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Phytoene Desaturase (PDS) Gene-Derived Markers Identify "A" and "B" Genomes in Banana (*Musa* spp.)

Fernanda dos Santos Nascimento [1,†], Marcelly Santana Mascarenhas [1,†], Samantha Costa Boaventura [2], Carla Catharina Hora de Souza [2], Andresa Priscila de Souza Ramos [3], Anelita de Jesus Rocha [1], Julianna Matos da Silva Soares [1], Leandro Eugenio Cardamone Diniz [4], Tiago Antônio de Oliveira Mendes [5], Claudia Fortes Ferreira [3] and Edson Perito Amorim [3,*]

1 Post-Graduate Program in Biotechnology, State University of Feira de Santana, Avenida Transnordestina, Novo Horizonte, Feira de Santana 44036-900, Bahia, Brazil; feel.20@hotmail.com (F.d.S.N.); marcelly.bio@hotmail.com (M.S.M.); anelitarocha@gmail.com (A.d.J.R.); juliannamatos91@gmail.com (J.M.d.S.S.)
2 Center for Agricultural, Environmental and Biological Sciences, Federal University of Recôncavo da Bahia, Rua Rui Barbosa, 710, Centro, Cruz das Almas 44380-000, Bahia, Brazil; samanthaboaventura.bio@gmail.com (S.C.B.); catharinahora13@gmail.com (C.C.H.d.S.)
3 Embrapa Mandioca e Fruticultura, Rua Embrapa, s/n, Cruz das Almas 44380-000, Bahia, Brazil; andresa.ramos@embrapa.br (A.P.d.S.R.); claudia.ferreira@embrapa.br (C.F.F.)
4 Embrapa Soja, Rodovia Carlos João Strass, Londrina 86085-981, Paraná, Brazil; leandro.diniz@embrapa.br
5 Department of Biochemistry and Molecular Biology, Federal University of Viçosa, Avenida Peter Henry Rolfs, s/n, Campus Universitário, Viçosa 36570-900, Minas Gerais, Brazil; tiagoaomendes@ufv.br
* Correspondence: edson.amorim@embrapa.br
† These authors contributed equally to this work.

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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" marker with 100% accuracy using MedCalc software. In this study, we used genotypes with A and B genomes that are used in the genetic improvement of bananas and an accession with the BT genome. It was not possible to differentiate the accession with the BT genome from the others, suggesting that the markers do not have the capacity to separate the T genome from the A and B genomes. 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 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 on 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.

Thus, this study provides a basis for the preliminary characterization of the genomic composition of banana accessions to predict agronomic, sensory, and resistance/tolerance traits that are desirable for breeding programs. We generated a fragment of 2166 bp for the marker "PDSMa" that was identified as polymorphic for genome A, while fragments of ~332 and ~225 bp were detected for the marker "PDSMb". This is the first report of a molecular marker derived from the PDS gene that can identify the "A" and "B" genomes in bananas with 99.33% and 100% accuracy, respectively. The objective of this study was to develop a marker based on the PDS gene capable of differentiating the A genome (*M. acuminata*) from the B genome (*M. balbisiana*) in bananas and to validate its potential by testing 150 banana accessions with different types of ploidy collected from the Embrapa germplasm bank.

2. Materials and Methods

2.1. Plant Material

We evaluated 150 banana accessions relevant to the genetic breeding program associated with different genomic groups and levels of ploidy (AA, AAA, AAAA, AB, BB, AAB, ABB, AAAB, AABB), including the Butuhan accession with the BT genome (*M. balbisiana* and *M. textilis*), 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/, accessed on 10 January 2024). "A" and "B" genome sequences were aligned to identify a conserved region in the "A" genome and a polymorphic region in the "B" genome, and vice versa, using Clustal Omega software v. 1.2 [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 were assessed on a 0.8% agarose gel in 0.5X TBE and a microvolume spectrophotometer (GE NanoVue Plus, Biochrom, Holliston, MA, USA). The samples were then diluted in TE, 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).

The samples were then amplified in a Veriti thermal cycler (Applied Biosystems, Waltham, MA, USA) with programming 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 GAGTT	CCTTTTGTTCCACACGAGATT	60 °C	110	[30]
PDS_AB	CAGCTAACTGAGATCAGTTT	AGATGGCTATATTTCGGTAC	55 °C	994	[26]
PDSMa	ATTGTGAAAGAGGTCGAGGA	TGCGGTAAAAGAAGCTTCAA	60 °C	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 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 the "A" genome, $y = -0.003x + 9.599$, and (ii) identification of the "A" and "B" genomes, $y = -0.0123 + 13.989$. MedCalc software Version 22.009 (https://www.medcalc.org/calc/diagnostic_test.php, accessed on 4 August 2023) [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/ accessed on 18 January 2024) 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/,accessed on 18 January 2024) to identify discriminatory/polymorphic regions between the "A" and "B" genomes (Figure 1).

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.

The cultivars Balbisiana Franca (BB), Butuhan (BT), *Musa balbisiana* (BB), BB Franca (BB), and Teparod (ABB) 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 $y = -0.003x + 9.5999$ ($R^2 = 0.90$). 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, B, and BT ploidy (Figure 3), which was based on the regression model $y = -0.0123 + 13.989$ ($R^2 = 0.96$). 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).

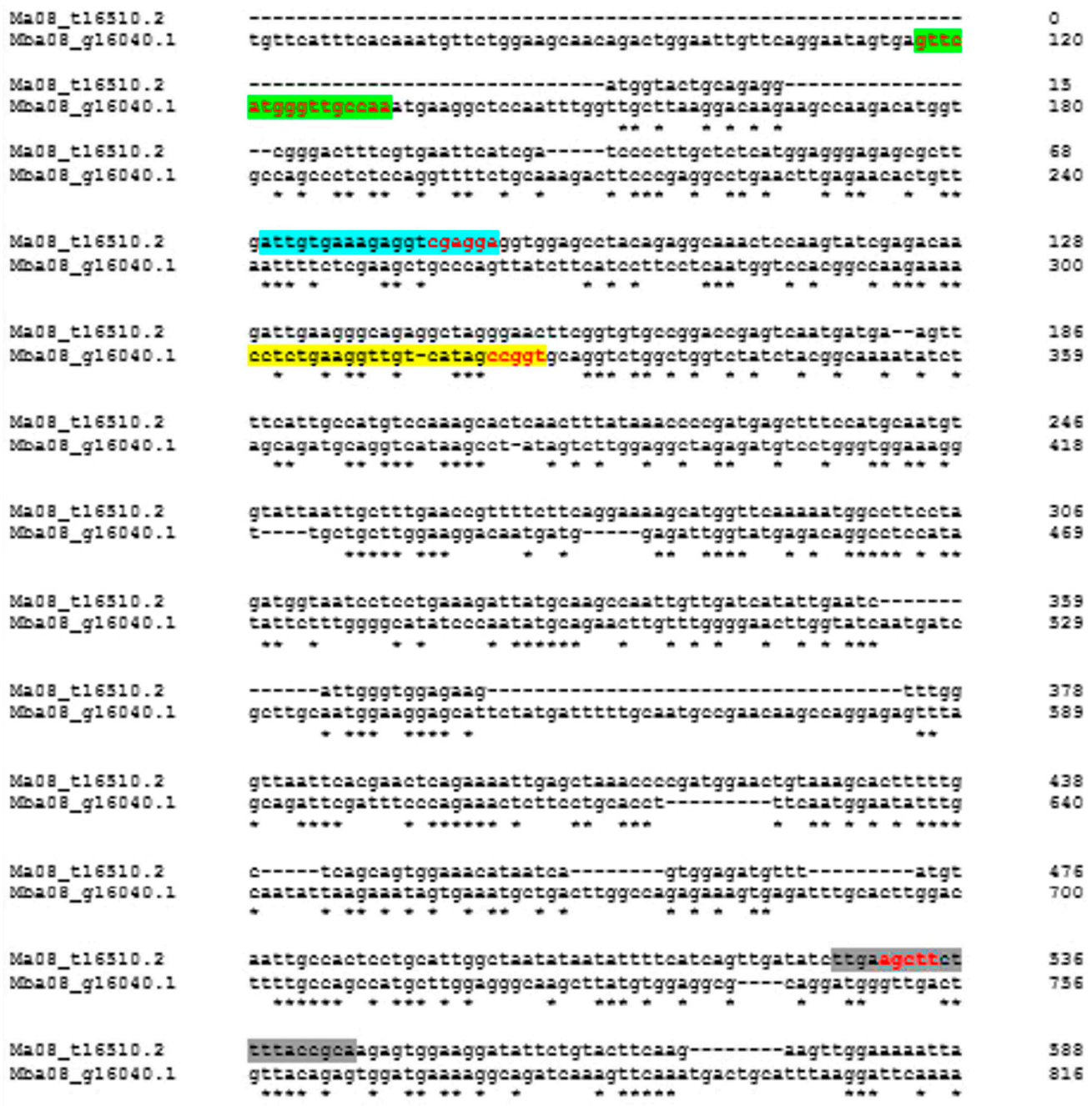

Figure 1. Partial alignment of the PDS gene of *Musa acuminata* (AA) and *M. balbisiana* (BB) for the construction of specific *M. acuminata* (PDSMa) and *M. balbisiana* (PDSMb) primers. Blue and gray markings correspond to the forward and reverse primers of PDSMa, respectively; green and yellow markings 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.

Table S1 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, BT, AB, AAB, ABB, AAAB, AABB. Table S1 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. It can also be observed that the Butuhan accession with the BT genome presented a banding pattern similar to that observed in A and B, i.e., the

markers PDSMa and PDSMb did not differentiate the T genome from A and B. This fact leads us to infer that, possibly, these markers do not have the potential to differ from other genomes of musaceae. This fact may be proven in future studies. 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.

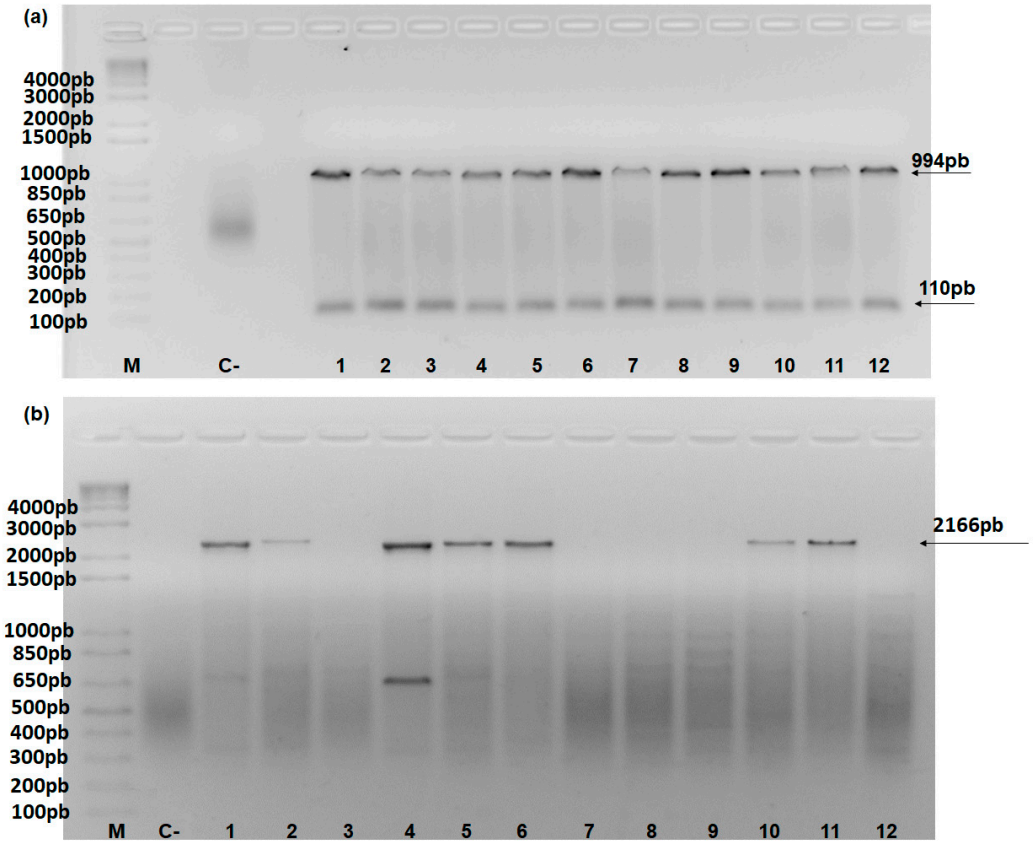

Figure 2. PCR amplification with. (**a**) PDS_AB (994 bp) and β-tubulin (110 bp) primers; (**b**) PDSMa (2166 bp) in accessions 1–12 from the Embrapa Mandioca e Fruticultura Germplasm Bank; (C) Control; (1) Gros Michel (AAA); (2) Prata Anã (AAB); (3) Balbisiana Franca (BB); (4) Zebrina (AA); (5) IAC 1 (AB); (6) Bucaneiro (AAAA); (7) Butuhan (BT); (8) Musa balbisiana (BB); (9) BB Franca (BB); (10) Pelipita (ITC 0472) (ABB); (11) FC06-02 (AABB); (12) Teparod (ABB), listed in Table 1. M = 1 kb marker (Invitrogen, Waltham, MA, USA). 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 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 S1).

MedCalc software Version 22.009 (https://www.medcalc.org/calc/diagnostic_test.php accessed on 4 August 2023) was used in the molecular analysis of the PDSMa and PDSMb markers. This software is used in the health sector for 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 the sensitivity, specificity, likelihood ratio (negative or positive), and accuracy of the polymorphic fragment found.

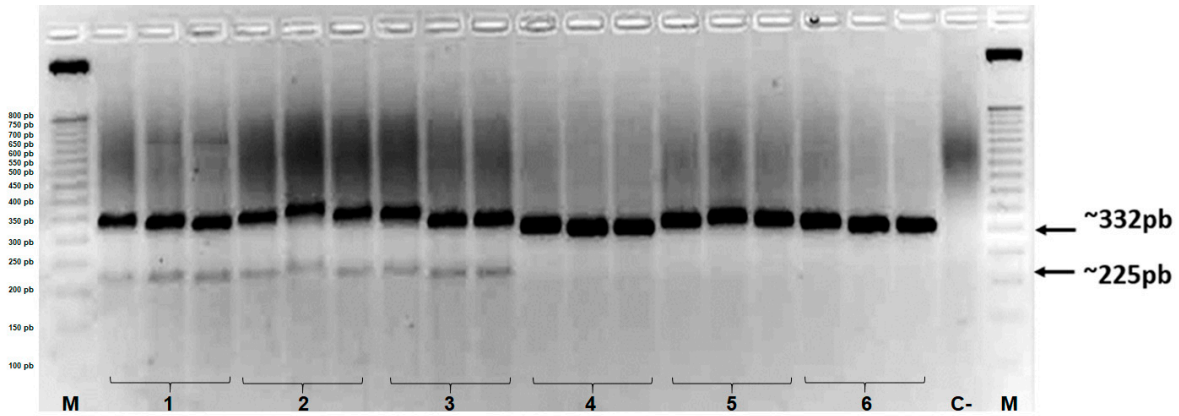

Figure 3. PCR amplification with the PDSMb primer on accessions with different ploidy types from the Embrapa Mandioca e Fruticultura Germplasm Bank, in triplicate: 1—Zebrina (AA), 2—Gros Michel (AAA), 3—Bucaneiro (AAAA), 4—IAC 1 (AB), 5—Balbisiana Franca (BB), 6—Butuhan (BT), listed in Table 1. M = 50 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.

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 (ABB), 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].

Although small differences in genome size between *M. acuminata*, *M. balbisiana*, and other *Musa* species are recognized, Jesus et al. [38] reported that the occurrence of chromosomal recombination and multivalent pairing during meiosis, leading to an imbalance in genome segregation, could generate a continuum in genome sizes between accessions, overlapping differences, and hindering the ability to distinguish the genomic constitution. The frequent occurrence of recurrent homologous exchanges indicates that genomes A and B are prone to recombination, making the genome of *M. balbisiana* a potentially useful source of variability for the development of new cultivars capable of responding to the numerous challenges of banana breeding. For example, bananas with the ABB genome are assumed to be more drought tolerant [40].

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 2).

Table 2. 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 has many advantages over morphological markers [41]. 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 [42] 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 [43] 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 [44], 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*). *M. balbisiana* contains endogenous banana streak virus sequences (eBSVs) that can, in the context of the interspecific genome, spontaneously release infectious viral genomes, and for this reason, 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 [9,45]. Thus, early identification of the "B" genome could be instrumental in enhancing crop management practices in the agricultural field.

Regarding the segregation of eBSV in interspecific crosses, Noumbissié et al. [46], when analyzing a triploid progeny from a cross between a tetraploid (AAAB) and diploid (AA) accessions, were able to demonstrate that (i) most of the alleles are found/transmitted at the frequency expected for the progeny with only 10% bias; (ii) 70% of the loci exhibited a segregation of tetrasomal alleles; and (iii) interspecific intra-chromosomal recombinations occurred for all chromosomal segments studied, suggesting the presence of a large translocation between genomes A and B involving chromosomes 1 and 3.

The presence of the eBSVs virus in the B genome is still the main restriction to the improvement of bananas and plantains, as well as in the exchange of genetic material in *Musa* species [6,9,45]. Despite this concern, Noumbissié et al. [46] proved in their studies that the development of offspring without eBSVs but with B chromosome segments, is still possible from AAAB × AA crosses.

The highly accurate PDS gene markers developed in this study to discriminate between the "A" and "B" genomes in bananas represent a useful new tool for the genetic improvement of Musaceae crops, particularly due to their 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.

4. 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.

In this study we used genotypes with A and B genomes which are used in the genetic breeding of bananas and one accession with BT genome. This accession presented a profile of bands similar to A and B. Thus, it can be inferred that markers PDSMa and PDSMb do not have the power to discriminate the T genome from the A and B genomes. Other research groups may validate our markers in species with S and T genomes, for example, or accessions such as *Musa basjoo* and *Musa intinerans* in order to observe the profile of the amplified bands in these species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10030294/s1, Table S1: 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.

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