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MOLECULAR CHARACTERIZATION OF THE SUPERIOR BANANA 'TORPEDO' MUTANTS GENERATED VIA SOMACLONAL VARIATION USING RAPD AND SCoT MARKERS

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SUMMARY

Tissue culture-somaclonal variation can generate desirable phenotypes in clonally propagated crops. In our previous study, inducing two horn banana (*Musa spp.*) mutants (Torpedo 1/TM1 and Torpedo 2/TM2) used benzyl aminopurine (BAP) that produced substantially enhanced fruit weight compared with their parental wild-type cultivars (Agung Semeru/PAS and Local Bogor/PLB). In this study, the employment of RAPD and SCoT markers with the sequencing of polymorphic amplicons sought to determine the genetic distinctiveness of these two mutants. RAPD analysis produced 68 loci up to 83% polymorphism, while SCoT markers generated 39 alleles with lower polymorphism (8%–14%). Sequencing of the 750 bp OPC-07 RAPD and SCoT-2 revealed SNPs and indels with extensive nucleotide variations. Although OPC-07 had a nearly identical band size, a mutation hotspot at positions 116–123 uniquely distinguished TM2 from others. Similarly, sequencing of SCoT-2 amplicons revealed numerous A–T, C–G, and A–G substitutions, several triallelic polymorphic sites, and small indels. The TM2 mutants were notable with point mutations, which revealed somaclonal variations, differentiating mutants from their wild types. The results disclosed RAPD and SCoT markers effectively distinguish the improved banana genotypes. Further characterization of these genetic differences will help in planning more precise and targeted banana breeding programs.

Keywords: Banana (*Musa spp.*), induced mutation, benzyl aminopurine (BAP), genetic characterization, plant tissue culture, mutants, Torpedo bananas

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Key findings: RAPD and SCoT markers distinguished the superior banana (*Musa spp.*) mutants Torpedo 1 and 2 from their wild type. Beyond detecting polymorphic bands, sequencing of selected amplicons revealed nucleotide variations in genes associated with organ formation and growth. Cluster analysis supported these findings by clearly separating the mutants Torpedo 1 and 2 from the wild type.

INTRODUCTION

Banana (*Musa spp.*) is a vital tropical fruit crop that holds considerable economic and social values in various developing countries, including Indonesia, which recorded a total production of 9.3 million tons in 2023 (FAO, 2023). Banana is a rich source of carbohydrates and numerous beneficial bioactive compounds, such as flavonoids, tannins, and vitamins (Kumari *et al.*, 2023), which eventually leads to a steady increase in global demand for bananas. The traditional method of propagating bananas using suckers was a widely practiced technique. However, this approach emerged to be inadequate due to the slow rate of plant regeneration and elevated risks of disease infection (Singh *et al.*, 2011). Tissue culture provides a more effective alternative, which utilizes disease-free planting materials and enables rapid multiplication. Exposure of genetic materials to chemical mutagens in the culture medium may increase mutation rates and elevate somaclonal variations (Bairu *et al.*, 2011). This consequently leads to genetic modifications that could contribute to improved crop traits (Krishna *et al.,* 2016).

A previous study reported that benzyl aminopurine (BAP) use in tissue culture successfully induced somaclonal variations, with enhanced traits in horn banana (*M. paradisiaca* L.) (Sianipar *et al.*, 2024). Banana mutant cultivars Torpedo 1 (TM1) and Torpedo 2 (TM2) produced three times heavier fruits than their wild-type counterparts. These newly developed banana cultivars have acquired a Plant Variety Protection (PVP) from the Indonesian Ministry of Agriculture under 00795/PPVT/S/2025 (TM1) and 00796/PPVT/S/2025 (TM2), emphasizing their distinct phenotypic characteristics. However, the underlying genetic variations at the molecular level in banana mutants TM1 and TM2 remain understudied.

Molecular markers have occurred as robust tools used to identify mutant plants resulting from somaclonal variations. For instance, random amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR)-based method using random primers to accurately identify the polymorphism among individuals without prior sequence information (Orbović *et al.*, 2008). Despite limitations in reproducibility and sensitivity to PCR conditions, RAPD has proven effective for exploring the genetic variability in plants derived from tissue culture (Abhang *et al.*, 2025). The start codon-targeted (SCoT) marker emerged to be a dominant marker that targets a short, conserved region flanking the ATG translation start codon in the available genes (Collard and Mackill, 2009). Both markers were relatively low-cost; however, the SCoT markers offer better specificity by targeting gene regions around the start codon instead of targeting random sequences. The conduct of the SCoT system has been successful in various in vitro propagated plant species to assess their genetic fidelity, resulting in high polymorphism and reproducibility (Rathore *et al.*, 2014; Mamgain *et al.*, 2022).

Based on the above discussion, the following study aimed to identify somaclonal variations in the mutant genotypes TM1 and TM2 compared with their wild-type counterparts using RAPD and SCoT markers, followed by sequencing. Employing Sanger sequencing further emphasized the genetic variations in banana mutants Torpedo 1 and Torpedo 2. Such types of investigations based on the genetic variations could uncover key genes responsible for desirable traits, which can enable harnessing for crop improvement in future breeding programs.

MATERIALS AND METHODS

Plant materials

This study used four banana cultivars comprising two tissue culture-derived mutants TM1 and TM2, obtained through somaclonal variation, and two wild-type cultivars Agung Semeru (PAS) and Local Bogor (PLB). Three plants from each cultivar entailed collection and cultivation in the banana-growing area at Bina Nusantara University, Western Jakarta, Indonesia (6.2197° S, 106.9998° E). Young, healthy leaves showing a light-green color and free from any visible disease symptoms or damage succeeded in their selection for DNA extraction. Leaves from one representative plant of each genotype underwent harvesting in April 2025 before storage at −20 °C until performing the DNA extraction.

Genomic DNA extraction

Extraction of genomic DNA used a modified CTAB protocol. Approximately 0.3 g of young and fresh leaf tissue from each banana genotype, ground into a fine powder in a sterile mortar, included 500 µL of extraction buffer (100 mM Tris-HCl [pH 8.0], 1.4 M NaCl, 20 mM EDTA [pH 8.0], and 2% [w/v] CTAB-cetyltrimethylammonium bromide). The homogenate, upon transferring into a 2 mL microtube, received an additional buffer to bring the total volume to 800 µL. Samples sustained incubation at 65 °C for 45 min, with gentle mixing every 15 min. Subsequently, adding 800 µL of chloroform: isoamyl alcohol (24:1) ensued, with the samples centrifuged at 13,000 rpm for 10 min at 20 °C. The supernatant's transfer to a new 1.5 mL microtube entailed mixing with 1/10 volume of 3 M sodium acetate (pH 5.2) and an equal volume of cold isopropanol.

The mixture, gently inverted, received the following process: incubation at -20 °C for 1 h, then centrifugation at 13000 rpm for 10 min. With the supernatant, the DNA pellet entailed washing with 500 µL cold 70% ethanol before centrifugation at 13000 rpm for 5 min. After removing the supernatant, the pellet underwent air-drying overnight at room temperature. Afterward, the pellet reached dissolving in 100 µL TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) containing RNase A (10 mg/mL) before incubating at 37 °C for 30 min. The DNA assessment qualitatively continued by electrophoresis on a 0.8% agarose gel in 1X TBE buffer and quantitatively used a Nano Drop 2000 spectrophotometer (Thermo Scientific, USA).

RAPD markers' amplification

Amplification proceeded using RAPD primers as listed in Table 1. The PCR succeeded in a 20 µL reaction containing 10 µL of 2x MyTaq™ HS Red Mix (Bioline, UK), 2 µL of genomic DNA (25 ng template), 0.8 µL of primer at a concentration of 10 µM, and nuclease-free water (Invitrogen, USA) to volume. Reactions performed employed a Bio-Rad T100 Thermal Cycler (Bio-Rad, USA), with an initial denaturation at 94 °C for 4 min, followed by 45 cycles of denaturation at 94 °C for 30 s,

Table 1. Random amplified polymorphic DNA (RAPD) primers used in the study.

No.	Primers	Sequence (5'-3')	Tm (°C)
1	OPA-07	GTCCCGACGA	32
2	OPA-04	AATCGGGCTG	34
3	OPD-07	TTGGCACGGG	34
4	OPC-05	GATGACCGCC	34
5	OPA-03	AGTCAGCCAC	34
6	OPB-12	CCTTGACGCA	32
7	OPE-01	CCCAAGGTCC	35
8	OPN-11	ACGGCGTATG	36
9	OPD-18	AGGTGACCGT	36
10	OPA-17	GACCGCTTG T	35.7

Table 2. Start codon targeted (SCoT) primers used in the study.

No.	Primers	Sequence (5'-3')	Tm (°C)
1	SCoT-33	CCATGGCTACCACCGCAG	58.9
2	SCoT-20BM	TAACCATGGCTACCMCG	52.8
3	SCoT-20	ACCATGGCTACCACCGCG	60.8
4	SCoT-2	CAACAATGGCTACCACCC	53.6
5	SCoT3-4	ACCATGGCTACCACCGCA	61

annealing at 35 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. The PCR products underwent electrophoresis on a 1.2% agarose gel in 1X TBE buffer (Tris-Borate-EDTA) before staining with Florosafe DNA stain (1st BASE). Using a 1 kb DNA ladder (Geneaid) served as a molecular size marker. Gels' imaging engaged a UV transilluminator (Syngene, USA). The RAPD assay, performed twice, continued to manual profile scoring. High-intensity bands (750 bp) produced by OPC-07 were selections subjected to Sanger sequencing in duplicate using the ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, USA).

SCoT markers' amplification

Five SCoT primers used in this study appear in Table 2 (Collard and Mackill, 2009; Igwe *et al*., 2022). The PCR carried out in a 20 µL reaction contained 10 µL 2× MyTaq™ HS Red Mix (Bioline, UK), 2 µL genomic DNA (25 ng), 0.8 µL 10 µM primer, and nuclease-free water (Invitrogen, USA). The cycling conditions were 95 °C for 5 min; 40 cycles of 94 °C for 30 s, 40 °C for one min, and 72 °C for one min; followed by a final extension at 60 °C for 15 min on a Bio-Rad T100 Thermal Cycler (Bio-Rad, USA). The PCR products' resolution ensued on a 1.5% agarose gel in 1× TBE buffer, stained with Florosafe DNA stain (1st Base), and visualized using a UV transilluminator (Syngene, USA). The SCoT amplification, performed in duplicate, had banding profiles scored manually. The SCoT-2 amplicons (750 bp) selected received sequencing twice by Sanger sequencing using an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, USA).

Data analysis

Consistent bands obtained from RAPD and SCoT marker amplifications received scores and transformation as binary data, with a score of 1 assigned for each observable band and 0 for absence of bands. Scoring data helped generate Jaccard's similarity coefficient. Subsequently, a phylogenetic dendrogram construction by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis used the NTSYS pc 2.0. Additionally, polymorphism information content (PIC) values also gained formulation using Power Marker 3.25 (Liu and Muse, 2005).

Sanger sequences bore manual trimming by inspecting chromatograms, then merging using the multiple sequence alignment (MSA) in Geneious Prime V.2025.1.2 (Biomatters, Ltd., Auckland, New Zealand). Amplicons from the four banana genotypes, viz., PAS, PLB, TM1, and TM2, entailed alignment using the ClustalW method to identify nucleotide variations. Subsequent selection of high-quality consensus regions proceeded for submission to the Banana Genome Hub (https://banana-genome-hub.southgreen.fr/blast) for BLAST analysis (Droc *et al.*, 2013).

RESULTS AND DISCUSSION

Polymorphism profile

RAPD primers totaled 10 that were used for genotyping four banana genotypes comprising two wild types (PAS and PLB) and two mutants (TM1 and TM2), generating 68 amplicons with ranges from 200 to 2000 bp. Two out of 10

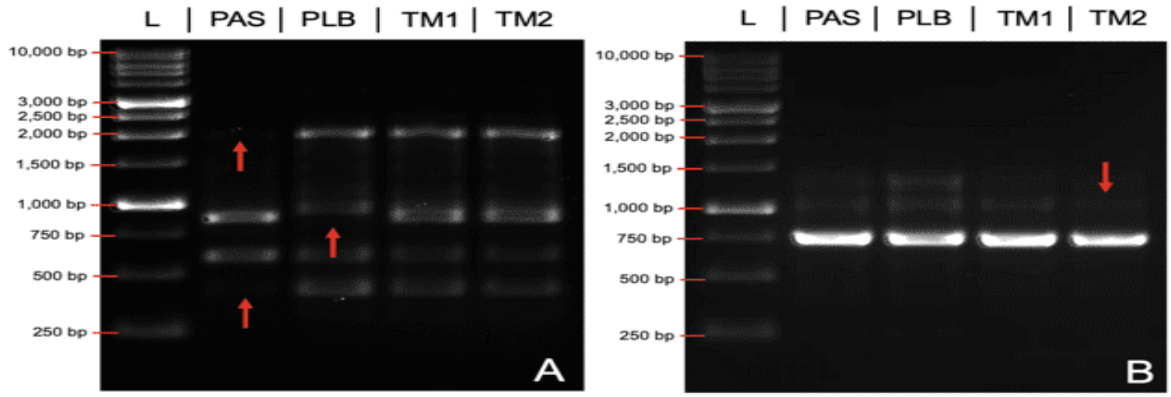

Figure 1. RAPD banding patterns of four banana genotypes generated by the OPN-11 (A) and OPC-07 (B) primers. Red arrows indicate the band showing variation among samples. L: 1 Kb Ladder (Geneaid), PAS: Agung Semeru, PLB: Local Bogor, TM1: Torpedo 1, and TM2: Torpedo 2.

Table 3. Polymorphism data of four banana genotypes amplified using RAPD markers.

Primers	Range of band size (bp)	Total number of bands	Major allele frequency	Gene diversity	PIC*	Percentage of polymorphism (%)
OPC-07	750–1500	5	0.75	0.325	0.2578	80
OPA-04	500–1000	8	0.000	0.000	0.000	0
OPD-07	500–2000	5	0.000	0.000	0.000	0
OPC-05	250–1000	7	0.8214	0.2321	0.1842	5
OPA-03	250–1500	11	0.000	0.000	0.000	0
OPB-12	500–1500	7	0.000	0.000	0.000	0
OPE-01	325–2000	7	0.9643	0.0536	0.0435	14
OPN-11	500–2000	7	0.9643	0.0536	0.0435	14
OPD-18	250–1500	5	0.8000	0.300	0.2438	80
OPA-17	500–2000	6	0.7083	0.3542	0.2773	83
Total		68				
Means		6.8	0.5008	0.1319	0.1050	27.6

*PIC = Polymorphic information content that measures the informativeness of the primers used in this study.

RAPD markers, OPN-11 and OPC-07 primers, displayed polymorphic bands, as shown by red arrows (Figures 1A and B). The primer OPN-11 polymorphisms differentiated the Torpedo bananas (TM1 and TM2) from the wild types (PLB and PAS). The banana genotypes PLB, TM1, and TM2 produced 2000 bp and ~400 bp bands, whereas the wild type PAS produced no bands. A ~875 bp band occurred in three genotypes—PAS, TM1, and TM2—but none of the bands resulted in PLB. OPC-07 produced a 1000-bp amplicon in all genotypes, except TM2 (Figure 1B). The highest polymorphism percentage of RAPD markers was evident in the primer OPA-17, whereas four primers (OPA-04, OPD-07, OPA-03, and OPB-12) showed no polymorphism (Table 3). High polymorphism observed for OPA-17, OPC-07, and OPD-18 supports earlier findings that identified these primers as highly informative for mutant differentiation and genetic diversity within species (Bukhari *et al.,* 2015; Shoagh *et al.,* 2025).

The PIC value measures the capability of a marker to identify genetic variations, ranging from 0 (OPA-04, OPD-07, OPA-03, and OPB-12) to 0.2773 (OPA-17). Generally, the PIC value greater than 0.5 tends to lean toward high polymorphism. From RAPD markers, the obtained PIC values were

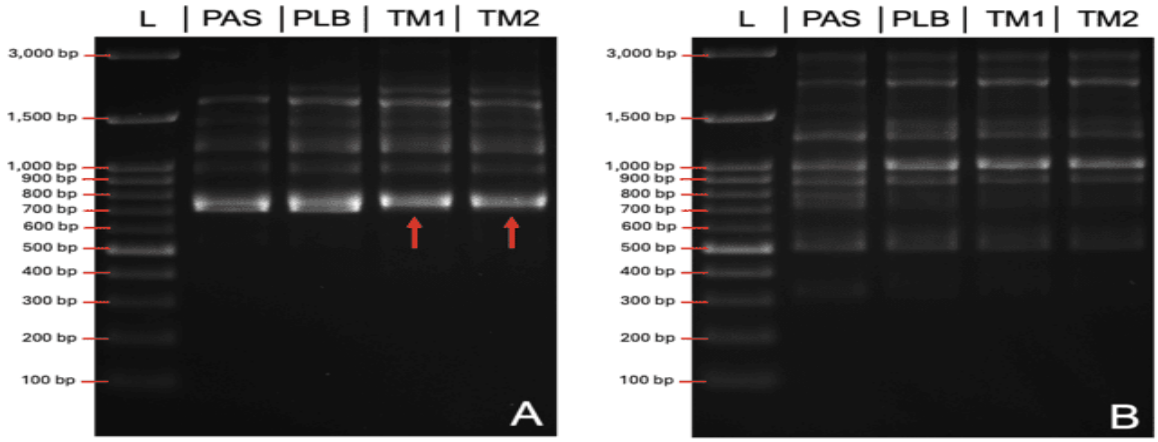

Figure 2. SCoT banding patterns of four banana genotypes generated by SCoT-2 (A) and SCoT-20 (B) primers. Red arrows indicate the band showing variation among samples. L: 100 bp Ladder (Geneaid), PAS: Agung Semeru, PLB: Local Bogor, TM1: Torpedo 1, and TM2: Torpedo 2.

Table 4. Polymorphism data of four banana genotypes amplified using SCoT markers.

Primers	Range of band size (bp)	Total number of bands	Major allele frequency	Gene diversity	PIC*	Percentage of polymorphism (%)
SCoT-33	800–3000	6	0.000	0.000	0.000	0
SCot20-BM	500–3000	7	0.000	0.000	0.000	0
SCoT-2	700–2250	7	0.9643	0.0536	0.0535	14
SCoT-20	800–3000	12	0.9583	0.0417	0.0313	8
SCoT-34	500–3000	7	0.000	0.000	0.000	0
Total		39				
Means		7.8	0.3845	0.0191	0.0170	4.4

*PIC = Polymorphic information content that measures the informativeness of the primers used in this study.

considerably moderately low (0–0.2773). However, low levels of polymorphism, expected as somaclonal variants, typically exhibit limited genetic variations with minor differences from their wild types. Several studies recorded low polymorphism in plants derived from tissue culture, such as between the mother plant and regenerants of *Dendrobium heterocarpum* (Longchar and Deb, 2022) and hybrid-derived plantlets of *Cymbidium giganteum* (Pradhan *et al.,* 2023).

In the DNA amplification of banana genotypes using five SCoT primers, it yielded 39 amplicons ranging from 500 to 3000 bp, and the genotypes revealed minor variations. The SCoT-2 primer produced a noticeably different banding pattern in the banana mutants (TM1 and TM2) compared with the wild types (Figure 2A). The mutants TM1 and TM2 generated slightly larger bands of approximately 800 bp, whereas the wild types PAS and PLB produced bands closer to 750 bp. Conversely, the SCoT-34 primer showed no detectable differences among all studied samples (Figure 2B). Out of five primers, the SCoT-2 and SCoT-20 revealed some degree of polymorphism, with values of 14% and 8%, respectively (Table 4). The SCoT primers exhibited low polymorphism, with SCoT-2 recording the highest PIC value (0.0535), followed by SCoT-20 (0.0313). The DNA base variations, substitutions, and small insertions or deletions generally do not produce detectable polymorphisms unless occurring within a primer binding site (Taylor *et al*., 1995). Although the SCoT markers targeted

regions near the ATG start codon and revealed no genetic variability between genotypes, unless somaclonal variation occurs in those specific regions. However, employing more primers could provide a much more comprehensive assessment about genetic differentiation (Orbović *et al.*, 2008).

Despite having moderate polymorphism rates, both RAPD and SCoT primers proved effective at distinguishing Torpedo bananas from their wild-type counterparts. Aligning with previous studies, the amplification profiles generated by the RAPD and SCoT markers have also successfully identified genetic variations in micropropagated plants (Ray *et al.*, 2006; Gurme *et al.*, 2018). Somaclonal variation can cause shifts in point mutation (Ngezahayo *et al.,* 2007) and somatic gene rearrangements (Krishna *et al.,* 2016). However, such variations may lead to different amplification patterns in banana genotypes PAS, PLB, TM1, and TM2. Further elucidating somaclonal variations in superior torpedo bananas requires employing an additional method of sequencing.

Comparative analysis of amplicon sequences

High-intensity bands sequenced resulting from the primer OPC-07 (750 bp) were successful through Sanger sequencing. The consensus region of 300 bp exhibited eight nucleotide variations, demonstrating a 2.67% sequence variation. However, all identified variations were single-nucleotide polymorphisms (SNPs), mainly A–T substitutions (Table 5). Most nucleotide substitutions came together between positions 116 and 123, where the banana mutant TM2 occurred consistently different from the other three cultivars. Likewise, the MSA of four genotypes from the 750 bp SCoT-2 amplicons showed that the 200 bp region surrounding the ATG start site was largely conserved, with several nucleotide variations among the samples. Comparable findings were reports released by Gajera *et al*. (2016), where unique SCoT fragments indicated sequence homology with ATG regions corresponding to biocontrol-related genes.

Identifying within the consensus region were 55 nucleotide variations, resulting in 27.5% variation among four banana genotypes. However, most variations were biallelic (A/T or C/T), and a few locations displayed triallelic polymorphism (Table 5). Missing bases (–) were also evident. The accumulation of these substitutions revealed an obvious set of point mutations responsible for the molecular differentiation of the mutants TM1 and TM2 from their wild types PAS and PLB. This supported the hypothesis that benzylaminopurine-induced tissue culture triggered somaclonal mutation that contributed to their superior phenotype. Ngezahayo *et al.* (2007) reported RAPD amplicons from rice (*O. sativa* L.) Nipponbare cultivars appeared identical in size, detecting variations in the nucleotide sequences.

Putative homologs of the consensus sequence OPC-07 primers had a successful identification using the Banana Genome Hub. Protein NINJA (Novel Interactor of JAZ) displayed 99.67% identity with an e-value of 1.0×10^{-244}. Additionally, an EAR (ethylene response factor–associated amphiphilic repression)–containing protein showed 91.67% identity with an e-value of 1.0×10^{-68}. The NINJA is a transcriptional repressor negatively regulating jasmonate responses (Pauwels *et al*., 2010). The EAR motif is a small but distinct regulatory sequence conserved in many plant transcriptional regulator (TR) proteins (Kagale and Rozwadowski, 2010). Both proteins appeared involved in stress and growth-related signaling cascades. Meanwhile, the SCoT-2 consensus region expressed homology to a single protein in *Musa acuminata* based on BLAST analysis, which was identified as a SCAR (suppressor of cAMP receptor) protein. The sequence provided 99.49% identity with an e-value of 2×10^{-74}. The SCAR, also known as WAVE (WASP family Verprolin homolog), is a conserved negative regulator of G-protein-coupled signaling. It plays a critical role in regulating the plant actin cytoskeleton (Wu and Li, 2024) and growth-related mechanisms, ultimately shaping the plant organ structure (Mathur *et al*., 2003).

Table 5. Nucleotide variations in the OPC-07 and SCoT-02 amplicon consensus region among the four banana genotypes determined by the multiple sequence alignment (MSA).

Marker	Position	PAS	PLB	TM1	TM2	Variation
OPC-07	112	G	C/G	G	G	SNP (C↔G)
	116	T	T	T	A/T	SNP (A↔T)
	120	T	T	T	A/T	SNP (A↔T)
	122	T	T	T	A/T	SNP (A↔T)
	123	T	T	T	A/T	SNP (A↔T)
	181	T	T	T	G/T	SNP (T↔G)
	385	G	G/T	G	G/T	SNP (T↔G)
	399	A/G	G	G	G	SNP (A↔G)
SCoT-2	10	G	A	A	A	SNP (A↔G)
	21	T	T	A	A	SNP (A↔T)
	42	C	G	C	C	SNP (C↔G)
	49	A	T	T	T	SNP (A↔T)
	51	G	C	G	G	SNP (C↔G)
	71	A	C	-	-	Deletion/ (A↔C)
	72	C	T	A	A	A/T/C
	73	G	A	C	C	G/A/C
	74	A	C	G	G	G/A/C
	76	C	G	A	A	A/T/C
	78	A	C	C	C	SNP (A↔C)
	88	G	G	A	A	SNP (A↔G)
	120	T	T	C	C	SNP (C↔T)
	127	A	A	T	T	SNP (A↔T)
	134	A	G	A	A	SNP (A↔G)
	137	A	A	G	G	SNP (A↔G)

Polymorphisms regulate plant growth regulation and structural development in these regions. Previous findings showed that TM1 and TM2 exhibited fruit weights approximately three times higher than wild-type fruits, averaging 644.90 g more than wild-type fruits, weighing 191.75 g each (Sianipar *et al*., 2024). These results suggest that sequence variations may have contributed to the increased fruit weight of banana mutants TM1 and TM2. For example, significant SNPs were evident between mature and young cucumber fruit lengths within the coding region of a homolog of the SCAR gene (Lin *et al.*, 2024). This study presents sequence variations that support the classification of TM1 and TM2 as putative mutants, still requiring additional targeted molecular validation. The RNA-based transcriptomic analysis would be particularly valuable, as it can reveal the gene expression patterns and protein biosynthesis pathways associated with growth and fruit development (Ban and Jung, 2023). Future work should incorporate gene-specific PCR amplification to verify the variations within candidate genes, as well as transcriptomic approaches to provide deeper molecular confirmation.

Similarity index and dendrogram analysis

The cluster analysis using the UPGMA method based on RAPD polymorphism grouped the four banana genotypes into three clusters at a similarity coefficient of 0.88 (Figure 3). Cluster 1 consisted of the wild types PAS and PLB, showing a genetic similarity of 0.96. Cluster 2 included the mutant TM1, while Cluster 3 contained the mutant TM2, revealing these genotypes were genetically distinct from the others. Similarly, the dendrogram constructed from SCoT polymorphism positioned the four banana genotypes into two distinct clusters at a threshold of 0.95 similarity (Figure 3). Cluster 1 comprised wild types PAS and PLB, while cluster 2 had the mutants (TM1 and TM2). The clustering pattern revealed the

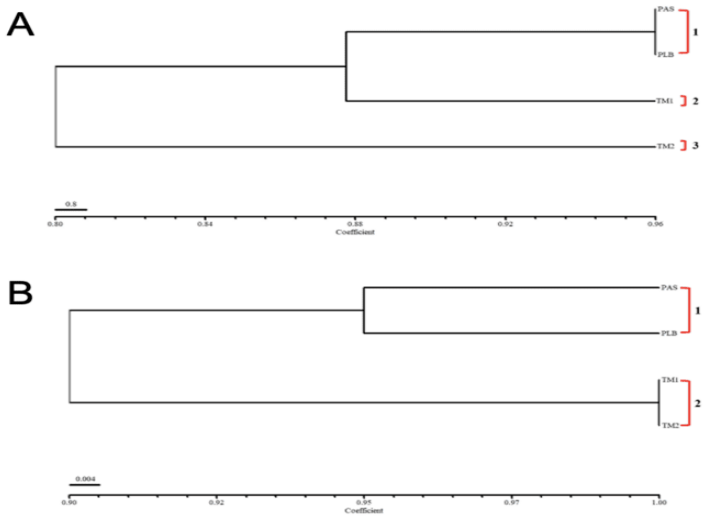

Figure 3. Dendrogram of four banana genotypes based on RAPD (A) and SCoT (B) markers. The red line indicates cluster grouping. PAS, PLB, TM1, and TM2 stand for Agung Semeru, Local Bogor, Torpedo 1, and Torpedo 2, respectively.

banana mutants TM1 and TM2 shared a greater genetic similarity, while the wild types PAS and PLB separately made the related pair. The clustering results suggested that the mutants Torpedo 1 and Torpedo 2 were genetically divergent from their wild types, probably due to somaclonal variation induced during in vitro culture, corroborating their morphological variations (Sianipar *et al.,* 2024). Previous studies have also stated the dendrogram analysis arranged mutant lines exhibiting comparable morphological features into the same cluster (Pillay *et al.*, 2001; Malek *et al.*, 2014).

CONCLUSIONS

RAPD and SCoT markers clearly detected the genetic divergence between the banana Torpedo mutants (TM1 and TM2) and their wild types (PAS and PLB). RAPD showed higher polymorphism, and primer OPA-17 generated 83% variation, while the SCoT markers displayed lower but consistent discriminatory power. Sequencing confirmed these differences by identifying eight SNPs in the OPC-07 RAPD fragment and 55 SNPs and indels in the SCoT-2 fragment. Although OPC-07 amplicons were uniform, a mutation hotspot at positions 116–123 and additional substitutions at positions 181, 385, and 399 consistently distinguished the banana mutant TM2 from PAS, PLB, and TM1. Cluster analyses using both marker systems separated the mutants TM1 and TM2 from the wild types, confirming that the Torpedo lines represent genetically distinct somaclonal variants.

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