the lowest survival rate (65.62%). All starch-based treatments were statistically superior to agar ($p < 0.05$).

Morphogenic responses further highlighted the suitability of starch-based gels. Callus initiation time differed slightly among treatments (ANOVA, $p < 0.05$), with agar, cassava, and sago starch media initiating callus within 55–56 days, while the starch blend required longer (58.25 days; Tukey's HSD, $p < 0.05$). Despite these differences, all treatments achieved a 100% callus formation rate. Shoot initiation did not vary significantly among treatments (32.50–34.50 days; ANOVA, $p > 0.05$). Shoot proliferation showed significant treatment effects (ANOVA, $p < 0.05$). Agar and cassava starch supported the highest proliferation (2.00 shoots per explant each), sago starch was intermediate (1.75), and the starch blend was lowest (1.25; Tukey's HSD, $p < 0.05$). Leaf development showed no significant variation among treatments, with 4.33–4.78 leaves per explant and 2.25–3.50 leaves per shoot (ANOVA, $p > 0.05$). Similarly, shoot height (4.38–5.00 cm) did not differ significantly across treatments (ANOVA, $p > 0.05$).

Table 1: Effects of Different Gelling Agents on *In Vitro* Growth, Morphogenesis, and Cost Efficiency in *M. textilis* cv. Agbayanon Tissue Culture

Parameters	Gelling Agents			
	Agar	Cassava Starch	Sago Starch	Cassava + Sago Starch
Survivability (%)	65.62 ^b	81.25 ^a	87.50 ^a	81.25 ^a
Number of days to callus formation	56.00 ^b	55.25 ^b	56.00 ^b	58.25 ^a
Callus formation rate (%)	100.00	100.00	100.00	100.00
Number of days to shoot formation	32.50 ^b	33.25 ^{ab}	33.50 ^{ab}	34.50 ^a
Number of shoots per explant	2.00 ^a	2.00 ^a	1.75 ^{ab}	1.25 ^b
Number of leaves per explant	4.73	4.78	4.33	4.35
Number of leaves per shoot	2.50	2.25	2.50	3.50
Shoot height	4.38	4.43	5.00	4.53
Cost analysis (Php)	400.12	397.41	397.53	397.47

A cost comparison of the culture media revealed slight but potentially meaningful differences mentioned in Table 1. Cassava starch-based medium was the most economical at ₱397.41 per liter, closely followed by cassava+sago (₱397.47) and sago starch (₱397.53). Agar, at ₱400.12 per liter, was the most expensive. At 30 mL per culture vessel, this equates to ₱12.00 per vessel for agar versus ₱11.92–11.93 for starch-based media. While the per-vessel difference is small, in large-scale propagation (e.g., 10,000 vessels), starch-based alternatives can reduce costs by several hundred pesos per cycle.

Discussion

The reduced clarity observed in starch-based media can be attributed to incomplete solubilization and gelatinization of starch granules, resulting in the presence of light-scattering particles that compromise transparency. This outcome concurs with earlier findings that cassava and sago starch produce more opaque matrices compared to conventional gelling agents such as agar and gelrite, which yield clearer, more homogenous gels (17, 18). Although this visual opacity may limit the ability to monitor explant responses or detect microbial contamination, it does not appear to compromise culture viability. Despite differences in clarity, cassava and sago starch formed gels of adequate firmness to support the establishment of explants. Gel strength is critical for ensuring mechanical support, nutrient diffusion, and aeration *in vitro*. Furthermore, the pH stability observed after autoclaving indicates that these starches do not disrupt the buffering capacity of the medium—an essential factor in maintaining nutrient solubility and optimal

enzyme function. These attributes highlight the practical suitability of cassava and sago starch as low-cost, locally available gelling alternatives, particularly in settings where the cost or availability of agar is prohibitive.

The enhanced survivability of *M. textilis* explants in starch-based media is likely due to the unique physicochemical properties of starch gels. Unlike agar, which forms relatively rigid structures, starch gels tend to be softer and more hydrated. This softer microenvironment may reduce physical stress on the explants, improve moisture availability, and facilitate nutrient and hormone diffusion during the critical early stages of micro propagation (11, 18, 19). These findings are in line with studies in 3D culture systems, where softer matrices have been shown to improve cell viability and function by enhancing mass transfer and reducing mechanical constraints (20).

The higher survival rates observed on cassava and sago starch-based media may also stem from their enhanced water-holding capacity and porous gel networks, which maintain favorable osmotic conditions and promote consistent metabolic activity. Given that osmotic balance is crucial in maintaining cell turgor and physiological stability (21), these starch-based matrices offer advantages beyond cost-effectiveness, particularly in the early acclimatization of explants.

Interestingly, while the cassava + sago starch blend yielded the highest percentage of callogenesis, it exhibited a delayed onset of visible callus formation. This delay may be linked to the increased gel density and reduced porosity of the mixed starch matrix, which could hinder the diffusion of critical signaling molecules such as auxins, known to drive dedifferentiation and callus

initiation (22). Previous reports have underscored the role of gelling agent physicochemical properties—including gel strength, water retention, and diffusion characteristics—in modulating explant responsiveness by altering hormone and nutrient availability (23).

Such findings have practical implications. While the cassava + sago combination may provide a conducive matrix for sustained callus proliferation, its delayed initiation may limit its application in time-sensitive tissue culture protocols such as somatic embryogenesis or rapid clonal propagation. Optimizing gelling formulations to balance strength and permeability is thus essential for tailored applications in micropropagation.

Uniform callus formation across all treatments suggests that the gelling agents, irrespective of origin, did not interfere with cellular dedifferentiation when exposed to an adequate hormonal stimulus. This observation underscores the sufficiency of 6-benzylaminopurine in inducing callogenesis in *Musa* explants, corroborating findings that highlight the dominant role of cytokinins in callus induction in *Musa* species (24). Consequently, the data confirm the functional viability of starch-based gelling agents as sustainable, cost-efficient alternatives to agar in tissue culture. Unlike earlier reports on abaca micropropagation that relied solely on agar as the gelling matrix, this study is the first to demonstrate the feasibility of sago, cassava, and their combination as cost-efficient and locally available alternatives, thereby establishing a novel basis for sustainable large-scale abaca propagation.

While all gelling agents supported shoot formation, the agar-based medium promoted more rapid shoot emergence. This difference, though not statistically significant, may reflect the superior physical matrix properties of agar—such as uniformity, porosity, and diffusion capacity—which enhance the bioavailability of cytokinins essential for shoot organogenesis (25, 26). In contrast, the denser matrix of the cassava + sago blend may impede the effective uptake of hormones, thus prolonging shoot initiation. For commercial applications where rapid turnover is critical, such subtle delays in morphogenic response could affect productivity.

Nevertheless, cassava starch alone supported shoot proliferation at levels comparable to agar, demonstrating its utility as a viable gelling agent.

The likely reason is its moderate gel firmness and permeability, which facilitate the diffusion of nutrients and growth regulators essential for axillary bud proliferation. These results align with prior studies that highlight cassava starch's potential as an effective substitute in tissue culture media (27, 28).

The lower shoot proliferation observed in the cassava + sago starch blend could be attributed to increased gel density and potential structural inconsistencies that restrict hormonal diffusion. Interactions between different starch types may also influence the internal gel matrix, altering the availability of nutrients and signaling compounds. Gelling agents, therefore, play a dual role—structurally supporting explants and influencing physiological responses through modulation of the culture microenvironment (29). These findings underscore the importance of optimizing starch concentrations and combinations when formulating mixed gelling systems.

Regarding foliar development, the number of leaves per shoot remained statistically consistent across all treatments. This suggests that starch-based gelling agents—whether used individually or in combination—can support leaf differentiation to a degree comparable to agar. The slightly higher leaf count observed in the cassava + sago treatment may reflect synergistic hydration and nutrient diffusion properties, although the variation was not significant. These results further reinforce the suitability of starch-based matrices in supporting shoot vigor and morphological completeness in *M. textilis*.

Leaf development is predominantly governed by endogenous hormone balance and is less sensitive to the gelling agent's origin, provided that the physical matrix allows adequate nutrient and water transport (30). Since all media were standardized for hormone content and cultured under uniform conditions, the consistency in leaf production across treatments affirms the reliability of alternative gelling agents in maintaining a favorable microenvironment for shoot development.

Similarly, shoot height was not significantly affected by the gelling agent, suggesting that all media types provided sufficient structural support and hydration to promote vertical elongation. Gel characteristics such as firmness and water potential directly impact nutrient diffusion and

turgor pressure, both critical for cell expansion and shoot growth. The comparable performance across treatments indicates that locally sourced starches can replicate the biophysical conditions necessary for shoot elongation, as documented in other studies (24).

The absence of hyperhydricity across all treatments further highlights the robustness of the current culture protocol. Hyperhydricity, a common physiological disorder in tissue culture, is often linked to poor gas exchange, excessive water availability, or imbalanced hormonal conditions (31). The lack of this anomaly in starch-based treatments confirms that these gelling agents do not disrupt the physical or osmotic balance within the medium. Moreover, the genotype of the explants (*M. textilis* cv. Agbayanon) may possess inherent resilience against hyperhydricity, which could be leveraged in future propagation efforts.

Finally, the economic evaluation revealed that starch-based media are more cost-efficient than agar-based formulations. While the per-liter cost differences were modest—cassava starch being the least expensive at ₱397.41 compared to ₱400.12 for agar—they become significant when extrapolated to large-scale operations. Significantly, these cost reductions did not compromise culture quality, as starch-based media supported comparable explant survival, shoot proliferation, and callus formation relative to agar. Taken together, the performance parameters evaluated in this study—namely survivability, shoot height, leaf development, callus formation, and the absence of hyperhydricity—demonstrate that cassava and sago starch are reliable alternatives to agar for *M. textilis* micropropagation. High survival rates and consistent morphogenic responses confirm that these starch-based matrices can sustain healthy explant growth without inducing physiological abnormalities, underscoring their robustness for routine culture applications. Notably, the comparable shoot proliferation and leaf development achieved on cassava starch highlight its potential to substitute agar without compromising plantlet quality. When combined with the economic advantages and widespread local availability of cassava and sago as renewable agricultural products in the Philippines, these findings suggest a practical and scalable approach for tissue culture operations, particularly in

resource-limited settings. Preliminary hardening and transfer of plantlets were not included in this study and are therefore acknowledged as limitations. Moreover, the rooting efficiency, physiological vigor, and acclimatization success of starch-based media remain to be validated. Addressing these aspects in future work will be essential to fully establish the commercial feasibility of starch-based alternatives. Nonetheless, the present results provide a strong foundation for considering starch-based media as cost-effective, sustainable, and locally accessible options for large-scale abaca micropropagation.

Conclusion

This study demonstrated that locally available starches—cassava and sago—are effective and economical alternatives to agar as gelling agents for the in vitro culture of *M. textilis* cv. Agbayanon. Both starches supported high explant survival, consistent callus formation, and robust shoot and leaf development without inducing hyperhydricity. Although agar promoted slightly earlier shoot initiation, cassava starch achieved comparable shoot proliferation, while sago starch supported the highest survival rates. The cassava + sago blend, while capable of sustaining growth, showed delayed callus initiation and reduced shoot proliferation, indicating that mixed starch matrices may require optimization before large-scale application. Importantly, starch-based media were more cost-efficient than agar, with even modest per-vessel savings translating to substantial reductions in large-scale propagation systems. Given their renewable supply and local availability in the Philippines, cassava and sago starch present sustainable alternatives that can reduce reliance on imported agar.

While this study established the viability of starch-based gelling agents for callus induction, shoot proliferation, and overall explant survival, further research should evaluate rooting efficiency, physiological vigor, and acclimatization success to fully validate these media for commercial micropropagation. Nevertheless, the present findings provide a solid foundation for developing low-cost, scalable, and sustainable tissue culture systems for abaca, supporting both conservation and industrial fiber production.

Abbreviations

ANOVA: Analysis of Variance, BAP: 6-Benzylaminopurine, HSD: Honest Significant Difference, RCBD: Randomized Complete Block Design, STAR: Statistical Tool for Agricultural Research.

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Author Contributions

Neil Adrian R. Alegria: conceptualization, conducted the investigation, data curation, performed the methodology and visualization, wrote original draft, edited the manuscript, Evelyn P. Vedasto: conceptualization, supervised the investigation, data curation, and methodology, reviewed and edited the manuscript.

Conflict of Interest

The authors declare that there are no financial or personal relationships that could have influenced, or appeared to influence, the research presented in this paper.

Declaration of Artificial Intelligence (AI) Assistance

The authors declare that no generative AI tools or AI-assisted technologies were used in the writing, data analysis, or preparation of this manuscript. All content, interpretations, and discussions are the result of the authors' independent work.

Ethics Approval

This study did not involve human participants or animal subjects and therefore did not require formal ethics approval. All plant materials were obtained in accordance with institutional guidelines for plant collection and handling.

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