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Micropropagation and genetic characterization of abaca (*Musa textilis* Nee) varieties

Micropropagación y caracterización genética de variedades de abacá (*Musa textilis* Née)

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ABSTRACT

Abaca (*Musa textilis* Nee) is a perennial plant of the Musaceae family, highly valued for the fibers obtained from its pseudostem. Costa Rica, the third-largest global producer of this fiber, faces limitations in propagation and variety selection due to its reliance on vegetative material and the limited genetic information available. In this study, efficient protocols were developed for the *in vitro* establishment and large-scale micropropagation of commercial abaca varieties cultivated in Costa Rica, as well as their genetic characterization using Simple Sequence Repeat (SSR) markers. Effective disinfection of explants derived from corms was achieved, and multiplication rates of 5 to 10 shoots per explant were obtained within 3 to 4 weeks, with plantlets ready for acclimatization in 8 to 12 weeks. Molecular characterization revealed high genetic variability among the analyzed varieties, based on differences in the number and size of the amplicons obtained with SSR markers. The R40 marker showed high discrimination power. These results demonstrate the potential of SSR markers for differentiating abaca genotypes and support their use in genetic improvement and conservation programs. The developed protocols represent a key tool for producing pathogen-free plant material and for strengthening commercial propagation and germplasm conservation strategies for abaca in Costa Rica.

Palabras clave:
Biotecnología
Planta textil
Diversidad genética
Microsatélites
Cultivo de tejidos

RESUMEN

El abacá (*Musa textilis* Née) es una planta perenne de la familia Musaceae, de gran relevancia por las fibras obtenidas a partir de su pseudotallo. Costa Rica, tercer productor mundial de esta fibra, enfrenta limitaciones en la propagación y en la selección de variedades debido a la dependencia de material vegetativo y a la escasa información genética disponible. En este estudio se desarrollaron protocolos eficientes para el establecimiento *in vitro* y la micropropagación a gran escala de variedades comerciales de abacá cultivadas en Costa Rica, así como su caracterización genética mediante marcadores de Secuencias Simples Repetidas (SSR). Se logró una desinfección efectiva de explantes provenientes de cormos y una multiplicación de 5 a 10 brotes por explante en un periodo de 3 a 4 semanas, alcanzando la formación de plántulas listas para aclimatación en 8 a 12 semanas. La caracterización molecular evidenció una amplia variabilidad genética entre las variedades analizadas, mediante diferencias en el número y tamaño de los amplicones obtenidos para los marcadores SSR. El marcador R40 mostró un alto poder discriminativo. Estos resultados demuestran el potencial de los SSR para diferenciar genotipos de abacá y respaldan su uso en programas de conservación y mejora genética. Los protocolos desarrollados representan una herramienta clave para la producción de material vegetal libre de patógenos y para el fortalecimiento de estrategias de propagación comercial y conservación del germoplasma de abacá en Costa Rica.

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Abaca (*Musa textilis* Nee) is an herbaceous perennial plant native to the Philippines and belonging to the Musaceae family (Araya-Gutiérrez et al. 2023; Araya-Salas et al. 2022; Lalusin and Villavicencio 2015). Morphologically, it resembles the banana plant, with slender pseudostems reaching heights between 1.58 and 1.90 m, and leaves measuring 0.98–1.20 m in length and 25–37 cm in width (Araya-Salas et al. 2022; Hastuti et al. 2019). However, unlike other members of the Musaceae family, the commercial value of abaca lies in its pseudostem, which yields high-quality fiber (Araya-Gutiérrez et al. 2023).

Robust pseudostems with sufficient diameter and height ensure a greater number of leaf sheaths, which are the primary source of fiber. Morphological characteristics that allow efficient separation of fiber bundles (e.g., relatively clean leaf sheaths) are preferable for mechanized processing (Palomares Dueña 2013). These fibers, which constitute approximately 5% of the pseudostem, are characterized by their high strength, flexibility, durability, buoyancy, and thermal stability (Araya-Gutiérrez et al. 2023; Karimah et al. 2021). Abaca fibers used for high-quality pulp production have been reported to contain about 13.2% lignin and low levels of extractives (e.g., waxes and resins). Raw abaca fibers contain approximately 23% non-cellulosic components, ~18% hemicellulose, and ~58% cellulose (Del Río and Gutiérrez 2006).

Historically used in the production of textiles, paper, and fishing nets, abaca fibers are currently employed as reinforcement materials in composites, such as fiberglass and high-density polyethylene (HDPE), enabling their integration into the automotive and aerospace industries (Sinha et al. 2021; Araya-Salas et al. 2022).

Costa Rica ranks as the third-largest abaca producer worldwide, although the cultivated area has decreased from approximately 4,200 to 1,500 hectares in recent years (Araya-Salas et al. 2022). Despite favorable environmental conditions and the availability of agricultural technologies, abaca cultivation in Costa Rica faces two major constraints: (1) a lack of genetic information regarding commercially cultivated varieties, which limits the identification and selection of superior genotypes; and (2) propagation relies heavily on vegetative material (buds and corms), while restrictions on the movement of Musaceae germplasm due to the risk of pathogens, such as *Fusarium oxysporum* f. sp. *cubense,* hinder their safe distribution (Araya-Gutiérrez et al. 2023).

In addition, sexual reproduction in abaca is not a viable propagation strategy due to the high degree of cross-compatibility among cultivated varieties and with other Musa species (Lalusin and Villavicencio 2015), resulting in highly heterogeneous progeny (Arias Aguilar et al. 2025). Moreover, abaca seed germination requires a highly specialized and technically demanding process, as reported by Arias Aguilar et al. (2025). Therefore, clonal propagation through *in vitro* culture remains the most reliable alternative for maintaining genetic uniformity and producing disease-free planting material (Arias Aguilar et al. 2025).

Despite abaca's growing economic relevance, no standardized protocols exist in Costa Rica for the *in vitro* establishment or large-scale micropropagation of *M. textilis*. Moreover, there are no reports on the genetic profiling of local abaca varieties. These gaps restrict both the production of clean planting material and the implementation of genotype-based selection strategies.

To address these challenges, this study aims to develop efficient protocols for the *in vitro* establishment and large-scale micropropagation of commercially cultivated abaca varieties in Costa Rica, alongside their genetic characterization using molecular markers, in order to evaluate their genetic diversity. The development of optimized micropropagation protocols and genetic profiling will enable the production of high-quality plant material and the differentiation of genotypes, respectively, thereby facilitating the selection of superior varieties for commercial cultivation.

MATERIALS AND METHODS

In vitro establishment of *Musa textilis* Nee

For the *in vitro* establishment of *Musa textilis* Nee, corms from the varieties Bungalanon, Tangongon, Maguindanao, and Bribri were utilized as the initial plant material. The outer leaf sheaths were removed to expose the apical region, and segments of approximately 3–5 cm containing the shoot apices were excised. These explanatory experiments were

first immersed in a commercial liquid detergent solution and subjected to agitation at 150 rpm for 10 minutes. Subsequently, the detergent solution was discarded, and the explants were treated with an agrochemical mixture composed of Agri-mycin 16.5 WP and Afungil 50 WP (5 g L^{-1} each). The explants were maintained in this solution under constant agitation (150 rpm) for 15 minutes. Following treatment, the agrochemical solution was removed, and the explants were rinsed twice with sterile distilled water.

The explants were subsequently transferred to a 5.25% (a.i.) sodium hypochlorite (NaOCl) solution and taken into a laminar flow cabinet, where they were disinfected under constant agitation (150 rpm) for 20 minutes. Following this step, the disinfectant solution was discarded, and the explants were rinsed three times with sterile distilled water. The segments were then trimmed to lengths of 2–3 cm and incubated in a 0.525% (a.i.) NaOCl solution supplemented with 0.5% (v/v) Tween® 20 to enhance surface wetting. After disinfection, the solution was discarded, and the explants were again rinsed three times with sterile distilled water. The explants were further reduced in size by carefully removing the outer leaf sheaths, exposing shoot apices measuring approximately 0.5–0.6 cm from base to distal tip. Finally, the apices were immersed in an antioxidant solution containing ascorbic acid (100 mg L^{-1}) for 10 minutes to minimize oxidative stress.

Following the 10-minute incubation in the antioxidant solution, the explants were longitudinally bisected to obtain symmetrical vertical sections of the shoot apices. These segments were then cultured in the initiation medium, which consisted of Murashige and Skoog (MS) salts and vitamins (Murashige and Skoog 1962), supplemented with 3% (w/v) sucrose, 2 mg L^{-1} 6-benzylaminopurine (BAP), 1 mg L^{-1} indole-3-butyric acid (IBA), and 3.3 g L^{-1} Gellan Gum®. Prior to autoclaving, the pH of the medium was adjusted to 5.7 using 1 N NaOH. All reagents were obtained from PhytoTech Labs® (Lenexa, KS, USA). Cultures were maintained at 25 °C in complete darkness for the first 7 days. Subsequently, explants were transferred to a photoperiod of 16 hours light / 8 hours dark, under an irradiance of 72 µmol m^{-2} s^{-1} and incubated for 3 to 4 weeks, depending on their morphogenic response.

Micropropagation and acclimatization of *Musa textilis* Nee *vitro* plants

Explants exhibiting sprouting after approximately four weeks of culture were sectioned at their base to stimulate the formation of multiple shoots. Each explant was bisected through the meristematic region prior to subculturing. The new culture medium consisted of Murashige and Skoog (MS) salts and vitamins (Murashige and Skoog 1962), supplemented with 3% (w/v) sucrose, 5 mg L^{-1} 6-benzylaminopurine (BAP), 2 mg L^{-1} α-naphthaleneacetic acid (NAA), and 3.3 g L^{-1} Gellan Gum®. Prior to autoclaving, the pH was adjusted to 5.7 using 1 N NaOH. All reagents were obtained from PhytoTech Labs® (Lenexa, KS, USA). Cultures were maintained at 25 °C under a 16-hour photoperiod with an irradiance of 72 µmol m^{-2} s^{-1} for 3 to 4 weeks, until the next subculture. During subsequent subcultures, newly formed shoots were separated and transferred to fresh medium of the same composition and maintained under the previously described environmental conditions.

In vitro-derived plantlets (vitroplants) cultured for 12 weeks and reaching heights of 10–15 cm were then transferred to greenhouse conditions for a two-week acclimatization period. Prior to transplantation, all residual culture medium was carefully removed from the root systems. The plantlets were then transplanted into plastic trays containing a sterile substrate composed of a 1:1 ratio of soil and peat moss. Irrigation was performed every 3 days. Additionally, during the first four weeks post-transplantation, plants received weekly applications of Fertiplant NPK® fertilizer (Seracsa, Tres Ríos, Costa Rica).

Characterization of abaca varieties through SSR markers

Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega Corporation©, Fitchburg, WI, USA), following the manufacturer's protocol with specific modifications to accommodate the high fiber content of *Musa textilis* leaves. Leaf tissue from greenhouse-grown plants of each variety was stored in a desiccator to maintain a dry and contaminant-free environment. For each variety, genomic DNA was extracted from three biological replicates, with each replicate corresponding to an independent greenhouse-

grown plant. The extraction protocol was modified as follows: (1) 30 mg of dried leaf tissue were used per sample; (2) all steps, including centrifugations, were carried out under cold conditions (4 °C), using chilled isopropanol and ethanol; (3) in step 6 of the protocol, 250 µL of protein precipitation solution were added, followed by centrifugation at 8,000 rpm for 5 minutes; and (4) in steps 9 and 10, centrifugation was performed at 5,000 rpm for 3 minutes.

The protocol for Simple Sequence Repeat (SSR) analysis via PCR was based on that proposed by Yllano et al. (2020). Table 1 details the SSR markers used. Thirty-microliter reactions contained 17 µL of 2× Master Mix, 2 µL of each primer (forward/reverse), 6 µL of nuclease-free water, and 3 µL of genomic DNA. The thermocycling program was: 95 °C for 3 min; 35 cycles of 95 °C for 30 s, Ta for 30 s, and 72 °C for 30 s; and the final extension at 72 °C for 5 min. Annealing temperatures actually used: R07=62 °C; R39=56 °C; R40=58 °C; R45=56 °C; R150=53 °C; R231=58 °C; and R264=57 °C. T_m values were obtained by a temperature-gradient PCR; gradient ranges and summary are shown in Table 2.

Table 1. Primers used for the amplification of Single Sequence Repeat (SSR) markers to identify abaca (*M. textilis* Nee) varieties.

SSR	Maximum Size (base pairs)	Forward Primer	Reverse Primer
R07	165	AACAACTAGGATGGTAATGTGTGGAA	GATCTGAGGATGGTTCTGTTGGAGTG
R39	400	AACACCGTACAGGGAGTCAC	GATACATAAGGCATCACATTG
R40	540	GGCAGCAACAACATACTACGAC	CATCTTCACCCCCATTCTTTTA
R45	275	TGCTGCCTTCATCGCTACTA	ACCGCACCTCCACCTCCTG
R150	270	ATGCTGTCATTGCCTTGT	GAATGCTGATACCTCTTTGG
R231	286	GCAAATAGTCAAGGGAATCA	ACCCAGGTCTATCAGGTCA
R264	274	AGGAGTGGGAGCCTATTT	CTCCTCGGTCAGTCCTC

The temperature gradient was used to determine the ideal annealing temperature (T_m) for each molecular marker, between the temperatures recommended by the manufacturer and those proposed by Yllano et al. (2020) (Table 2). The tested annealing gradients were as follows: 53–62 °C for R07, 52–57 °C for R39, 54–60 °C for R40, 52–57 °C for R45, 51–57 °C for R150, 55–60 °C for R231, and 55–62 °C for R264. These temperature intervals were selected to evaluate amplification efficiency and specificity under variable stringency conditions.

To obtain higher quality and resolution in the gel (agarose 1.5% w/v), both the loading buffer and the molecular weight marker were prepared in advance. The first was prepared by adding 1 µL of Gel Red (Gold Biotechnology, St. Louis, MO, USA) and 100 µL of 6X loading buffer; for this, Gel Red was not added during gel preparation, to ensure better band resolution. Meanwhile, the molecular weight marker was prepared by adding 2 µL of nuclease-free water, 1 µL of 6X loading buffer, and 1 µL of molecular weight marker solution. For the analysis of PCR samples, the QIAxcel

Table 2. Annealing temperatures (T_m) recommended for the amplification of different Simple Sequence Repeat (SSR) markers in abaca (*M. textilis* Nee).

SSR	R07	R39	R40	R45	R150	R231	R264
T_m recommended by the manufacturer (°C)	60	55	55	54	57	60	62
T_m*(°C)	53	52	54	57	54	55	53

* Yllano et al. (2020).

Advance system and QIAxcel ScreenGel v1.6 software were used. This analysis was carried out using the AL420 method, a 15-1,500 bp molecular weight marker, and a 15-3,000 bp annealing marker.

Genetic diversity analysis

Alleles were called from QIAxcel electropherograms (AL420) as discrete fragment sizes per accession and locus after binning to integer base pairs with a global tolerance of ±1–2 bp to account for sizing jitter, and filtering stutter/low-intensity peaks (retaining peaks with S/N $\geq$ 3 and height $\geq$ 10% of the highest peak in that trace, discarding ladder-like stutter within ±1–4 bp of a main allele if height <15% of the main peak). Assuming diploidy, genotypes were limited to $\leq$ 2 alleles per accession.

For each SSR, the following parameters were computed: Na (number of distinct alleles observed), Ho (fraction of accessions that are heterozygous at the locus), and He (Nei's gene diversity, $He=1-\sum i\, pi^2$, with pi representing the allele frequency based on counts across 2N gene copies). Given the small sample size, PIC was approximated as PIC $\approx$ He (a standard practice for multiallelic codominant SSRs under limited N). The exact PIC value can be recalculated when a larger panel becomes available.

RESULTS AND DISCUSSION

***In vitro* establishment and micropropagation**

Micropropagation of species within the Musaceae family represents a fundamental strategy for the large-scale production of pest- and disease-free planting material (Shah et al. 2020). Moreover, this biotechnological approach enables the rapid multiplication of genetically uniform and stable plantlets, which is essential for commercial propagation systems (Abdalla et al. 2022; Cardoso et al. 2018). However, the establishment and maintenance of *in vitro* culture systems can be cost-prohibitive due to the need for specialized infrastructure, consumables, and skilled labor (Abdalla et al. 2022). Therefore, the development and implementation of optimized and cost-effective micropropagation protocols is crucial to increase efficiency and reduce production costs, as emphasized by Abdalla et al. (2022).

In this context, the results obtained in the present study demonstrate that the micropropagation protocol originally developed for banana can be successfully applied to abaca, achieving a high multiplication rate and a significant increase in the number of shoots per explant. This finding highlights the adaptability and efficiency of the protocol across related species, offering an alternative that is both technically feasible and cost-effective for the large-scale propagation of abaca. The ability to obtain a greater amount of plant material under similar culture conditions suggests that this approach can contribute to reducing production time and optimizing resource use in commercial and research settings for abaca.

According to Abdelnour-Esquivel et al. (2020), prior to *in vitro* establishment, superficial disinfection of the explant material is essential due to the ubiquitous presence of microbial contaminants. The application of fungicidal, bactericidal, and antibiotic agents has been shown to significantly reduce both fungal and bacterial contamination in donor tissues, as reported by Ray and Ali (2017) and Orlikowska et al. (2017). Among the various disinfectants available, sodium hypochlorite (NaOCl) is one of the most commonly used biocides, owing to its broad-spectrum antimicrobial activity, ease of availability, and low cost (Abdelnour-Esquivel et al. 2020; Orlikowska et al. 2017).

The disinfection, establishment, and *in vitro* multiplication protocols implemented for *Musa textilis* explants resulted in successful shoot induction and plant development (Figure 1). Figures 1A, 1B, and 1C depict the preparation of apical explants from processed corms, including size reduction and transverse sectioning prior to culture initiation. Figure 1D shows an apical segment one week after culture initiation, exhibiting signs of greening and metabolic activation. Subsequent stages of shoot induction and plantlet formation are illustrated in Figures 1E through 1H. The described protocols proved effective, producing between 5 and 10 shoots per explant within 3–4 weeks. Under the established conditions of temperature, light, and culture medium composition, complete *in vitro* plantlet production was achieved within 8 to 12 weeks.

Rooting occurred successfully without the need for supplementary additives in the multiplication medium. Furthermore, from the twelfth week onward, the *in vitro* plantlets exhibited morphological and physiological characteristics appropriate for acclimatization and subsequent cultivation under greenhouse conditions (Figure 1I).

In this context, Shah et al. (2020) and Sugiyono et al. (2021) report that benzylaminopurine (BAP) is the most suitable cytokinin for *in vitro* culture due to its high

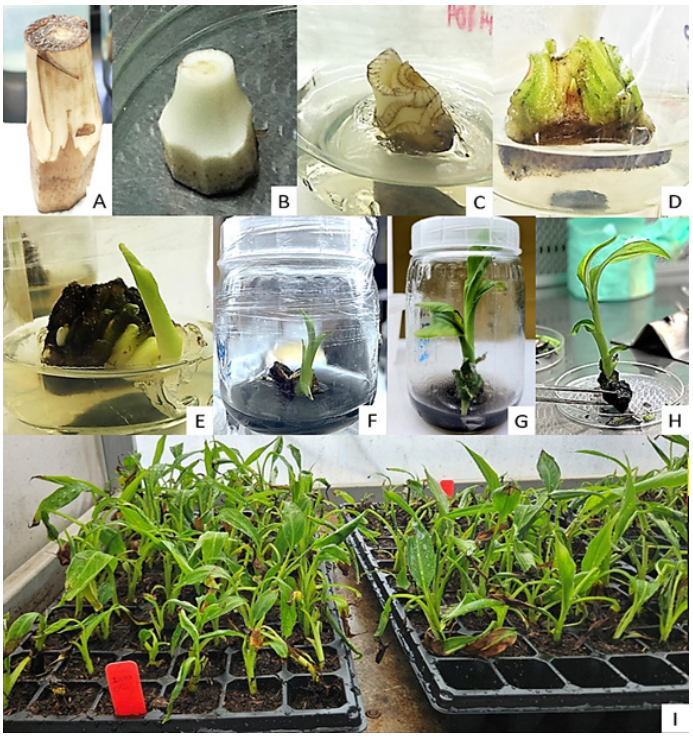

Figure 1. Stages of the micropropagation process of abaca (*Musa textilis* Nee). **A.** Explant prior to disinfection. **B.** Size-reduced explant prepared for culture initiation. **C.** Explant recently inoculated onto semisolid culture medium. **D.** Explant 1-week post-inoculation, showing signs of greening and metabolic activation. **E** and **F**. Explants at 4 weeks post-inoculation, exhibiting the emergence of initial shoots. **G** and **H.** Plantlets after eight weeks of culture in multiplication medium, and **I.** Acclimatized plants grown under greenhouse conditions.

chemical stability. Furthermore, BAP plays a critical role in shoot induction and proliferation (Priyanka 2020). Consistent with the present study, previous research has demonstrated that BAP concentrations ranging from 4.0 to 6.0 mg L^{-1} in the culture medium effectively promote shoot emergence in Musaceae species (Khatun et al. 2017; Ngomuo et al. 2013; Priyanka 2020; Uzaribara et al. 2015). Nevertheless, the balanced and combined application of cytokinins and auxins enhances shoot development, delays tissue senescence, and facilitates root formation (Priyanka 2020; Sugiyono et al. 2021). Kalimuthu et al. (2007) and Priyanka (2020) further emphasize that the concomitant use of BAP and α-naphthaleneacetic acid (NAA) in *in vitro* cultures of Musaceae, as implemented in this study, results in improved shoot proliferation and rooting.

Characterization of abaca varieties through SSR analysis

Comparing annealing temperatures is essential for determining the optimal conditions to amplify each molecular marker (Dewangan et al. 2020). Furthermore, it is essential, as small variations can significantly influence the efficiency of amplification and the specificity of PCR (Ishii and Fukui 2001). To determine the optimal annealing temperatures for the amplification of SSR markers in *Musa textilis*, a gradient PCR protocol was implemented, applying temperature ranges adjusted to the physicochemical characteristics and melting temperatures of each primer pair.

In most cases present in this study, the ideal temperatures obtained during the PCR gradient repetitions were close to those recommended by the manufacturer. However, some discrepancies were identified, such as in the case of the R39 marker, where the ideal temperature is slightly lower than that recommended by the manufacturer. These discrepancies could be due to specific factors related to the abaca samples or to variations in the laboratory conditions (Porta and Enners 2012).

Following electrophoretic analysis, the optimal annealing temperatures were determined as 62 °C for R07, 56 °C for R39, 58 °C for R40, 56 °C for R45, 53 °C for R150, 58 °C for R231, and 57 °C for R264. These annealing values were subsequently employed in all downstream amplifications to ensure consistent PCR performance, high resolution of allelic variants, and robust marker reproducibility across the different abaca varieties analyzed.

In the analysis of the R40 marker, a significant variation in the length of the obtained amplicons was observed, compared to the expected 540 bp (Figure 2A). For the Tangongon variety, a 207 bp fragment was obtained; meanwhile, for the Maguindanao variety, two amplicons of 214 and 231 bp were detected, indicating variability in the length of the PCR products. For the Bungalanon material, a 215 bp fragment was obtained; meanwhile, the Bribri material showed three amplicons with lengths of 178, 212, and 228 bp.

For the R07 marker (165 bp), divergent results were observed in the analyzed abaca samples (Figure 2B). The Tangongon material presented two amplicons of 133 and 177 bp. In the Maguindanao variety, 120 and 133 bp fragments were detected. For the Bungalanon material, 133 and 176-bp fragments were observed. Lastly, the Bribri material showed two amplicons of 106 and 135 bp.

Regarding the R231 marker, a 286 bp fragment was expected; however, the results revealed shorter amplicons (Figure 2C). The Tangongon material showed an amplification of 223 bp, while the Maguindanao material had an amplification of 224 bp. The Bungalanon and Bribri varieties each presented an amplicon of 226 bp.

In the case of the R45 marker, a 275 bp amplicon was expected, but slightly larger fragments were observed (Figure 2D). The Tangongon material presented an amplicon of 277 bp, and in the Maguindanao variety, a fragment of 276 bp was detected. In the Bungalanon material, a 280 bp fragment was detected, while in the Bribri material, a 278-bp amplicon was obtained.

For the R39 marker, fragments of nearly 400 bp were expected (Figure 2E). The Tangongon material presented two amplicons with lengths of 324 and 355 bp. In the Maguindanao variety, a 327 bp fragment was detected, while the Bungalanon material showed two amplicons of 324 and 356 bp. Two amplicons were obtained from the Bribri material, with lengths of 318 and 352 bp.

For the R264 marker, a 274 bp amplicon was expected, but shorter fragments were detected in all abaca samples (Figure 2F). The Tangongon material presented three different amplicons (180, 270, and 255 bp), while the Maguindanao variety showed four different fragments with lengths of 186, 210, 230, and 248 bp. In the Bungalanon and Bribri varieties, three amplicons (189, 210, and 228 bp) and two amplicons (211 and 230 bp) were detected, respectively.

Finally, in the case of the R150 marker, a 270 bp fragment was expected, but longer fragments were detected in all abaca samples (Figure 2G). The Tangongon and Maguindanao materials presented a single fragment of 355 bp. In the Bungalanon and Bribri varieties, a single amplicon was also detected, with lengths of 354 and 356 bp, respectively.

The results of this study reveal notable differences in the length of the amplicons obtained for each variety using the same molecular markers. This variability suggests the presence of significant genetic differences among the analyzed materials and supports the hypothesis that it is possible to differentiate abaca varieties through molecular marker analysis. In addition, the detection of multiple amplicons in some varieties is significant, as it suggests greater genetic complexity in these genotypes (Chen et al. 2020). For example, the R40 marker revealed multiple amplifications in the Bribri variety, with lengths of 178, 212, and 228 bp. These differences in the length and number of amplicons within the same variety indicate significant genetic variability. Beyond qualitative size shifts, the SSR panel exhibited clear multi-allelic polymorphism across accessions (Table 3). Marker informativeness was highest for R40, R39, and R264 ($He \approx PIC \geq 0.80$), while R231 and R150 showed moderate informativeness ($PIC \approx 0.63$). Observed heterozygosity (Ho) peaked at R40 and R264 (Ho=1.00), and was null for R231, R45, and R150, consistent with their predominantly single-band profiles. These indices

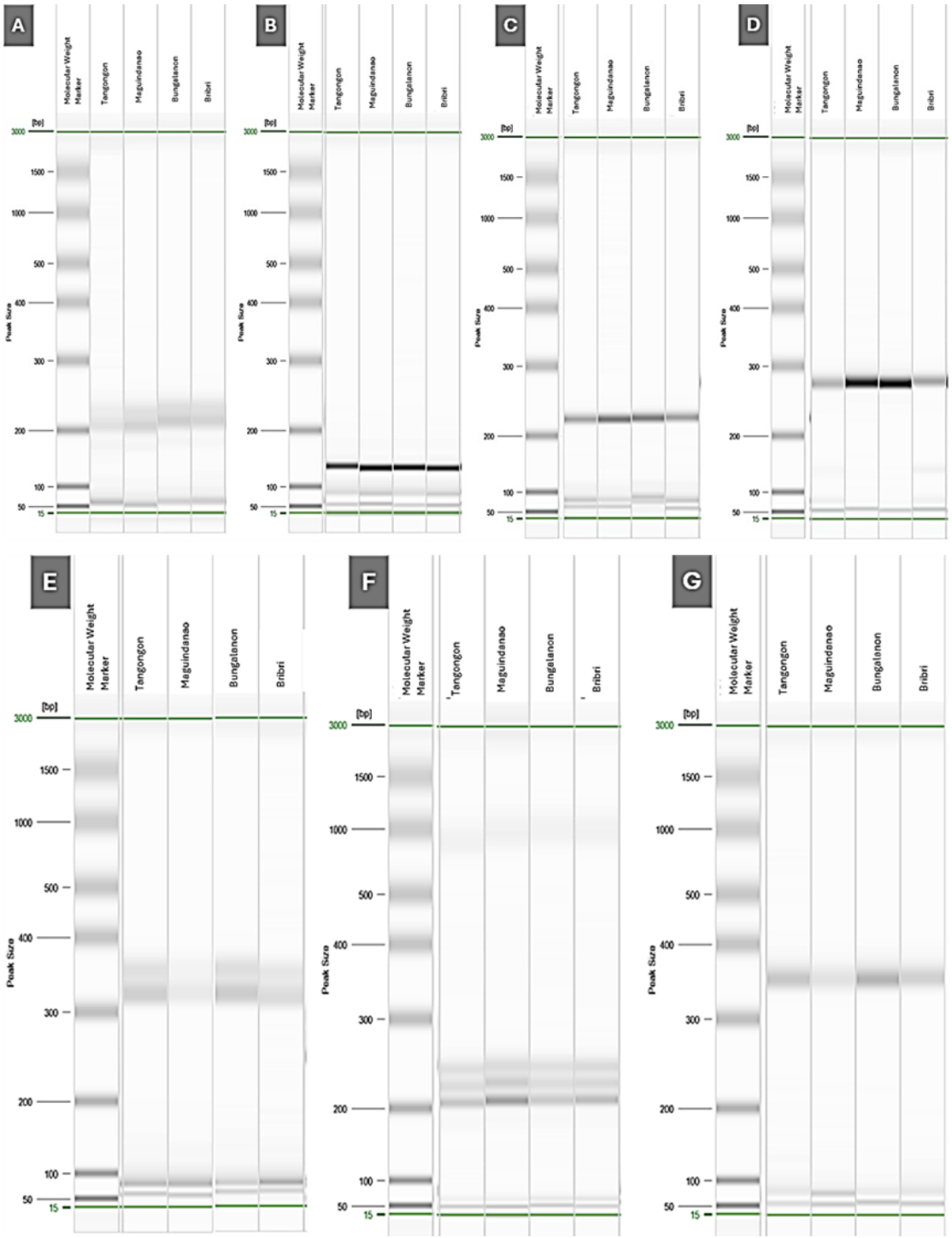

Figure 2. Results of capillary electrophoresis coupled to fluorescence for SSR markers: **A.** R40, **B**. R07, **C.** R231, **D**. R45, **E.** R39, **F.** R264, and **G.** R150.

quantitatively support the genetic differentiation already apparent from the amplicon-size patterns.

Furthermore, the greater the differences in these amplicons, the stronger the evidence of genetic differentiation among varieties (Patil et al. 2021). However, it is important to acknowledge the limitations of using SSR markers alone for genetic differentiation, as they may not capture the full extent of genomic diversity (Adamek et al. 2023). Additional analyses, such as whole-genome sequencing, could provide a more comprehensive understanding of genetic variation among abaca varieties (Jagadeesan et al. 2019). Within the analyzed panel, R40 emerged as one of the most informative loci (Table 3) and represents a valuable tool for differentiating abaca varieties, given its ability to detect significant genetic differences (Marimuthu Somasundaram et al. 2019). In contrast, markers that generate more homogeneous profiles are less useful for varietal discrimination because they offer limited resolution and produce simpler amplicon patterns (Gaikwad et al. 2023).

Although the SSR panel differentiated the accessions evaluated in this study, the inference space is constrained by the limited varietal breadth (four varieties). With such a small number of varieties, resolution among closely related genotypes and the stability of allele-frequency metrics (e.g., PIC/He in Table 3) remain limited; therefore, these values should be interpreted as panel-level indicators rather than population parameters. Replicability was ensured by analyzing three biological replicates per variety (independent greenhouse-grown plants) and by repeating PCR and capillary electrophoresis. Under the adopted binning and stutter-filter criteria, allele bins were consistent across replicates, supporting the technical repeatability of genotype calls. Regarding applicability to *in vitro* explants, the same SSR primers (including R40) can be used to assess genetic diversity and clonal fidelity during micropropagation. Nevertheless, potential sources of error—such as somaclonal variation, occasional chimerism, and allele dropout related to DNA quality or quantity—should be anticipated and minimized through biological replication and standardized allele-calling thresholds.

Table 3. Marker-level diversity indices estimated from QIAxcel runs.

Marker	Na	Ho	He	PIC*
R40	8	1.00	0.875	0.875
R07	5	0.75	0.688	0.688
R231	3	0.00	0.625	0.625
R45	4	0.00	0.750	0.750
R39	6	0.75	0.812	0.812
R264	6	1.00	0.812	0.812
R150	3	0.00	0.625	0.625

CONCLUSION

The methodologies implemented for the disinfection and micropropagation of abaca (*Musa textilis* Nee) enabled the successful development of sprouts and *in vitro* plantlets. The selected disinfectant agents proved effective, although the highest microbial load was initially observed in the corms. The culture protocols applied were efficient, resulting in multiplication rates of 5–10 sprouts per explant within 3–4 weeks. The materials and procedures used minimize dependence on field-derived propagules and reduce the risk of spreading pathogens, such as *Fusarium oxysporum* f. sp. *cubense*.

Furthermore, the presence of differences in PCR product length and the detection of multiple amplicons with the same molecular markers among abaca accessions support their differentiation through molecular analysis. Molecular markers that reveal significant variation in amplicon size and genetic complexity hold potential for future applications in the identification and classification of *M. textilis* varieties, thus contributing to conservation and genetic resource management.

The SSR (Simple Sequence Repeat) analysis conducted on the abaca (*Musa textilis*) varieties revealed the presence

of genetic variability even within a single variety. This was evidenced by the detection of multiple amplicons of different lengths for the same SSR markers, particularly in the Bribri variety, which exhibited three distinct fragments for the R40 marker (178, 212, and 228 bp). Such intravarietal polymorphism indicates a considerable degree of genetic heterogeneity among plants classified under the same varietal name. However, while this internal variability suggests that there is some genetic basis for selection and improvement within a given variety, the results also indicate that the diversity detected by SSR markers alone may be insufficient to support a comprehensive genetic improvement program. SSR markers provide valuable insights into allelic variation, but they do not capture the full genomic complexity of *Musa textilis*. Therefore, to broaden the genetic base and enhance the potential for crop improvement, the inclusion of new genetic material—possibly from other abaca varieties or related *Musa* species—would be advisable. Complementary genomic tools, such as whole-genome sequencing or SNP-based genotyping, could further refine the understanding of the genetic relationships and variability necessary for an effective breeding program. These protocols represent a promising tool for the development of certified plant material and for the design of *in situ* and *ex situ* germplasm preservation strategies, as well as for commercial-scale propagation efforts.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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