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First Application of *FLORICAULA* second intron (*FLint2*) as a Phylogenetic Marker in Bananas (Musaceae)

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Abstract

The utility of potential low-copy genes as molecular markers is becoming more practical for phylogenetic study because they often outperform the multi-copy genes. The application of a low-copy gene, *FLORICAULA* second intron (*FLint2*), that contributes to early flowering as a phylogenetic marker in bananas (Musaceae), has never been reported. Yet, inflorescence remains a dominant distinguishing characteristic in bananas and its closely related species. Hence, this study aimed to determine the potential phylogenetic utility of *FLint2* compared with *Internal Transcribed Spacer* (*ITS*) as a multicopy gene in bananas and closely related species. Results showed that *FLint2* was easily amplified under optimized PCR conditions. The *FLint2* amplicons were found to be medium length (450-500 bp) in bananas and significantly differ from its closely related species of Heliconiaceae and Strelitziaceae (1200-1300 bp). The sequences were GC-rich and highly variable at 89.11%. Individual maximum-likelihood phylogenetic trees of both markers were mostly congruent. The *FLint2* provides an alternative internal topology within bananas and is better at separating the cultivar genome groups, although weak in bootstrap support. The combined sequences improved the tree topology and strong-supported clades. In conclusion, *FLint2* was proven to have high potential phylogenetic utility to intraspecific and genomic levels in bananas and it is also suggested to be suitable for use in closely related species within the Zingiberales order.

Keywords: *FLORICAULA*, *LEAFY*, phylogeny, polymorphism, Musaceae, Zingiberales

1. Introduction

The tropical fruit of bananas (Musaceae) is a globally important cash crop and nutritious food source. More than 1,000 cultivated varieties (cultivars) were recorded, mainly produced and consumed for local home consumption, and a few of them contributed to international trade (Pareek, 2016). Most of today's cultivated bananas presumably come from diploid wild species of *Musa acuminata* (genome A) and *Musa balbisiana* (genome B). Hybridizations that occur among and within those species were generated progenies with various levels of ploidy and genome groups such as AA, AB, BB (diploids); AAA, AAB, ABB (triploids); and AAAA, AAAB, AABB (tetraploids) (De Jesus *et al*., 2013). Furthermore, sterility, parthenocarpy, and human selection have complicated the domestication of bananas (De Langhe *et al*., 2009). Hence, evolutionary study through phylogenetic analysis of *Musa* genetic resources is significant as the basis information for further banana breeding programs.

In plants, molecular markers are mainly derived from nuclear and plastid genomes. The nuclear ribosomal *Internal Transcribed Spacer* (*ITS*) is a multi-copy gene that has been popularly used as a molecular marker in land plants with high resolving power and more informative results than plastid markers such as *trn*L, *mat*K, *rbc*L, *atp*B-*rbc*L, *rps16*, *rpo*C, and others (Ranibala *et al*., 2018; Ha *et al*., 2022; Amer *let al*., 2022; Salih, 2023). The *ITS* has high phylogenetic utility as an intron (non-coding region) because evolution may occur more neutrally. However, multiple divergent *ITS* sequence types and paralogues were detected due to the complex and several phylogenetic scenarios of rRNA loci in hybrids, particularly in allopolyploid bananas. It promotes bad signaling and has a negative effect on phylogenetic inference. Therefore, plasmid cloning is necessary before sequencing (Hřibová *et al*., 2011; Hapsari *et al*., 2018).

The utility of potential low-copy genes as molecular markers is becoming more practical for phylogenetic study because they often outperform the multi-copy genes. The *FLORICAULA*, or *LEAFY*, is one of the low-copy nuclear genes, comprising three exons and two introns. It is one of the main regulatory genes that contributed to controlling the change from vegetative to generative phase, initiating and developing flowers (Yang *et al*., 2017). Particularly, the second intron of the *FLORICAULA* gene (*FLint2*) is considered a recommended marker for phylogenetic

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studies at lower taxonomic levels of flowering plants (Angiosperms) (Grob *et al*., 2004). Several studies have utilized *FLint2* for phylogenetic analyses of various plant species, including *Amorphophallus* (Nikmah *et al*., 2016), orchids (Schlüter *et al*., 2007), papaya (Yu *et al*., 2005), *Brassica* (Pankin *et al*., 2008), *Citrus* (Yingzhi *et al*., 2007), and *Cinnamomum* (Huang *et al*., 2016).

Until today, the phylogenetic study using *FLint2* in bananas has not been investigated. Meanwhile, inflorescence remains a dominant distinguishing characteristic in bananas (Jaitrong and Manthey, 2018; Inta *et al*., 2023) and its closely related species such as the bird of paradise plants (Iles *et al*., 2017; Kholqiyah *et al*., 2024) and gingers (Kress *et al*., 2002; Záveská *et al*., 2016), *Canna* (Sultana *et al*., 2019), and others. This makes *FLint2* allegedly suitable for phylogenetic studies. Both *FLint2* and *ITS* are introns (untranslated gene regions of genomic DNA that are spliced out in the formation of mature RNA molecules) and present in the nuclear genome (Creer *et al*., 2007); thus, a phylogenetic study for the combination sequences is possible. Hence, the purpose of this study was to provide a new opportunity for the potential utility of a low-copy *FLint2* gene in comparison with a multi-copy *ITS* gene as a molecular marker to study the phylogenetic study at the lower taxonomic level of bananas. The finding of this study may become the basis reference of molecular evidence to support further breeding programs and proposing a genetic conservation strategy in bananas, and possibly in its closely related species within the Zingiberales order.

2. Materials and Methods

2.1. Plant Materials

Nine living plant accessions of bananas (*Musa* spp., Musaceae) from East Java, Indonesia, were used as the study material's ingroup. It comprised five cultivars representing four genome groups and four wild species representing their two putative ancestral parents. Two closely related species from the order of Zingiberales, namely Heliconiaceae and Strelitziaceae, were used as the outgroup (Table 1, Figure 1).

Table 1. Plant study material of bananas and closely related species

Code	Species name	Local name	Genome group	Family
M1	*Musa balbisiana*	Pisang Klutuk Ijo	BBw	Musaceae
M2	*Musa balbisiana*	Pisang Klutuk Wulung	BBw	Musaceae
M3	*Musa acuminata* var. *rutilifes*	Pisang Cici	AAw	Musaceae
M4	*Musa acuminata* var. *alasensis*	Pisang Monyet	AAw	Musaceae
M5	*Musa acuminata*	Pisang Gading	AAcv	Musaceae
M6	*Musa acuminata*	Pisang Nangka	AAA/AAB	Musaceae
M7	*Musa x paradisiaca*	Pisang Ongkap	AAB	Musaceae
M8	*Musa x paradisiaca*	Pisang Saba Landa	ABB	Musaceae
M9	*Musa x paradisiaca*	Pisang Ebung	ABB	Musaceae
H1	*Heliconia wagneriana*	Pisang Hias	-	Heliconiaceae
S1	*Ravenala madagascariensis*	Pisang Kipas	-	Strelitziaceae

Figure 1. Inflorescence morphology of bananas and closely related species.

2.2. DNA Isolation, PCR and sequencing

Whole genome DNA was isolated from fresh young leaves using an isolation kit (Promega), following the manufacturer's protocol for plants. PCR was performed using a thermocycler (Bio-rad) with primer pair, i.e., *FLint2*F1 5'-CTTCCACCTCTACGACCAGTG-3' and *FLint2*R1 5'-TCTTGGGCTTGTTGATGTAGC-3' (Grob *et al*., 2014). PCR reactions comprised 30 cycles with an initial denaturation at 94°C for 4 minutes, followed by denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds, extension at 72°C for 30 seconds and final

extension at 72°C for 7 minutes (Nikmah *et al*., 2016). The amplifications were evaluated by electrophoresis 1.5% agarose gel. The PCR products were directly sequenced with ABI Sequencer (Applied Biosystems) at 1stBASE Lab. Sdn Bhd (Malaysia). The *FLint2* sequences from this study have been deposited for open access to the NCBI GenBank with accession numbers OL690520 to OL690530 (Table 1).

2.3. Data analysis molecular polymorphism and phylogenetic tree reconstruction

Raw sequences were evaluated using Seqscanner v.10. Polymorphism and phylogenetic analyses were carried out on separate *FLint2*, and *ITS*, and combined sequences. The *ITS* sequences of the same samples from previous studies (Hapsari *et al*., 2018) were retrieved from the NCBI GenBank for further comparison phylogenetic study (Table 2). Multiple sequence alignments were conducted in ClustalW (Thompson *et al*., 1994). DNA polymorphisms were analyzed with DnaSP 6.12.03 (Librado and Rozas, 2009). Phylogenetic tree reconstructions were performed in MEGA X by Kimura 2 model maximum likelihood, and 1000 bootstraps (Kumar *et al*., 2018). Bootstrap supports were categorized as strong (>85%), moderate (70-85%), weak (50-69%), and very weak (<50%) (Kress *et al*., 2002). Visual comparisons of tree topologies were performed to determine the congruence of phylogenetic signal and combinability of *FLint2* and *ITS* datasets.

3. Results

3.1. FLint2 amplicons

The *Flint2* amplification successfully yielded a single band at an annealing time of 62 °C. The *FLint2* amplicon length in bananas was approximately 450-500 bp, which is significantly different from the outgroup Heliconiaceae and Strelitziaceae (1200-1300 bp) (Figure 2). Furthermore, direct sequencing on *FLint2* amplicons of 9 banana samples yielded raw nucleotides with a length of 520-534 bp, as for *H. wagneriana* at 1,300 bp and *R. madagascariensis* at 1,291 bp. In comparison, *ITS* amplicons in bananas were longer, around 643-651 bp, yet shorter in *H. wagneriana* and *R. madagascariensis*, i.e., 569 bp and 602 bp, respectively. Meanwhile, the GC content of *Flint2* sequences in bananas was high, 56.40-60.30%, but lower in *H. wagneriana* (44.10%) and *R. madagascariensis* (43.20%). Nonetheless, the GC content of *ITS* was higher than *FLint2* (Table 2).

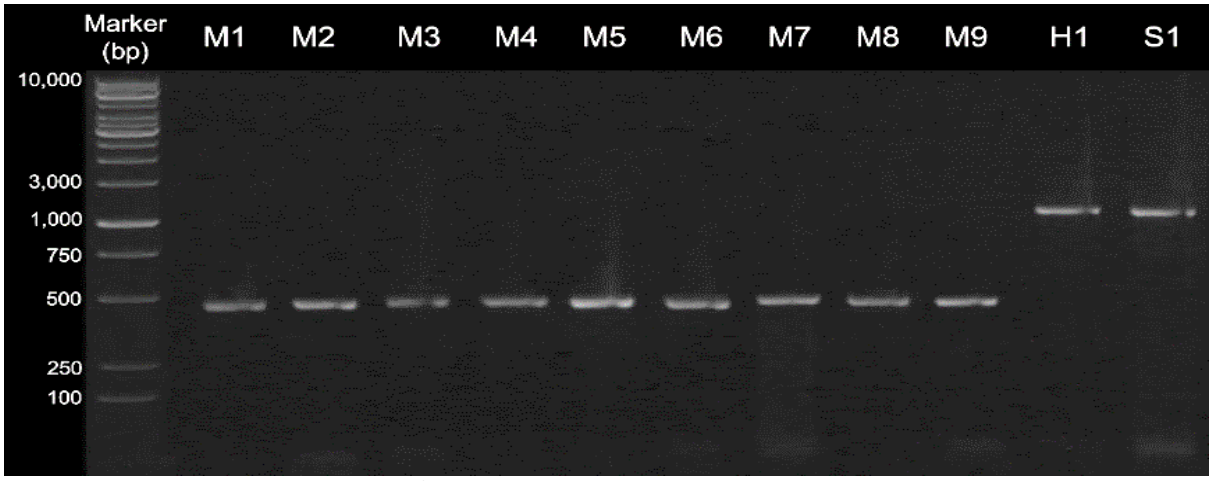

Figure 2. Electrophoregram *FLint2* amplicons of bananas and closely related species.

Table 2. Statistic sequences of *FLint2* and *ITS* in bananas and closely related species

Code	Operational Taxonomic Unit	NCBI acc. number		Seq. length (bp)		GC (%)	
		FLint2	*ITS*	*Flint2*	*ITS*	*Flint2*	*ITS*
M1	*M. balbisiana* Klutuk Ijo	OL690520	KT696444	532	650	58.9	63.7
M2	*M. balbisiana* Klutuk Wulung	OL690521	KT696445	524	651	58.4	63.6
M3	*M. acuminata* var. *rutilifes*	OL690522	KT696459	533	648	59.5	62.9
M4	*M. acuminata* var. *alasensis*	OL690523	KT696462	525	647	58.7	60.7
M5	Pisang Gading (AAcv)	OL690524	KT696473	534	650	59.3	62.0
M6	Pisang Nangka (AAA/AAB)	OL690525	KT696477	526	648	56.4	62.7
M7	Pisang Ongkap (AAB)	OL690526	KT696457	532	650	60.3	62.9
M8	Pisang Saba Landa (ABB)	OL690527	KT696449	520	643	59.5	63.6
M9	Pisang Ebung (ABB)	OL690528	KT696452	520	649	57.7	63.1
H1	Pisang Hias	OL690529	KY215128	1300	569	44.1	70.3
S1	Pisang Kipas	OL690530	FJ428107	1291	602	43.2	73.2

3.2. DNA polymorphisms of FLint2 compared to ITS

The comparison of DNA polymorphism analyses of *FLint2* compared to *ITS* and the combined sequences of both regions in bananas and closely related species are presented in Table 3. The results showed that *FLint2* was highly polymorphic at 89.11%, with a total number of mutations reaching up to 906 events. Further, the mutation events consisted of 44 singleton variables (19 variants, 18 three variants, 7 four variants) and 414 informative parsimonies (105 two variants, 202 three variants, and 107 four variants). The *FLint2* and *ITS* regions were high in

GC content, with *FLint2* having 57.20% GC and *ITS* having 65.10% GC. The *ITS* region was much more conserved (polymorphic 36.90%, monomorphic 63.10%) than *FLint2*. Meanwhile, the combined sequences yielded moderate variations (40.33%) yet relatively conserved (59.67%) ones.

Table 3. Comparison of polymorphisms data of *FLint2*, *ITS* and combined sequences in bananas

Polymorphisms	***FLint2***	***ITS***	**Combined sequences**
Number of all aligned sites	1308	1179	2496
Number of sites with alignment gaps	794	599	1400
Number of sites in the final dataset	514	580	1096
Number of polymorphic sites	458 (89.11%)	214 (36.90%)	442 (40.33%)
Number of monomorphic sites	56 (10.89%)	366 (63.10%)	654 (59.67%)
G+C content	57.20%	65.10%	61.30%
Total number of mutations (Eta)	906	251	506
Number of parsimony informative sites	414	113	311
Number of singleton sites	44	101	131
Haplotype (gene) diversity (Hd±SD)	1.000± 0.039	0.982±0.046	0.982±0.046
Nucleotide diversity (π±SD)	0.480±0.076	0.113±0.029	0.131±0.039
Genetic similarities (%)	38.16-99.82	64.45-100	57.21-100
Number of haplotypes	11	10	10
Number of haplogroups	0	1 (M1+M2)	1 (M1+M2)
Number of clades in all taxa	3	3	3
Number of subclades in Musaceae	4	4	5
Number of clades and subclades in Musaceae with ≥70% BS	2	5	6

Remarks: G=Guanine, C=Cytosine, BS=Bootstrap Support, SD=Standard Deviation, BS=Bootstrap

Due to the high DNA polymorphisms of *FLint2*, haplotype analysis resulted in haplotype gene diversity at maximum level (1.000±0.039) with high nucleotide diversity (0.480±0.076). Further, it was separated into 11 haplotypes with none of the haplogroups. The bananas and closely related species examined had a high genetic variation of *FLint2*, with similarities ranging from 38.16% to 99.82% (Table 3). Meanwhile, the *ITS* and the combined sequences resulted in lower haplotype and nucleotide diversity but were still categorized as high (Hd>0.5; π>0.5%). They resulted in 10 haplotypes with one haplogroup, *i.e*. Pisang Klutuk Wulung and Pisang Klutuk Ijo (*M. balbisiana*), with 100% genetic similarity (Table 2).

3.3. Phylogenetic trees of FLint2, ITS and combined sequences

Individual phylogenetic reconstruction in bananas using *FLint2* and *ITS* resulted in phylogenetic trees, which are primarily congruent. It comprised two main clades in which the Heliconiaceae and Strelitziaceae were separated from Musaceae and served as an outgroup with a strong bootstrap support. Specific to the Musaceae only (ingroup), the phylogenetic tree of *Flint2* was clustered into three clades. The *FLint2* tree demonstrated an alternative internal topology among and within the genome group of bananas but supported by weak to moderate bootstraps. Banana cultivars with two or more A genomes (AAw, AAcv, AAA, and AAB) were clustered in clade 1. Two wild bananas, *M. balbisiana* (BBw), were separated in clade 2, and banana cultivars with ABB genome were separated in clade 3 (Figure 3).

Meanwhile, the *ITS* phylogenetic tree resulted in only two clades. The first clade is similar to *Flint2* and was comprised of banana genome groups AAw, AAcv, AAA, and AAB. The second clade consists of wild *M. balbisiana* and ABB as sisters supported by strong bootstraps (Figure 4). Likewise, the combined analysis of *FLint2* and *ITS* shows an improvement in tree topology and increases the number of clades with solid bootstrap compared to individual analysis. The tree topology of combined sequences was much more congruent with *ITS* than *FLint2* (Table 3, Figure 5).

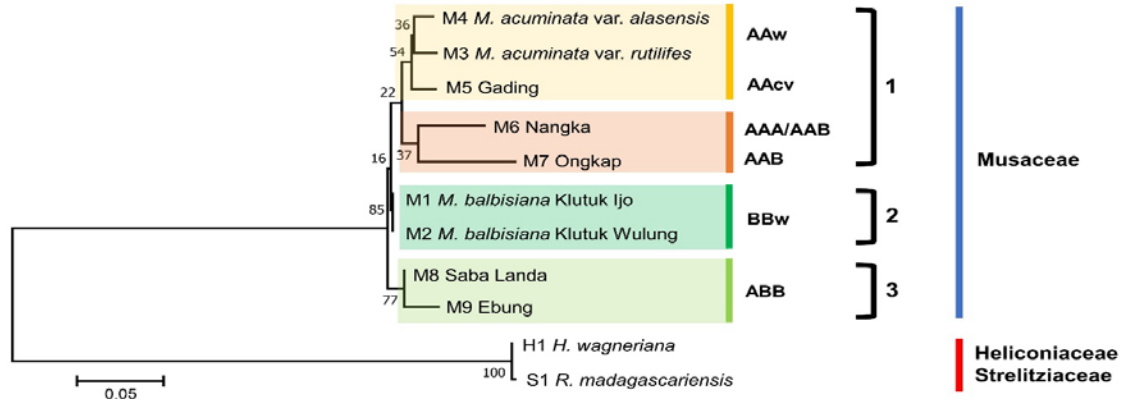

Figure 3. Maximum likelihood phylogenetic tree of *FLint2*

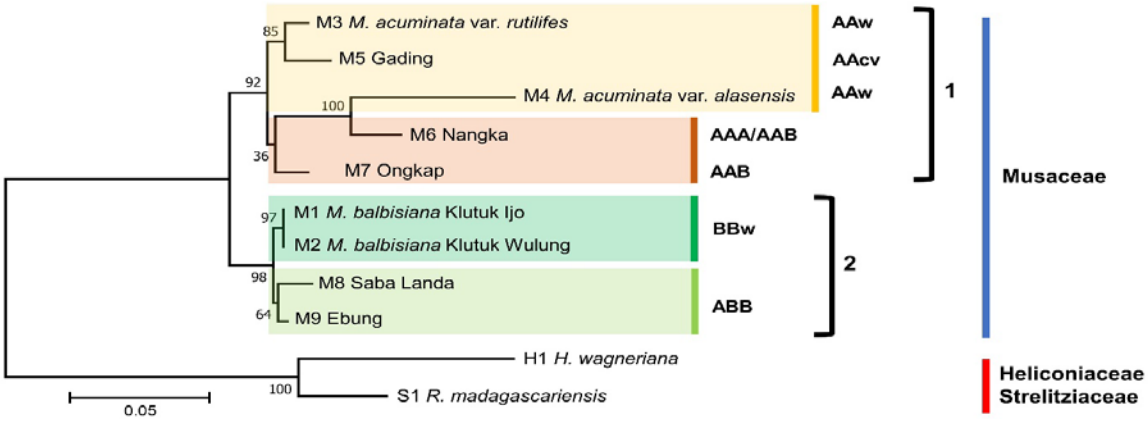

Figure 4. Maximum likelihood phylogenetic tree of *ITS*

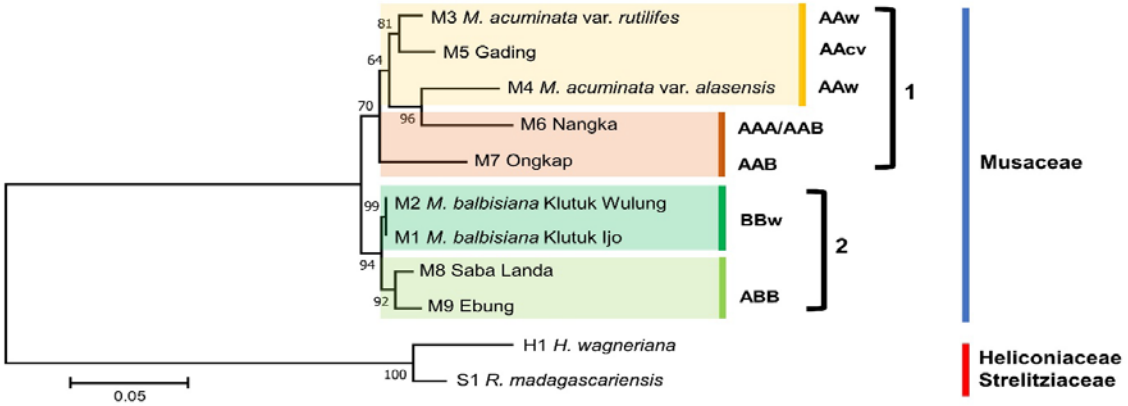

Figure 5. Maximum likelihood phylogenetic tree of combined *FLint2* and *ITS*

4. Discussion

The *FLint2* primers were found to be easily amplified to the whole genome DNA of bananas and closely related species under optimized PCR conditions. The amplifications successfully yielded single bands (Figure 2), thus allowing direct sequencing. In this current study, the *FLint2* primers in bananas need high annealing temperatures to produce single bands at 62 °C; meanwhile, *ITS* needs a low annealing temperature at 53 ºC (Hapsari *et al*., 2018). The *FLint2* primers were known to be strongly temperature-dependent (Nikmah *et al*., 2016). At lower annealing temperatures, the templates resulted in multiple bands, possibly due to nonspecific priming (Ruiz-Villalba *et al*., 2017).

Interestingly, the *FLint2* amplicon length in bananas significantly differs compared to Heliconiaceae and Strelitziaceae. The amplicon's length gaps were about 600-700 bp. Heliconiaceae was found to have the same amplicon size as Strelitziaceae (Figure 2). These results are supported by a previous report by Pankin *et al*. (2008), which stated that *FLint2* was considered highly varied in size, even between genera under the same family. In comparison, *ITS* amplicons in bananas were longer than *Flint2* in wild species and cultivars yet shorter in *H. wagneriana* and *R. madagascariensis* (Table 2). In general, there is no significant difference in the length of the *ITS* amplicon in Angiospermae, which is around 600-700 bp (CBOL, 2009; Hřibová *et al*., 2011).

Furthermore, the *FLint2* sequences in bananas from this study were considered medium in size and sufficient for phylogenetic analysis. BLAST NCBI analysis has

confirmed the generated sequences homologous to partial cds *Floricaula/Leafy* of some species from the Zingiberales order, such as *Curcuma* spp. with a similarity of 91.84%-97.62%, *Globba* sp. with a similarity of 93.33%-95.84% and *Zingiber* spp. with similarity of 91.11%-93.33% (Záveská *et al*., 2016).

Both *FLint2* and *ITS* regions are introns with high GC content. A higher GC content level indicates a higher mutation and recombination events become mutation hotspots (Amit *et al*., 2012; Kiktev *et al*., 2018). Interestingly, comparative DNA polymorphism analysis showed that *FLint2* has higher polymorphism and mutation occurrence than *ITS* and thus will provide better phylogenetic information (Kress *et al*., 2002). Furthermore, haplotype analysis of *FLint2* also resulted in higher haplotype gene diversity than *ITS*. A haplotype is a specific allele or a cluster of DNA sequences of closely linked genes on a chromosome passed down from a common ancestral (Garg *et al*., 2021).

This study found that *FLint2* sequences in bananas and closely related species were highly variable and informative; therefore, they were powerful in differentiation at lower taxonomic levels. This finding is also supported by the fact that the Zingiberales order is classified as a group of highly diverse species, varieties, hybrids, and cultivars, which are distinguished mainly based on morphology, including the flowering organ, which is important for taxonomic differentiation (Kress *et al*., 2002; Iles *et al*., 2017).

Particularly in bananas, about 13 out of 15 distinguishing morphological characters among genome groups are dominated by inflorescence characteristics, including peduncle texture, pedicel length, ovules arrangement, bract curling behavior, bract shape and color, and male flower shape and color (Jaitrong and Manthey, 2018; Gusmiati *et al*., 2018; Inta *et al*., 2023) (Figure 1). Therefore, polymorphisms in *Flint2* sequences that contributed to early flowering allegedly may affect the inflorescence phenotype characteristics of the individual. Polymorphisms commonly occur in nature and are often associated with biodiversity, genetic variation, and adaptation processes (Chung *et al*., 2023).

Maximum likelihood is an accurate method commonly used for phylogenetic analysis. This method searches for the best tree topology with the highest probability or likelihood of character state changes from a precise evolutionary model (Lin *et al*., 2013). The phylogenetic trees of individual and combined sequences resulted in phylogenetic trees which are mostly congruent. It comprised two main clades in which the Heliconiaceae and Strelitziaceae were separated from Musaceae and served as an outgroup with a strong bootstrap support. An outgroup clade provides a time arrow for the historical sequence polarization of all subsequent evolutionary events (Graham *et al*., 2002). Hence, this study supports that Heliconiaceae and Strelitziaceae were primitive relatives of bananas.

Specific to the Musaceae only (ingroup), the phylogenetic tree of *Flint2* was clustered into three clades; meanwhile, in *ITS* and combined sequences, it was separated into two clades. However, the taxon members of the clade were quite similar (Figures 3, 4, 5). Although supported by weak to moderate bootstraps, the *FLint2* phylogenetic tree demonstrated an alternative internal topology and is better at separating the cultivar genome groups. Furthermore, the combined analysis of *FLint2* and *ITS* shows an improvement in tree topology and increases the number of clades with strong bootstrap compared to individual analysis (Table 3, Figure 5). In addition, the phylogenetic trees from this study supported the banana cultivar domestication theory (De Langhe *et al*., 2009) that diploid AAcv first came from intersubspecific hybridization of wild *M. acuminata* (AAw); then, triploid AAA appeared, followed by AAB, and next ABB from hybridization of edible AAcv with wild *M. balbisiana* (BBw). However, due to continuous evolution resulting from genetic variation and mutations, the AAcv and AAA genome groups cannot be separated (Hariyanto *et al*., 2021).

5. Conclusion

The present study is the first report of the application of *Flint2* as a phylogenetic marker in bananas (Musaceae). The finding of this study indicates that *FLint2* has a high potential phylogenetic utility at lower taxonomic levels to intraspecific and genomic levels in bananas. *FLint*2 was easy to amplify and yielded a single product suitable for direct sequencing. It resulted in medium-sized amplicons in bananas but larger in their closely related species of different genera. The *FLint2* sequences were highly variable and informative; therefore, they were powerful enough to differentiate at lower taxonomic levels. The phylogenetic analysis of *FLint2* provides an alternative internal topology within bananas and is better at separating the cultivar genome groups; it also well supports the banana domestication theory. Further research suggests that studies at the intra and interspecific levels of the closely related species in the Zingiberales order should be done using this *FLint2* marker by involving more taxonomically diverse samples.

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References

Amer WM, Shoulkamy MA, Abd El-Baset HD and Faried AM. 2022. Infraspecific identity of the wild *Brassica nigra* (L.) Koch. using morphological, cytogenetics, and molecular (nuclear and chloroplast) approaches. *Jordan J Biol Sci*., **15(3):** 489-500.

Amit M, Donyo M, Hollander D, Goren A, Kim E, Gelfman S, Lev-Maor G, Burstein D, Schwartz S, Postolsky B, Pupko T and Ast G. 2012. Differential GC content between exons and introns establishes distinct strategies of splice-site recognition. *Cell Rep*., **1(5):** 543-556.

CBOL Plant Working Group. 2009. A DNA barcode for land plants. *Proc Natl Acad Sci USA.*, **106(31):** 12794-12797.

Chung MY, Merilä J, Li J, Mao K, Lopez-Pujol J, Tsumura Y and Chung MG. 2023. Neutral and adaptive genetic diversity in plants: An overview. *Front Ecol Evol*., **11**: 1-14.

Creer S. 2007. Choosing and using introns in molecular phylogenetics. *Evol Bioinform*., **3**: pp. 99-108.

De Jesus ON, de Oliveira e Silva E, Amorim EP, Ferreira CF, de Campos JMS, de Gaspari Silva G and Figueira A. 2013. Genetic diversity and population structure of *Musa* accessions in *ex-situ* conservation. *BMC Plant Biol.*, **13**: 41.

De Langhe E, Vrydaghs L, de Maret P, Perrier X and Denham T. 2009. Why bananas matter: An introduction to the history of banana domestication. *Ethnobot Res Appl*., **7**: 165-177.

Garg S. 2021. Computational methods for chromosome-scale haplotype reconstruction. *Genome Biol.,* **101**: pp. 1–24.

Graham SW, Olmstead RG and Barrett SC. 2002. Rooting phylogenetic trees with distant outgroups: a case study from the commelinoid monocots. *Mol Biol Evol*., **19(10):** 1769-1781.

Grob GBJ, Gravendeel B and Eurlings MCM. 2004. Potential phylogenetic utility of the nuclear *FLORICAULA/LEAFY* second intron: comparison with three chloroplast DNA regions in *Amorphophallus* (Araceae). *Mol Phyl Evol*., **30(1):** 13-23.

Gusmiati LH, Hapsari L and Wahyudi D. 2018. Morphological diversity and clustering of 10 cooking bananas (*Musa* cv. Group ABB) collection of Purwodadi Botanic Garden–LIPI. *Floribunda*, **5(8):** 299-314.

Ha DC, Cao PB, Nguyen VD and Le CT. 2022. Molecular phylogeny and deep origins of the hybrid Mokara Dear Heart (Orchidaceae). *Jordan J Biol Sci*., **15(5):** 805-811.

Hapsari L, Azrianingsih R and Arumingtyas EL. 2018. Genetic variability and relationship of banana cultivars (*Musa* L.) from East Java, Indonesia based on the *Internal Transcribed Spacer* region nrDNA sequences. *J Trop Biol Conserv*., **15**: 101-120.

Hariyanto S, Ainiyah RK, Utami ESW and Hapsari L. 2021. Genetic diversity and network within dessert bananas (*Musa acuminata* cv. AA andAAA) inferred by newly designed *matK* marker. *Int J Conserv Sci*., **12(2):** 585-598.

Hřibová E, Jćížková J, Christelová P, Taudin S, de Langhe E and Doleżel J. 2011. The *ITS1-5.8S-ITS2* sequence region in the Musaceae: structure, diversity, and use in molecular phylogeny. *PLoS ONE*, **6**: e17863.

Huang JF, Li L, Conran JG and Li J. 2016. Phylogenetic utility of *LEAFY* gene in *Cinnamomum* (Lauraceae): Gene duplication and polymerase chain reaction-mediated recombination. *J Syst Evol*., **54(3):** 238–249.

Iles WJD, Lagomarsino L, Benson-martin G and Driscoll H. 2017. The phylogeny of *Heliconia* (Heliconiaceae) and the evolution of floral presentation. *Mol Phyl Evol*., **117**: 150-167.

Inta W, Traiperm P, Ruchisansakun S, Janssens SB, Viboonjun U and Swangpol SC. 2023. Evolution and classification of Musaceae based on male floral morphology. *Plants*, **12(8):** 1602.

Jaitrong S and Manthey JA. 2018. Male bud characteristics of diploid, triploid, and tetraploid bananas. *Act Hort*., 1210: pp. 171–176.

Kholqiyah SF, Wahyudi D and Hapsari L. 2024. Genetic diversity and relationship among and within the bird of paradise plants of Strelitziaceae and Heliconiaceae. *Jordan J Biol Sci*., **17(1):** 163-171.

Kiktev DA, Sheng Z, Lobachev KS and Petes TD. 2018. GC content elevates mutation and recombination rates in the yeast *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA*., **115(30):** E7109-E7118.

Kress WJ, Prince LM and Williams KJ. 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): Evidence from molecular data. *Am J Bot*., **89(10):** 1682–1696.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol*., **35(6):** 1547-1549.

Librado P and Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatic*s, **25(11):** 1451-1452.

Lin Y, Hu F, Tang J and Moret BM. 2013. Maximum likelihood phylogenetic reconstruction from high-resolution whole-genome data and a tree of 68 eukaryotes. *Pac Symp Biocomp*., 285-296.

Nikmah IA, Azrianingsih R and Wahyudi D. 2016. Genetic diversity of porang populations (*Amorphophallus muelleri* Blume) in Central Java and West Java based on *LEAFY* second intron marker. *J Trop Life Sci*., 6(1): 23-27.

Pankin AA, Vorobiev VA and Khavkin EE. 2008. Polymorphism of intron 2 of the *FLORICAULA/LEAFY* gene in *Brassica* plants. *Russ J Plant Phyiol*., **55**: 565-570.

Pareek S. 2016. Nutritional and biochemical composition of banana (*Musa* spp.) cultivars. In: Simmonds MSJ and Preedy VR. Nutritional composition of fruit cultivars, Academic Press, Massachusetts, USA. p. 49–81.

Ranibala G, Singh SS, Singh WR and Khan ML. 2018. Molecular identification and phylogeny of *Musa* species from North-Eastern India by *Internal Transcribed Spacer* 2 of nuclear DNA. *Bionature*, **38(4):** 266-276.

Ruiz-Villalba A, Van Pelt-verkuil E, Gunst QD and Ruijter JM. 2017. Biomolecular detection and quantification amplification of nonspecific products in quantitative polymerase chain reactions (qPCR). *Biomol Detect Quant*., **14**: 7-18.

Salih RHM. 2023. Comparative analysis of Cichorieae tribe (Asteraceae) chloroplast genomes: insight to structure, repetitive DNA, and phylogeny. *Jordan J Biol Sci*., **16(3):** 477-502.

Schlüter PM, Kohl G, Stuessy TF and Paulus HF. 2007. A screen of low-copy nuclear genes reveals the *LFY* gene as phylogenetically informative in closely related species of orchids (*Ophrys*). *Taxon*, **56(2):** 493-504.

Sultana N, Akhi SS, Hassan MA and Rahman MO. 2019. Morphological and anatomical investigation among six variants of *Canna indica* L. *Bangladesh J Plant Taxon.*, **26(2):** 219-230.

Thompson JD, Higgins DG, and Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.,* **22(22):** 4673-80.

Yang T, Du M, Guo Y and Liu X. 2017. Two *LEAFY* homologs *ILFY1* and *ILFY2* control reproductive and vegetative developments in *Isoetes* L. *Sci Rep*., **7(1):** 225.

Yingzhi L, Yunjiang C, Nengguo T and Xiuxin D. 2007. Phylogenetic analysis of mandarin landraces, wild mandarins, and related species in China using nuclear *LEAFY* second intron and plastid *trnL-trnF* sequence. *J am Soc Hort Sci*., **132(6):** 796-806.

Yu Q, Moore PH, Albert HH, Roader AHK and Ming R. 2005. Cloning and characterization of a *FLORICAULA/LEAFY* ortholog, *PFL*, in polygamous papaya. *Cell Res*., **15(8)**: 576–584.

Záveská E, Fér T, Šída O, Marhold K and Leong-Škorničková J. 2016. Hybridization among distantly related species: Examples from the polyploid genus *Curcuma* (Zingiberaceae). *Mol Phyl Evol*., **100**: 303-321.