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Abstract: Phytoene desaturase (PDS) is a plant enzyme involved in carotenoid biosynthesis. The PDS gene has been used as a selective marker for genome editing in several plant species, including banana (*Musa* spp.). Its knockout promotes dwarfism and albinism, characteristics that are easily recognizable and highly favorable. In *Musa* spp., the A genome increases fruit production and quality, whereas the B genome is associated with tolerance to biotic and abiotic stresses. The objective of this study was to identify a molecular marker in the PDS gene to easily discriminate the A and B genomes of banana. A 2166 bp fragment for the 'PDSMa' marker was identified as polymorphic for the A genome (identification accuracy of 99.33%), whereas ~332 and ~225 bp fragments were detected for the 'PDSMb' primer with 100% accuracy using MedCalc software. To the best of our knowledge, this is the first study to use the PDS gene to determine doses of the A genome and identify the B genome in *Musa* spp., which will aid in evaluating the genomic constitution of banana hybrids and accessions at the seedling stage, and accelerating their classification in crop genetic improvement programs.

Keywords: gene composition; phytoene desaturase; molecular marker; genetic improvement; *Musa* spp.

1. Introduction

Bananas and plantains (Musaceae) are grown in all tropical and subtropical regions worldwide. They are the fourth largest food crop on the global market, after rice, wheat, and maize [1].

Most commercial banana cultivars originated from crosses between the wild subspecies *Musa acuminata* Colla (2n = 2x = 22; genome A) and *Musa balbisiana* Colla (2n = 2x = 22; genome B), which

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produced a series of diploid, triploid, and tetraploid bananas. The genomic groups resulting from these crosses are classified as AA, AB, AAA, AAB, ABB, AABB, AAAB, and ABBB [2]. The genome sequence of *M. acuminata* ssp. *malaccensis*, derived from a double haploid Pahang accession, represents the A genome (n = 11) [3,4], whereas that of *M. balbisiana*, derived from a Pisang Klutuk Wulung accession, represents the B genome (x = 11) [5,6].

The A genome is mostly related to improved production, yield, and fruit quality attributes, whereas the B genome lends robustness and tolerance/resistance to abiotic and biotic stresses [3,5,6]. The "B" genome is associated with the *banana streak virus* (*BSV*) [7], which influences the exchange of accessions between germplasm banks, field management, and in vitro cultivation. The virus has two forms of endogenous sequences (eBSV) in the "B" genome [8,9]: i) incomplete sequences that are considered evolutionary relics from previous infections and do not cause the disease, and ii) complete sequences that are initially dormant and activated to promote pathogenesis when the plant is challenged by biotic/abiotic stresses [10,11].

The genomic composition of banana is unpredictable, even in controlled crosses, owing to "unbalanced meiosis" and homologous recombination between "A" and "B" genomes, leading to a different number of sets or segments of each parent genome [12–14].

Ploidy is determined in banana using several methods, including morphological markers [15]. However, morphological markers are sensitive to environmental factors and are imprecise and impractical to measure at a large scale [13,16]. The use of molecular markers to distinguish the doses of "A" and "B" genomes in *Musa* spp. has been evaluated [13,15,17], however, despite their advantages over morphological markers, molecular markers are vulnerable to co-amplification with fungal DNA, if present, leading to misidentification, multiple copies, and ultimately, low accuracy.

Breeding programs seek effective and long-lasting techniques to improve crop characteristics, but are limited by the complex inheritance of most agronomic traits and strong genotype–environment interaction [18]. Recently, the CRISPR/Cas9 system has been widely used to induce specific genome mutations in several plant species, which has greatly contributed to the study of gene function in crop genetic improvement programs. This technique facilitates gene editing by cutting and replacing or adding sequences to the DNA of a given genotype [19]. To validate the use of CRISPR/Cas9 for tolerance to biotic and abiotic stresses in banana, the literature proposes initially using the knockout of the PDS (Phytoene desaturase) gene as a proof of concept [20–22].

The PDS gene has been widely used as a molecular marker for genome editing in several plant species, including bananas [20,21]. This gene plays a fundamental role in the carotenoid biosynthesis pathway, as it is highly conserved and has similar catalytic properties. PDS is a key enzyme in the carotenoid biosynthesis pathway, catalyzing the desaturation of phytoene (a transparent compound) into ζ-carotene, which is subsequently converted into lycopene, a colored compound [23]. PDS knockout affects photosynthesis, gibberellin production, and carotenoid biosynthesis, which leads to dwarfism and albinism in plants [24–26], suggesting that PDS can be a selective marker for the development of genetic engineering products.

The objective of this study was to develop a marker from the PDS gene capable of differentiating the A genome (*M. acuminata*) from the B genome (*M. balbisiana*) in banana. To validate its potential, the gene marker was tested on 150 banana accessions with different ploidy types collected from the Embrapa Mandioca e Fruticultura Germplasm Bank. This is the first report of a PDS gene-derived molecular marker that can identify "A" and "B" genomes in banana with 99.33% and 100% accuracy, respectively. Our study provides a foundation for the preliminary characterization of the genomic composition of banana accessions to predict agronomic, sensory, and resistance/tolerance characteristics that are desirable for genetic improvement programs.

2. Materials and Methods

2.1. Plant material

We evaluated 150 banana accessions associated with different genomic groups and levels of ploidy (AA, AAA, AAAA, AB, BB, AAB, ABB, AAAB, AABB, and ABBB), which were procured from

the Banana Germplasm Bank of Embrapa Mandioca e Fruticultura, Cruz das Almas, Bahia, Brazil (12°40′48.03″S and 39°05′20.91″W). The collection consisted of four plants per accession, spaced 2.0 m between rows × 1.7 m between plants, and irrigation by micro-sprinklers was performed according to plant needs. This approach would minimize water stress, even during dry periods.

2.2. Primer construction

To design specific primers for *M. acuminata* and *M. balbisiana*, PDS gene sequences from the "A" genome (*M. acuminata*, gene Ma08_t16510.2) and "B" genome (*M. balbisiana*, gene Mba08_g16040.1) were initially obtained from the SouthGreen-Banana Genome Hub platform (https://banana-genome-hub.southgreen.fr/). "A" and "B" genome sequences were aligned to identify a conserved region in the "A" genome and polymorphic region in the "B" genome, and vice versa, using Clustal Omega software [27].

After identifying the conserved PDS regions of *M. acuminata* and *M. balbisiana*, specific PDS gene primers were constructed using Oligo Explorer software (version 1.2.) (PDSMaF ATTGTGAAAGAGGTCGAGGA, PDSMaR TGCGGTAAAAGAAGCTTCAA; PDSMbF GTGAGTTCATGGGTTGCCAA, PDSMbR ACCGGCTATGACAACCTTCA). The discriminatory power of the primers was assessed by *polymerase chain reaction* (PCR) analysis.

2.3. DNA extraction and PCR conditions

DNA was extracted from the young leaves of the 150 banana accessions as described by Doyle and Doyle [28], with modifications proposed by Ferreira et al. [29]. The samples of young banana leaves (300 mg) were immediately placed in plastic bags (20 × 10 cm) with 2 mL extraction buffer (2% CTAB; 100 mM Tris-HCl, pH 8.0; 50 mM EDTA, pH 8.0; 1.4 M NaCl; 2% PVP-40; and 1% sodium sulfite). They were then macerated in a drill press to release nuclear DNA until a homogeneous solution was obtained.

The quantity and quality of genomic DNA was assessed on 0.8% agarose gel in 0.5X TBE (90 mM tris, 90 mM boric acid, 2.5 mM EDTA, pH 8.3) and a microvolume spectrophotometer (GE NanoVue Plus, Biochrom, Holliston, MA, USA). The samples were then diluted in TE (1 M tris-HCl 1 M, pH 8.0; and 0.5 M EDTA, pH 8.0), and standardized to 5 ng/µL.

The DNA samples obtained from each genotype were amplified by PCR using the reference genes, *β-tubulin*, PDS_AB [26], PDSMa, and PDSMb primers (Table 1). Amplification reactions were conducted with 1.5 µL PCR buffer (10×), 1.5 µL MgCl2 (50 mM), 1.2 µL dNTP (2.5 mM), 1.0 µL of the F and R primers (10.0 µM), and 0.2 µL *Taq* DNA polymerase (5 U/µL), with a final volume of 20 µL.

The samples were then amplified in a Veriti thermal cycler (Applied Biosystems, Waltham, MA, USA) under the following programming conditions: one cycle at 95 °C for 15 min; 34 cycles at 94 °C for 30 s; one cycle at 55 °C for the PDS_AB primer, 60 °C for β-tubulin and PDSMa, and 65 °C for PDSMb for 30 s for primer annealing, and one cycle at 72 °C for 1 min; and a final cycle at 72 °C for 10 min (adapted from Ntui et al. [26]. Primer sequences and annealing temperatures are shown in Table 1.

Table 1. Primers used in the validation study of the PDS gene-derived PDSMa and PDSMb molecular markers of *M. acuminata* and *M. balbisiana*.

	Sequence				
Primers	**F* (5′- 3′)**	**R* (5′- 3′)**	**At***	**pb***	**Reference**
β-tubulin	ACATTGTCAGGT GGG	GAGTTCCTTTTGTTCCACACGAGATT	60 ºC	110	[30]
PDS_AB	CAGCTAACTGAGATCAGTTT	AGATGGCTATATTTCGGTAC	55 ºC	994	[26]
PDSMa	ATTGTGAAAGAGGTCGAGGA	TGCGGTAAAAGAAGCTTCAA	60 ºC	2166	
PDSMb	GTGAGTTCATGGGTTGCCAA	ACCGGCTATGACAACCTTCA	65 °C	332	

The amplification products were separated by electrophoresis in 2% and 3% agarose gel (PDSMb) at 70 V in TBE buffer for 4 h and stained with Gel red (1 µg/mL). The amplified products

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were visualized and photographed under ultraviolet light in an (L-Pix Touch documentation system (Loccus, Cotia, Brazil).

2.4. Data analysis

The data obtained through electrophoresis was recorded as band presence or absence. The PDS gene marker for the "A" and "B" genomes (994 bp; [26]) was used to compare the efficiency of the new primers in identifying the "A" and "B" genomes, and the β-tubulin primer (110 bp) was used as an endogenous control for *Musa* spp. [30]. The base pairs of the fragments amplified by the PDS gene-derived primers were calculated on the log scale with the following regression models: i) identification of "A" genome, y = –0.003x + 9.599 and ii) identification of "A" and "B" genome, y=–0.0123+13.989. MedCalc software (https://www.medcalc.org/calc/diagnostic_test.php) [31] was used for statistical analysis. This software provides statistical information regarding the sensitivity, specificity, *likelihood ratio* (negative or positive), and accuracy of the polymorphic fragment detected by the *screening* of a set of genotypes.

3. Results and Discussion

The complete PDS gene sequences of *M. acuminata* (AA) and *M. balbisiana* (BB) were downloaded from the Banana Genome Hub (https://banana-genome-hub.southgreen.fr/) on the SouthGreen platform. The PDS gene (Ma08_g16510) of *M. acuminata* has 27 944 bp and 14 exons, and the PDS gene (Mba08_g16040.1) of *M. balbisiana* has 21 262 bp and 11 exons. The alignment of these sequences shared 96.40% nucleotide homology from the start to stop codons.

The PDS gene has often been used as a concept marker/proof in CRISPR/Cas9 gene editing experiments in many plant species such as maize [32], *Arabdopsis* [33], tomato [34], rice [35], and banana [26].

After downloading the material to construct the PDSMa- and PDSMb-specific markers, the coding regions of the PDS gene were aligned using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) to identify discriminatory/polymorphic regions between the "A" and "B"genomes (Figure 1).

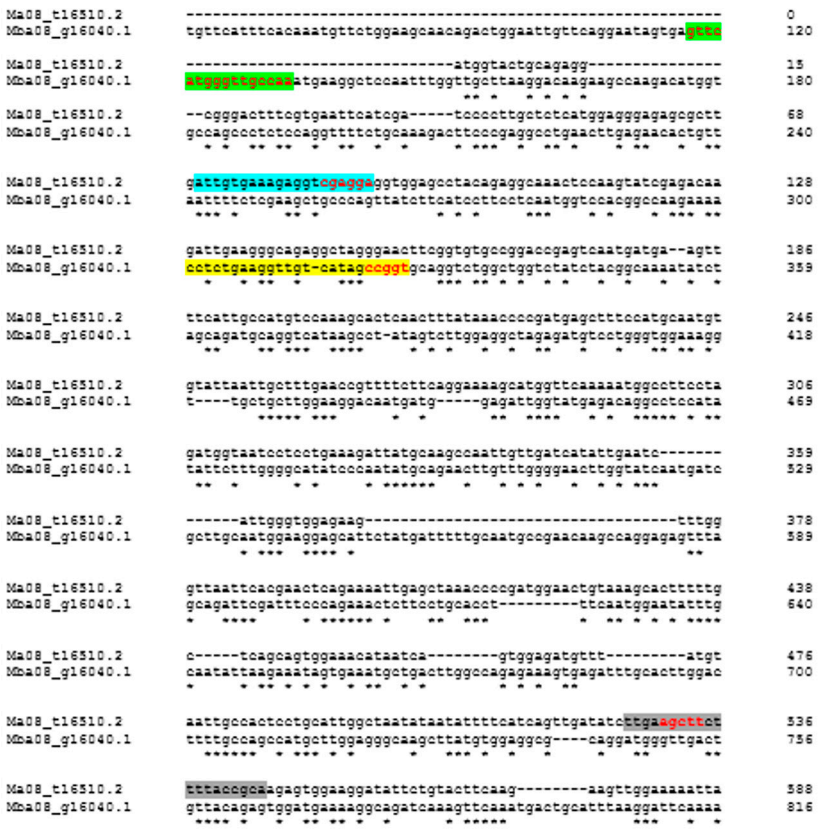

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Figure 1. Partial alignment of the PDS gene of *Musa acuminata* (AA) and *M. balbisiana* (BB) for the construction of specific *M. acuminata* (PDSMa) and *M. balbisiana* (PDSMb) primers. Blue and gray markings correspond to the forward and reverse primers of PDSMa, respectively; green and yellow markings to the forward and reverse primers of PDSMb, respectively. Red marking represents the polymorphic region of the F and R primers between the "A" and "B" genomes.

The specific primers for PDSMa and PDSMb were evaluated by PCR. Two primers were used as controls—*β-tubulin*, as an endogenous gene, and the primer developed by Ntui et al. [26] to amplify the PDS gene in both the "A" and "B" genomes (PDS_AB) (Figure 2A). The amplification of β-tubulin, PDS_AB [26], and PDSMa in 12 banana samples with representative genomes of different ploidy types (Germplasm Bank of Embrapa Mandioca e Fruticultura) is shown in Figure 2A.

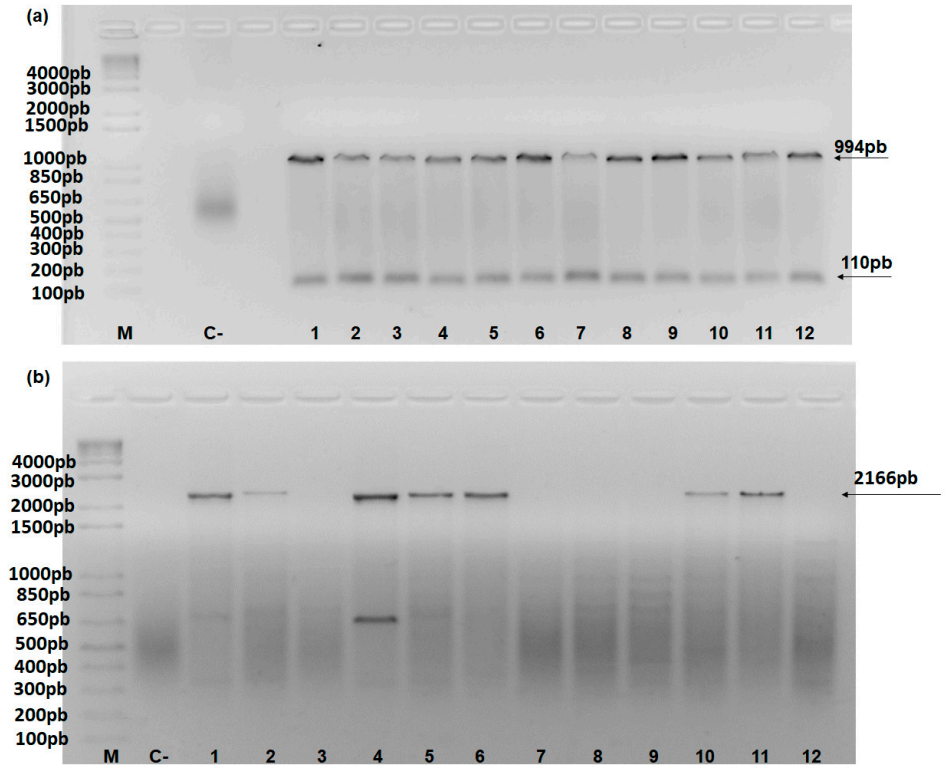

Figure 2. PCR amplification with. (A) PDS_AB (994 bp) and β-tubulin (110 bp) primers; (B) PDSMa (2166 bp) in accessions 1–12 from the Embrapa Mandioca e Fruticultura Germplasm Bank; (C) Control; 1) Gros Michel (AAA); 2) Prata Anã (AAB); 3) Balbisiana Franca (BB); 4) Zebrina (AA); 5) IAC 1 (AB); 6) Bucaneiro (AAAA); 7) Butuhan (BB); 8) Musa balbisiana (BB); 9) BB Franca (BB); 10) Pelipita (ITC 0472) (ABB); 11) FC06-02 (AABB); 12) Teparod (ABBB), listed in Table 1. M = 1 kb marker (Invitrogen). Arrows of 110, 994, and 2166 bp correspond to the β-tubulin, PDS_AB, and PDSMa primers, respectively. The 500-bp fragment in genotype 4 for the PDSMa primer (B) corresponds to the non-specific band that only appeared in this case.

The cultivars Balbisiana Franca (BB), Butuhan (BB), *Musa balbisiana* (BB), BB Franca (BB), and Teparod (ABBB) showed band amplification only for the PDS_AB and *β-tubulin* primers, confirming the discriminatory power of the PDSMa primer (Figure 2B).

The PDSMa primer has 476 bp and was constructed without intron regions. The amplification of this primer in all "A" genome *Musa* accessions produced a 2166 bp fragment (Figure 2B) based on the regression model y = –0.003x + 9.5999 (R2 = 0.90). This band size reflects our use of total genomic DNA, which contains introns. The PDS_AB and *β-tubulin* primers produced fragments of 994 and 110 bp, corroborating the PDS gene sizes reported by Ntui et al. [26] and Podevin et al. [30], respectively.

The amplification of the PDSMb primer in six banana samples with different ploidy types is shown in Figure 3. A fragment of approximately 332 bp was observed in all samples with A and B

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ploidy (Figure 3), which was based on the regression model y = –0.0123 + 13.989 (R2 = 0.96). In addition to the ~322 bp band, the cultivars Zebrina (AA), Gros Michel (AAA), and Bucaneiro (AAAA) presented the amplification of a second specific band of ~225 bp (based on the same regression model), indicating that this band pattern only occurred in specimens with 100% A ploidy (Figure 3).

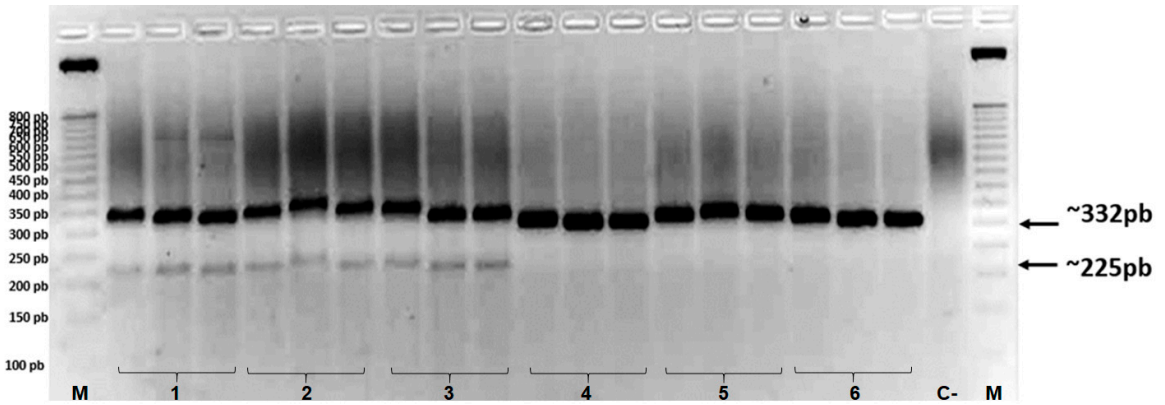

Figure 3. PCR amplification with the PDSMb primer on accessions with different ploidy types from the Embrapa Mandioca e Fruticultura Germplasm Bank, in triplicate: 1- Zebrina (AA), 2- Gros Michel (AAA), 3- Bucaneiro (AAAA), 4- IAC 1 (AB), 5- Balbisiana Franca (BB), 6- Butuhan (ABBB), listed in Table 1. M = 50 bp marker (Promega). Arrows indicating ~332 bp fragments correspond to bands present in all samples, and ~225 bp fragments correspond only to A-genome accessions.

Table 2 shows the banana accessions from the Embrapa Mandioca e Fruticultura Germplasm Bank and their respective PCR amplification results with the four primers. The 150 genotypes evaluated were represented by different ploidy types: AA, AAA, AAAA, BB, AB, AAB, ABB, AAAB, AABB, and ABBB. Table 2 shows cultivars with bands amplified using the primers *β-tubulin*, PDS_AB, PDSMa, and PDSMb. All genotypes were positive for the primers *β-tubulin* and PDS_AB. For the PDSMa primer, amplification did not occur in genotypes with >75% of genome "B", and all 100% "A" ploidy genotypes had amplified bands of ~225 bp in PDSMb.

Banana genetic improvement is based on crossing wild or improved diploids with commercial cultivars to generate hybrids resistant/tolerant to biotic and abiotic stresses and with agronomic characteristics consistent with market demands [36,37]. Nwakanma et al. [15], suggested that early determination of the banana genome composition can aid breeders in predicting the occurrence of useful agronomic characteristics and developing new varieties.

The development of molecular markers capable of discriminating high doses of the "B" genome in bananas is essential for determining gene composition and inferring important characteristics in hybrids [22]. The PDSMb primer developed in this study proved to be useful for detecting the ploidy of cultivars developed in the Embrapa breeding program. This marker effectively identified the "B" genome in the gene composition of the different accessions; even if the sample has only 25% of the B genome in its ploidy, the primer will not detect and not reveal the second ~225 bp band (Figure 3, Table 2).

Table 2. Banana accessions from the Embrapa Mandioca e Fruticultura Germplasm Bank used in "A" and "B" genome differentiation by PCR with the β-tubulin, PDS_AB, PDSMa, and PDSMb primers.

Accessions			Primer				
Nº	Genotype/Cultivar	Genomic Group	Tubulin	PDS_AB	PDSMa	PDSMb ~320pb	PDSMb ~225pb
1	Gros Michel	AAA	+	+	+	+	+
2	Prata Anã	AAB	+	+	+	+	-
3	Balbisiana Franca	BB	+	+	-	+	-
4	Zebrina	AA	+	+	+	+	+

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5	IAC 1	AB	+	+	+	+	-
6	Bucaneiro	AAAA	+	+	+	+	+
7	Butuhan	BB	+	+	-	+	-
8	Musa balbisiana	BB	+	+	-	+	-
9	BB França	BB	+	+	-	+	-
10	Pelipita	ABB	+	+	+	+	-
11	FC06-02	AABB	+	+	+	+	-
12	Teparod	ABBB	+	+	-	+	-
13	028003-01	AA	+	+	+	+	+
14	M53	AA	+	+	+	+	+
15	Pisang Jaran	AA	+	+	+	+	+
16	Malbut	AA	+	+	+	+	+
17	Calcutta 4	AA	+	+	+	+	+
18	PA Rayong	AA	+	+	+	+	+
19	Buitenzorg	AA	+	+	+	+	+
20	Khai Nai On	AA	+	+	+	+	+
21	Microcarpa	AA	+	+	+	+	+
22	Mambee Thu	AA	+	+	+	+	+
23	Malaccensis	AA	+	+	+	+	+
24	Pisang Tongat	AA	+	+	+	+	+
25	Pisang Pipit	AA	+	+	+	+	+
26	SF0751	AA	+	+	+	+	+
27	Pisang Rojo Uter	AA	+	+	+	+	+
28	Tong Dok Mak	AA	+	+	+	+	+
29	Pisang Jari Buaya	AA	+	+	+	+	+
30	Pisang Lilin	AA	+	+	+	+	+
31	M48	AA	+	+	+	+	+
32	NBA 14	AA	+	+	+	+	+
33	Pisang Lidi	AA	+	+	+	+	+
34	Khai	AA	+	+	+	+	+
35	Pisang Berlin	AA	+	+	+	+	+
36	Khi Maeo	AA	+	+	+	+	+
37	Niyarma Yik	AA	+	+	+	+	+
38	Pisang Jari Buaya	AA	+	+	+	+	+
39	Tuu Gia	AA	+	+	+	+	+
40	Tjau Lagada	AA	+	+	+	+	+
41	Pisang Mas	AA	+	+	+	+	+
42	Ouro	AA	+	+	+	+	+
43	M61	AA	+	+	+	+	+
44	Birmanie	AA	+	+	+	+	+
45	Borneo	AA	+	+	+	+	+
46	PA Musore 2	AA	+	+	+	+	+
47	Pisang Cici	AA	+	+	+	+	+
48	42049004	AA	+	+	+	+	+
49	42052004	AA	+	+	+	+	+
50	42079006	AA	+	+	+	+	+
51	42079013	AA	+	+	+	+	+
52	42085002	AA	+	+	+	+	+
53	50012002	AA	+	+	+	+	+
54	58054003	AA	+	+	+	+	+

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55	73041001	AA	+	+	+	+	+
56	86079009	AA	+	+	+	+	+
57	86079010	AA	+	+	+	+	+
58	86094015	AA	+	+	+	+	+
59	86094020	AA	+	+	+	+	+
60	89087001	AA	+	+	+	+	+
61	91079003	AA	+	+	+	+	+
62	91087002	AA	+	+	+	+	+
63	91094004	AA	+	+	+	+	+
64	SH3263	AA	+	+	+	+	+
65	SH3362	AA	+	+	+	+	+
66	TH03001	AA	+	+	+	+	+
67	42052003	AA	+	+	+	+	+
68	1916001	AA	+	+	+	+	+
69	13004006	AA	+	+	+	+	+
70	BRS SCS Belluna	AAA	+	+	+	+	+
71	N´Jok Kon	AAB	+	+	+	+	-
72	CNPMF 0557	AA	+	+	+	+	+
73	CNPMF0496	AA	+	+	+	+	+
74	CNPMF0513	AA	+	+	+	+	+
75	CNPMF0519	AA	+	+	+	+	+
76	CNPMF0536	AA	+	+	+	+	+
77	CNPMF0534	AA	+	+	+	+	+
78	CNPMF0542	AA	+	+	+	+	+
79	CNPMF0565	AA	+	+	+	+	+
80	CNPMF0572	AA	+	+	+	+	+
81	CNPMF0612	AA	+	+	+	+	+
82	CNPMF0731	AA	+	+	+	+	+
83	CNPMF0767	AA	+	+	+	+	+
84	CNPMF0811	AA	+	+	+	+	+
85	CNPMF0037	AA	+	+	+	+	+
86	CNPMF0038	AA	+	+	+	+	+
87	CNPMF 1102	AA	+	+	+	+	+
88	CNPMF 0993	AA	+	+	+	+	+
89	CNPMF 1323	AA	+	+	+	+	+
90	CNPMF 1105	AA	+	+	+	+	+
91	CNPMF 0998	AA	+	+	+	+	+
92	CNPMF 1272	AA	+	+	+	+	+
93	CNPMF1286	AA	+	+	+	+	+
94	BRS Pacoua	AAAB	+	+	+	+	-
95	Yangambi Km 5	AAA	+	+	+	+	+
96	Nanicão Cena 225	AAA	+	+	+	+	+
97	Nanicão Jangada	AAA	+	+	+	+	+
98	IAC 2001	AAA	+	+	+	+	+
99	IAC 504	AAA	+	+	+	+	+
100	IAC 505	AAA	+	+	+	+	+
101	Nanicão Viana	AAA	+	+	+	+	+
102	Rabo de Égua	AAA	+	+	+	+	+
103	Yangambi Nº2	AAB	+	+	+	+	-
104	Saney	AAB	+	+	+	+	-

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105	Thap maeo	AAB	+	+	+	+	-
106	Samura 'B'	AAB	+	+	+	+	-
107	Prata Jussara	AAB	+	+	+	+	-
108	Prata IAC	AAB	+	+	+	+	-
109	Tai	AAB	+	+	+	+	-
110	Pacovan	AAB	+	+	+	+	-
111	Walha	AAB	+	+	+	+	-
112	Prata Ponta Aparada	AAB	+	+	+	+	-
113	Prata Comum	AAB	+	+	+	+	-
114	Prata Manteiga	AAB	+	+	+	+	-
115	Terrinha	AAB	+	+	+	+	-
116	Curare Enano	AAB	+	+	+	+	-
117	Red Yade	AAB	+	+	+	+	-
118	D'Angola	AAB	+	+	+	+	-
119	Mongolo	AAB	+	+	+	+	-
120	Terra ponta aparada	AAB	+	+	+	+	-
121	Pinha	AAB	+	+	+	+	-
122	Tipo Velhaca	AAB	+	+	+	+	-
123	Terra maranhão	AAB	+	+	+	+	-
124	Trois Vert	AAB	+	+	+	+	-
125	Prata Baby	AAB	+	+	+	+	-
126	Prata graúda	AAB	+	+	+	+	-
127	Namwa Daeng	ABB	+	+	+	+	-
128	Ice Cream	ABB	+	+	+	+	-
129	Pitogo	ABB	+	+	+	+	-
130	Saba Honduras	ABB	+	+	+	+	-
131	Saba	ABB	+	+	+	+	-
132	Espermo	ABB	+	+	+	+	-
133	Poteau Naine	ABB	+	+	+	+	-
134	Figo	ABB	+	+	+	+	-
135	Fhia 02	AAAA	+	+	+	+	+
136	Platina IAC	AAAB	+	+	+	+	-
137	Fhia 18	AAAB	+	+	+	+	-
138	Prata Maçã	AAAB	+	+	+	+	-
139	BRS Japira	AAAB	+	+	+	+	-
140	BRS Pioneira	AAAB	+	+	+	+	-
141	BRS Garantida	AAAB	+	+	+	+	-
142	BRS Preciosa	AAAB	+	+	+	+	-
143	BRS Princesa	AAAB	+	+	+	+	-
144	BRS Vitoria	AAAB	+	+	+	+	-
145	YB 4203	AAAB	+	+	+	+	-
146	Fhia 21	AAAB	+	+	+	+	-
147	PA 42038	AAAB	+	+	+	+	-
148	PA 42028	AAAB	+	+	+	+	-
149	PA 42019	AAAB	+	+	+	+	-
150	Fhia 03	AABB	+	+	+	+	-

(+): presence of a band; (-): absence of a band.

MedCalc software (https://www.medcalc.org/calc/diagnostic_test.php) was used in the molecular analysis of the PDSMa and PDSMb markers. This software is used in the health sector for

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disease diagnosis and can be adapted for use in plants. In the program you need to fill in information about true positives, false negatives, false positives and true negatives. This way, it is possible to extract statistics on sensitivity, specificity, likelihood ratio (negative or positive) and accuracy of the polymorphic fragment found.

Of the 150 accessions subjected to PCR, 145 showed bands at 2166 bp for the PDSMa primer, indicating the presence of the "A" genome, and 92 showed bands at ~225 bp for the PDSMb primer. These samples were classified as true positive in the MedCalc analysis.

Only one sample, the Teparod genotype (ABBB), was identified as a false negative for the PDSMa primer, because the "A" genome in its composition was not identified by band amplification in this region. This result corroborates the occurrence of homologous recombination between "A" and "B" genome cultivars, suggesting that, in this specific case, the Teparod genotype may not be carrying the full complement of the "A" genome [13,38,39]. There were no false negatives for PDSMb, as band amplification (~225 bp) occurred in all cultivars with 100% "A" ploidy.

None of the samples with the "B" genome showed the 2166 bp fragment in PDSMa, nor the second band of ~225 bp in PDSMb, representing a false positive. Four samples containing the "B" genome showed no amplification with PDSMa, and 58 with "B" ploidy genotypes showed no amplification for the second band with PDSMb, making them true negatives. We calculated the static parameters of the PDSMa and PDSMb markers, which showed 99.32% and 100% sensitivity, 100% specificity, 100% positive predictive value, 80% and 100% negative predictive value, and 99.33% and 100% accuracy, respectively, indicating that the PDSMa marker is highly effective in discriminating "B" genome doses >75% in banana genotypes and that the PDSMb marker can identify accessions with 100% of the "A" genome in their ploidy (Table 3).

Table 3. Statistical analysis, using MedCalc software, of the sensitivity, specificity, positive and negative predictive value, and accuracy of the specific markers for the PDS gene of *Musa acuminata* and *M. balbisiana*.

Statistic	Value-PDSMa	Value-PDSMb	95% CI- PDSMa	95% CI- PDSMb
Sensitivity	99.32%	100.00%	96.24% to 99.98%	96.07% to 100.00%
Specificity	100.00%	100.00%	39.76% to 100.00%	93.84% to 100.00%
Positive Likelihood Ratio				
Negative Likelihood Ratio	0.01	0.00	0.00 to 0.05	
Disease prevalence (*)	97.33%	61.33%	93.31% to 99.27%	53.05% to 69.16%
Positive Predictive Value (*)	100.00%	100.00%		96.07% to 100.00%
Negative Predictive Value (*)	80.00%	100.00%	36.19% to 96.58%	93.84% to 100.00%
Accuracy (*)	99.33%	100.00%	96.34% to 99.98%	97.57% to 100.00%

The use of molecular markers to determine the genomic composition of *Musa* cultivars and other crops have many advantages over morphological markers [40]. Several studies have used molecular methods to identify the genomes of *M. acuminata* and *M. balbisiana*. Nwakanma et al. [15] and Jesus et al. [13] identified molecular markers based on internal transcribed spacers (ITS), which discriminated "A" from "B" genomes in bananas, but not very accurately. Hollingsworth [41] showed that markers based on ITS regions were vulnerable to co-amplification with fungal DNA, leading to misidentification and multiple, possibly divergent, ITS copies in a single specimen.

Mabonga and Pillay [42] developed a 500 bp SCAR marker based on a RAPD marker to identify the "A" genome in bananas and plantains. Although the marker was useful for identifying the "A" genome, a 700 bp fragment hybridized with all the genotypes and impeded the differentiation of "A" and "B" genomes. Many primers have been obtained by converting RAPD markers into SCAR markers. However, this conversion generally leads to a decreased level of polymorphism [43], particularly with different genetic backgrounds.

The identification of genotypes with B genome doses based on the absence of a band is also valuable for predicting BSV disease onset, which is mainly caused by three virus species: *Goldfinger*

(*eBSGFV*), *Imovè* (*eBSIMV*), and *Obinol'Ewai* (*eBSOLV*). Because of viral introgression in the B genome (*eBSV*), caution must be exercised in the cultivation of "B"genome accessions since disease onset can be stimulated by the several *in vitro* subcultures required by the crop, which is vegetatively propagated, and external plant stresses, such as low temperatures [9,44]. Thus, early identification of the "B" genome could be instrumental in enhancing crop management practices in the agricultural field.

The highly accurate PDS gene markers developed in this study to discriminate the "A" and "B" genomes in bananas represents a useful new tool for the genetic improvement of Musaceae crops, particularly due to its origin from a highly conserved gene with few copies. These markers can potentially be used in the molecular characterization of germplasm collections and new accessions to expand the genetic diversity of the crop, which would be useful in discriminating between controlled and uncontrolled crosses and providing information for seedling exchange. These activities constitute the basis of genetic improvement programs for bananas.

5. Conclusions

The PDSMa and PDSMb markers derived from the PDS gene enable early prediction of genotypes with "A" and "B" genomes in banana hybrids. These markers represent useful tools for genetic improvement strategies, aimed at developing varieties with higher fruit quality, yield, and disease resistance, and could potentially predict BSV disease onset triggered by environmental conditions and biotic stresses. For CRISPR/Cas9-based genetic editing of banana crops in Brazil, the sequences of the PDSMa and PDSMa markers will be used in future studies as guide RNA for Cas9 in the CRISPR knockout of the PDS gene, which is involved in the biosynthesis of carotenoids and is associated with albinism. Subsequent studies will focus on the identification and manipulation of genes for resistance/tolerance to biotic and abiotic stresses.

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