

## ***In silico* Comparisons of the *Ethylene Response Factor 1 (ERF1)* Gene Between Malaysian Wild Banana (*Musa acuminata* ssp. *malaccensis*) and Pisang Klutuk Wulung (*Musa balbisiana*)**

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### **ABSTRACT**

*Musa balbisiana* (B genome) has been observed to have a higher tolerance of biotic and abiotic stresses than *Musa acuminata* (A genome). *Ethylene Response Factor 1 (ERF1)* is a gene activator for pathogenesis-related proteins (PR proteins) such as basic chitinases and beta-1,3-glucanase. There are numerous *ERF1* gene studies about *Oryza sativa*, but information about the banana *ERF1* gene, especially in the B genome (*Musa balbisiana* “Pisang Klutuk Wulung”), has still not been explored thoroughly. Using annotated genomic data in an A genome (*Musa acuminata* ssp. *malaccensis*) and genomic data in a B genome (*Musa balbisiana* “Pisang Klutuk Wulung”), research on the *ERF1* gene can be conducted at the gene sequences and amino acid sequences levels. The *Musa acuminata* (A genome) *ERF1* gene nucleotide sequence was retrieved from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The *Musa balbisiana* (B genome) *ERF1* gene nucleotide sequence was identified with the nucleotide Basic Local Alignment Search Tool (BLASTn) using an A genome *ERF1* gene sequence as a query. Both *ERF1* gene nucleotide

sequences and amino acid sequences in the A and B genomes were annotated and compared. Seven annotated genome *ERF1* gene sequences from the A and B genomes were identified with the probability that these genes were actively transcribed in cell activity. *ERF1* gene comparisons between the A and B genomes showed that nucleotide composition, gene structure, gene position in each respective chromosome, *ERF*

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clusterization, identified motif, and amino acid composition in each of the identified motifs have similar characteristics.

*Keywords:* AP2/ERF domain, comparative genomics, ethylene response factor 1, sequence annotation

## INTRODUCTION

Banana plants are commonly grown in tropical and subtropical countries. They can be classified into two groups based on their genomic composition: *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) (Simmonds, 1959; Sumardi & Wulandari, 2010). The Cavendish banana cultivar (AAA) covers 90% of globally planted bananas and reduces diversity, especially at the plantation site (Drenth & Kema, 2021; Food and Agriculture Organization of the United Nations [FAO], 2019). Low diversity at plantation sites may cause problems in plants like susceptibility to a particular disease, such as the devastating Fusarium wilt (also known as “Panama Disease”), which attacked the cultivar Gros Michel (de Bellaire et al., 2010; Marín et al., 2003).

The ethylene hormone regulates the plant’s defense response against the pathogen through signal transduction. The first contact in this signal transduction is the ethylene response (ETR) receptor. Next, the ETR receptor activates a signal transduction cascade by releasing the block exerted by the CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) on *Ethylene Insensitive 2* (*EIN2*) (Karlova et al., 2014). Finally, this release will actuate *EIN3/EIN3-like* (*EIL*) primary transcription factor genes (Tieman et al.,

2001), leading to the activation of ethylene response factors (ERF) (Adams-Phillips et al., 2004; Bapat et al., 2010).

The ERF family, part of the AP2/ERF superfamily, is the most widely studied transcription family in plants (Riechmann & Meyerowitz, 1998). The *ERF* gene family is the gene activator for many genes (Pirrello et al., 2012). In Nakano et al. (2006), *ERF* genes were divided into groups I–X based on identified motifs besides the AP2/ERF domain. Therefore, ERF groupings based on Nakano et al. (2006) can be used as a reference to identify motifs besides AP2/ERF domain and determine the function of the sequences acquired in this study. The *ERF1* gene has been thoroughly studied in the *Arabidopsis thaliana*. It acts as a gene activator for pathogenesis-related proteins (PR proteins), such as basic-chitinases and beta-1,3-glucanase. In a previous study by Lakhwani et al. (2016), a genome-wide analysis was conducted to identify members of the AP2/ERF family in *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) as well as changes leading to neofunctionalization of genes. However, information about the *ERF1* gene in the *Musa balbisiana* genome remains unexplored.

The study aimed to compare the *ERF1* genes in the A (*Musa acuminata* “DH Pahang”) and B genomes (*Musa balbisiana* “Pisang Klutuk Wulung”), including their gene structure (exon-intron architecture), gene position on the chromosome, and gene function (protein clustering and motifs). Therefore, the *ERF1* gene study in *Musa*

*balbisaniana* “Pisang Klutuk Wulung” can be conducted using the annotated genomic information data of *Musa acuminata* “DH Pahang” (D’Hont et al., 2012).

## MATERIALS AND METHODS

### ***ERF1* Gene Nucleotide Sequences and Amino Acid Sequences Identification**

A genome *ERF1* gene sequences were retrieved from KEGG (Kyoto Encyclopedia of Genes and Genomes) ([https://www.genome.jp/dbgetbin/www\\_bget?K14516+K14517](https://www.genome.jp/dbgetbin/www_bget?K14516+K14517)) (Kanehisa & Goto, 2000). BLAST (Basic Local Alignment Search Tool) on the banana genome hub site (<https://banana-genome-hub.southgreen.fr/blast>) was used to identify *ERF1* gene sequences from the B genome with the highest similarity approach (Eisen, 1998). Translated protein sequences from identified *ERF1* genes of both genomes were classified with the phylogenetic tree approach. Identified *ERF1* genes and 128 amino acid *Oryza sativa* sequences (Nakano et al., 2006) was used as a phylogenetic tree construction dataset. Phylogenetic tree construction was based on Nakano et al.’s (2006) study on the platform Molecular Evolutionary Genetics Analysis (MEGA-X) (version 10.1.5) (Kumar et al., 2018).

### ***ERF1* Gene Nucleotide Comparison in *Musa acuminata* ssp. *malaccensis* and *Musa balbisaniana***

Seven nucleotide sequences were retrieved and analyzed in pairs between the *MaERF1* A and B genome genes with pairwise sequence alignment and Needleman-Wunsch as the

algorithm (Needleman & Wunsch, 1970) on the European Bioinformatics Institute site (EMBL-EBI) ([https://www.ebi.ac.uk/Tools/psa/emboss\\_needle/](https://www.ebi.ac.uk/Tools/psa/emboss_needle/)) (Madeira et al., 2019).

### ***ERF1* Gene Structure Prediction and Visualization**

Seven *ERF1* of B genome gene structures were predicted using the FGENESH+ program (Solovyev, 2007). Each predicted *ERF1* gene on both A and B genomes was visualized using the CLC Sequence Viewer (version 8.0). The location of the genes in chromosomes for both *Musa acuminata* ssp. *malaccensis* and *Musa balbisaniana* were retrieved from the BLAST search and visualized using MS Paint (version 11.2201.22.0).

### ***ERF1* Motif Identification and Comparison in *Musa acuminata* ssp. *malaccensis***

*ERF1* amino acid sequences in both *Musa acuminata* ssp. *malaccensis* and *Musa balbisaniana* motifs were identified with Multiple Expectation maximizations for Motif Elicitation (MEME) suite (Bailey & Elkan, 1994) using *ERF* group IX consensus motifs from Nakano et al. (2006) as motif targets. The identified motif in the *Musa acuminata* ssp. *malaccensis* and *Musa balbisaniana* amino acid sequences were visualized using the Weblogo3 program with default parameters (<http://weblogo.threeplusone.com/create.cgi>) (Crooks et al., 2004).

## RESULTS AND DISCUSSION

### *ERF1* Genes Identification in *Musa balbisiana* and *Musa acuminata*

Through searching and selections from BLASTn *ERF* gene results, seven *ERF1* genes in *Musa acuminata* (A genome) were retrieved from the KEGG database with the following gene IDs from NCBI (National Centre for Biotechnology Information): “103971653” (*MaERF1\_1*), “103972093” (*MaERF1\_2*), “103973681” (*MaERF1\_3*), “103981246” (*MaERF1\_4*), “103981564” (*MaERF1\_5*), “103983138” (*MaERF1\_6*), “103985947” (*MaERF1\_7*). Seven *ERF1* genes in *Musa balbisiana* (B genome) were identified with gene identification: *MbERF1\_1*, *MbERF1\_2*, *MbERF1\_3*, *MbERF1\_4*, *MbERF1\_5*, *MbERF1\_6*, and *MbERF1\_7*. *ERF1* genes in A and B genomes had a similarity of above 90% (Supplementary 1).

### *ERF1* Genes Structure and Composition in *Musa acuminata* and *Musa balbisiana*

The *ERF1* genes in *Musa acuminata* ssp. *malaccensis* and *Musa balbisiana* have no introns. The longest *ERF1* gene was *MaERF1\_7* in the A genome and *MbERF1\_7* in the B genome. The shortest *ERF1* gene was *MbERF1\_5* in the B genome and *MaERF1\_5* in the A genome (Figure 1). Like relatively short sequences, including the intron, coding sequences, and exon compared to other genes, these are the housekeeping genes' typical genomic features (Eisenberg & Levanon,

2003; Vinogradov, 2004). M. Liu et al.'s study (2019) also showed that 79.3% of *FtERF* genes had no introns. Thus, there is a probability that these *ERF1* genes are transcribed actively in cell activities. Furthermore, the nucleotide compositions of all seven *ERF1* genes in both genomes showed a similarity percentage above 95%, the data for which have been presented in Supplementary 1. These results showed that *ERF1* genes of *Musa acuminata* ssp. *malaccensis* and *Musa balbisiana* have a close evolutionary relationship because the nucleotide varieties were minimal.

### *ERF1* Genes Location in *Musa acuminata* and *Musa balbisiana* Chromosomes

The *ERF1* genes' positions in A and B genome chromosomes were similar: *ERF1\_1* (for A and B genomes) in chromosome 2, *ERF1\_2* in chromosome 11, *ERF1\_4* and *ERF1\_5* in chromosome 4, *ERF1\_6* in chromosome 1, and *ERF1\_7* in chromosome 5. *ERF1\_3* gene in both genomes was not identified in the genome database and identified as uncategorized in the chromosome in the Banana Genome Hub database (Figure 2). Chromosome 4 has the most identified *ERF1* genes, with two in the A (*MaERF4* and *MaERF5*) and B genomes (*MbERF4* and *MbERF5*). Multiple *ERF1* genes in both banana genomes resulted from gene replication, also identified in Tartary buckwheat (*Fagopyrum tataricum*) (M. Liu et al., 2019).

*In silico* Comparisons of *ERF1* Gene Between Bananas

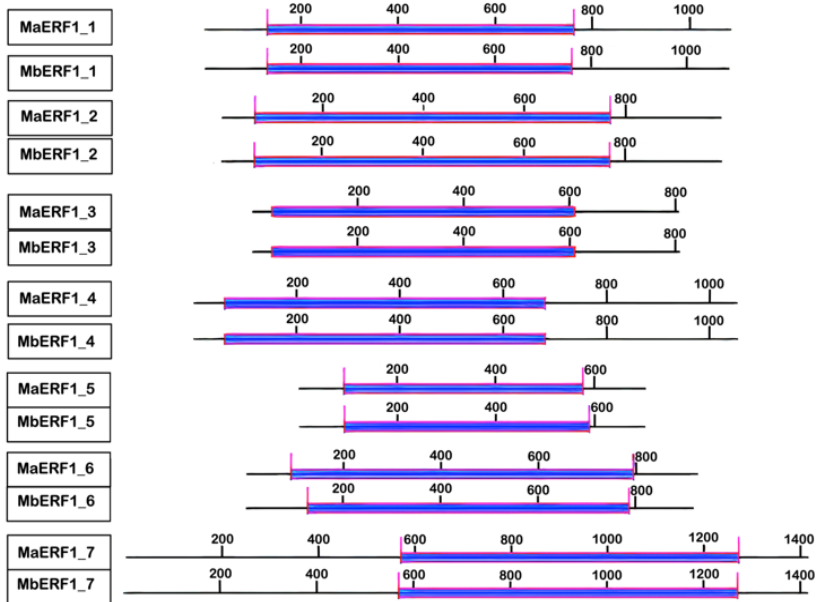


Figure 1. The structure of the *ERF1* gene in *Musa acuminata* (A genome) and *Musa balbisiana* (B genome). Bars are marked in the base pair (bp). The figure was visualized using the CLC Sequence Viewer (version 8.0)

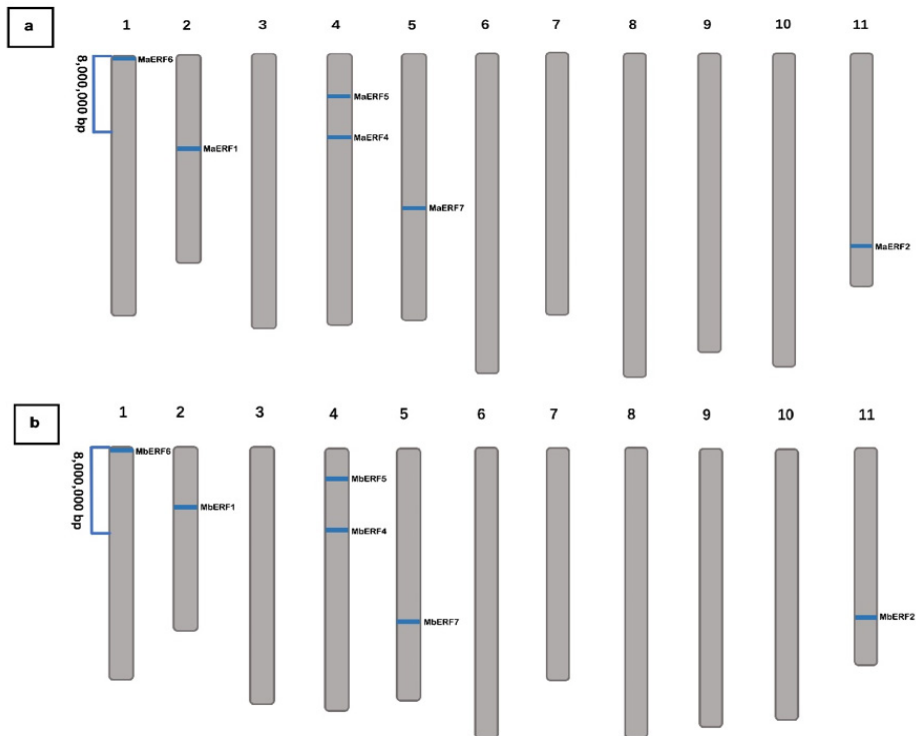


Figure 2. Position of *ERF1* genes in (a) *Musa acuminata* (A genome) and (b) *Musa balbisiana* (B genome) chromosomes. The figure was visualized using MS Paint (version 11.2201.22.0)



### Phylogenetic Tree Analysis

Phylogenetic tree analysis (Figure 3) showed that MaERF1 and MbERF1 amino acid sequences (red-colored area) were in one clade with the *Oryza sativa* ERF group IX (blue-colored area). *Oryza sativa* was used as a comparison species because its ERF gene database was already established in a previous study by Nakano et al. (2006). *Oryza sativa* is monocotyledonous like the *Musa* species. So, based on the data, *Oryza sativa* is a widely accepted model for monocots that gives evidence of the similarities and differences between the two major groups of higher plant species and has a close lineage with *Musa* (Goff et al., 2002; Izawa & Shimamoto, 1996). The MaERF1 and MbERF1 amino acid

sequences within group IX were grouped with *Oryza sativa* ERF group IXc. of the other genes, *MaERF1\_1* grouped as sister taxa with *MbERF1\_1*, *MaERF1\_2* with *MbERF1\_2*, *MaERF1\_3* with *MbERF1\_3*, *MaERF1\_4* with *MbERF1\_4*, *MaERF1\_5* with *MbERF1\_5*, *MaERF1\_6* with *MbERF1\_6*, and *MaERF1\_7* with *MbERF1\_7*. Phylogenetic tree analysis results showed that all *MaERF1* and *MbERF1* were ERF IXc based on ERF classification by Nakano et al. (2006), who also explained that the ERF gene in group IX has disease resistance roles in tomato and tobacco (Fischer & Dröge-Laser, 2004; Huang et al., 2004). ERF1 in the A and B genomes were closely related because they are grouped as sister taxa.

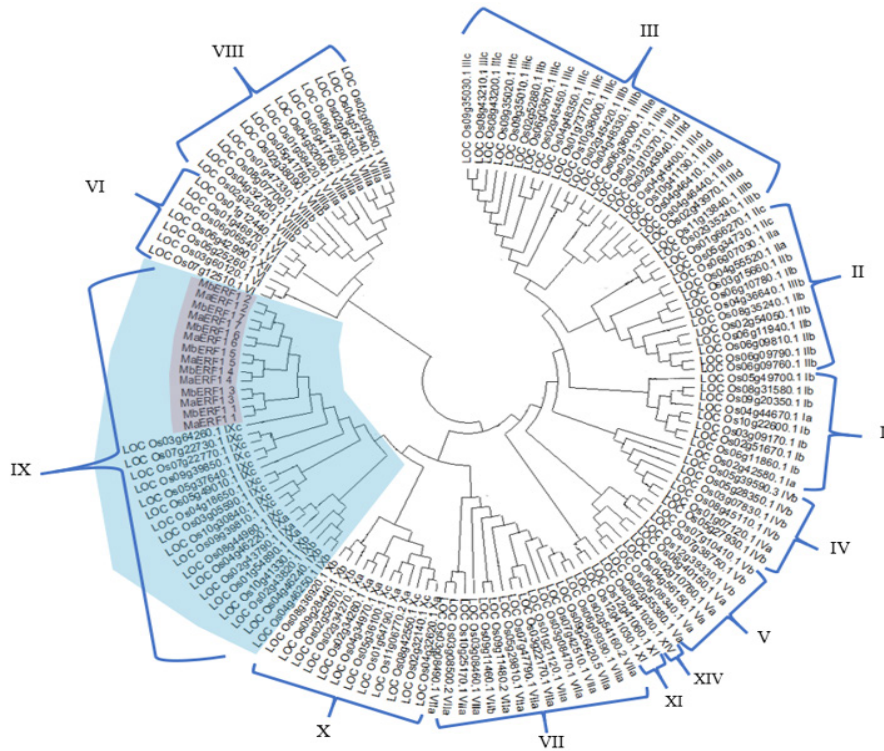


Figure 3. Phylogenetic tree of *Musa acuminata* (A genome), *Musa balbisiana* (B genome), and *Oryza sativa* ERF genes. The figure was constructed using MEGA-X software (version 10.1.5)

### Amino Acid Motif Composition Comparison

There were four motifs in total in the MaERF1\_1, MbERF1\_1, MaERF1\_2, MbERF1\_2, MaERF1\_3, MbERF1\_3, MaERF1\_4, MbERF1\_4, MaERF1\_5, MbERF1\_5, MaERF1\_7, and MbERF1\_7 amino acid sequences (Figure 4). There was one domain AP2/ERF (red box) and three motifs which consisted of CMIX-1 (cyan box), CMIX-3 (purple box), and CMIX-4 (orange box). In addition, three motifs were detected in MaERF1\_6 and MbERF1\_7. AP2/ERF, CMIX-4, and CMIX-1 were

detected, while in MbERF1\_6, AP2/ERF, and CMIX-4 motifs were detected. On the other hand, domain AP2/ERF was detected in all MaERF1 and MbERF1 amino acid sequences, indicating that these amino acid sequences are classified as the ERF subfamily (Riechmann & Meyerowitz, 1998). Motifs besides AP2/ERF are transcription factors likely to have similar essential functions (Rashid et al., 2012; Reyes et al., 2004) that consist of transcription factors' activities, interactions between proteins, and nuclear localization (L. Liu et al., 1999).

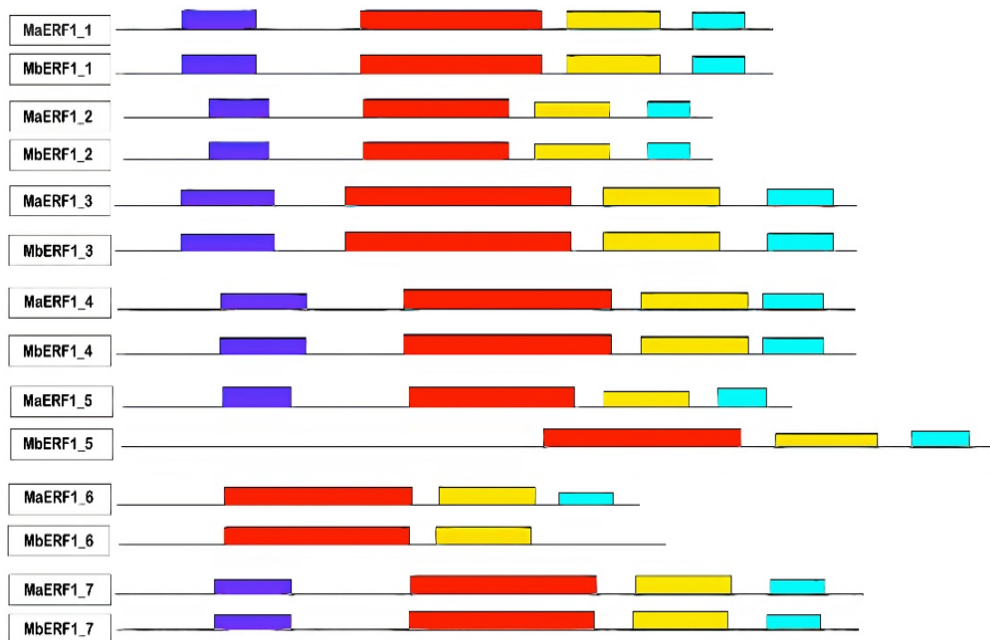


Figure 4. The amino acid motif of ERF sequences compares *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) chromosomes. The box shows domain AP2/ERF (red box) and three motifs, which consist of CMIX-1 (cyan box), CMIX-3 (purple box), and CMIX-4 (orange box). The figure was visualized using the MEME suite program

Each identified amino acid motif of MaERF1 and MbERF1 was also analyzed. Overall, all the identified motifs between MaERF1 and MbERF1 have high similarities. For example, in Figure 6, both

MaERF1 and MbERF1 have similar CMIX-4 motifs with slight amino acid composition differences detected at positions 4, 9, 19, and 22.

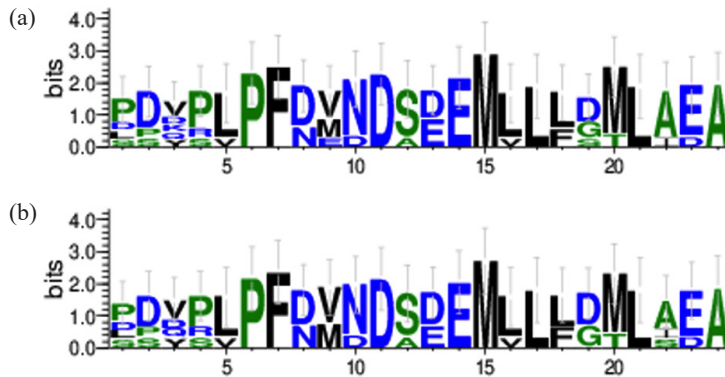


Figure 5. CMIX-3 motif sequences of (a) *Musa acuminata* (A genome) and (b) *Musa balbisiana* (B genome). The figure was visualized using the Weblogo3 program

In addition to the CMIX-4 motif, the amino acid composition of the CMIX-3 motif varied, but both generally have the same amino acid consensuses at each position (Figure 5). Based on the analysis, the CMIX-1 motif has the shortest amino acid sequence compared with other identified motifs, and variation between MaERF1 and MbERF1 in this motif was relatively low (Figure 7). On the other hand, the AP2/ERF motif was the longest in both genomes, with 58 amino acids. This result confirmed the previous study by Wessler (2005), which showed that AP2/ERF length was around 60–70 amino acids. Besides its length,

domain motifs AP2/ERF in both genomes were conserved. There were two conserved amino acids in both genomes, YRG in position numbers 1–3 and RAYD in position numbers 39–42. YRG conserved motif was the rich basic hydrophilic amino acids located at N-terminus and has a function in DNA binding (Okamuro et al., 1997). On the other hand, the RAYD conserved domain has an essential function in domain structure and function. However, in both genomes, the AP2/ERF domain has L (leucine) in position number 39 rather than R (arginine) (Okamuro et al., 1997).



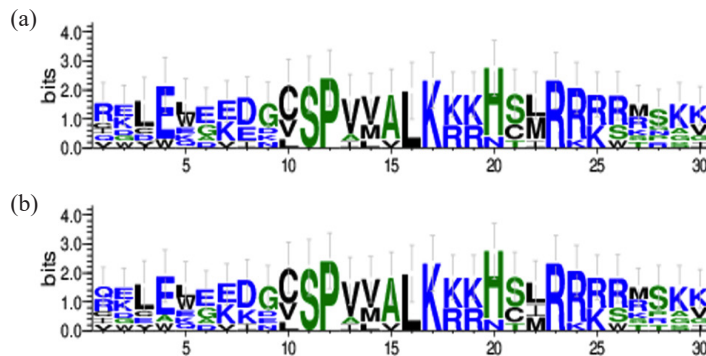


Figure 6. CMIX-4 motif sequences of (a) *Musa acuminata* (A genome) and (b) *Musa balbisiana* (B genome). The figure was visualized using the Weblogo3 program

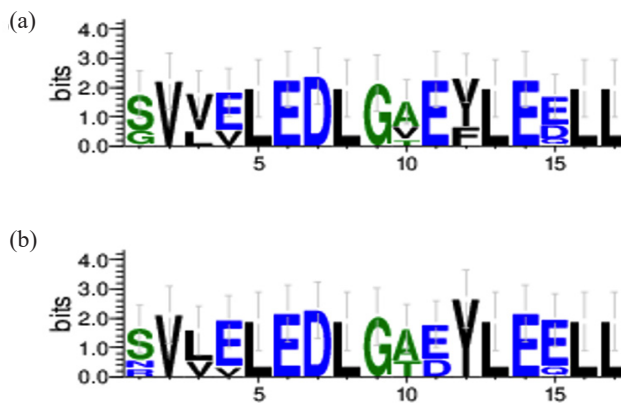


Figure 7. CMIX-1 motif sequences of (a) *Musa acuminata* (A genome) and (b) *Musa balbisiana* (B genome). The figure was visualized using the Weblogo3 program

Based on this study’s comparison with Lakhwani et al. (2016), there were several differences in identified motifs outside the AP2/ERF domain. In Lakhwani et al. (2016), the identified motif in group IX of the *ERF* gene was only the LNFP motif. On the other hand, this study identified three motifs: CMIX-1, CMIX-3, and CMIX-4. Furthermore, Lakhwani et al. (2016) showed that the identified motif outside AP2/ERF domain was named the LNFP motif, but based on Fujimoto et al. (2000), the LNFP motif is the part of the AP2/ERF domain

amino acid residues. Considering both studies used the *in silico* approach, further confirmation is needed to elucidate the differences.

## CONCLUSION

Based on this study, the *ERF1* genes of *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) showed high similarities in their nucleotide sequences, gene structures, and positions in the chromosome, phylogenetic clustering, and the motif predicted in the protein sequences.

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## AUTHORS' CONTRIBUTIONS

GK, FMD, and HN designed the study. GK analyzed the data. RRP reviewed and edited the manuscript. All authors wrote, read, and approved the final version of the manuscript.

## CONFLICT OF INTEREST

All authors declare no conflict of interest.

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*In silico* Comparisons of *ERF1* Gene Between Bananas

```

#-----
#
# Aligned_sequences: 2
# 1: MaERF1_2
# 2: MbERF1_2
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 992
# Identity:   967/992 (97.5%)
# Similarity: 967/992 (97.5%)
# Gaps:       2/992 ( 0.2%)
# Score: 4732.5
#
#-----
MaERF1_2      1  GCTCGAGAAGAAGCAAGGAGGTGGGCGAAACCTGCGCTCTGCCTCGTTC      50
      |||
MbERF1_2      1  GCTCGAGAAGAAGCAAGGAGGTGGGCCAAACCTACGCTCTGCCTCGTTC      50
      |||
MaERF1_2     51  CTCCTTTTCTCCCTATGGACTACTACCTCTTCGACTCCCTGAACCACGA     100
      |||
MbERF1_2     51  CTCCTCTCTCCCTATGGACTACTACCTCTTCGACTCCCTGAACCACGA     100
      |||
MaERF1_2    101  ACACTCGCCGGAATCCTCCACCGGTTCCACCGAGCCCTTCCATGGGCCG     150
      |||
MbERF1_2    101  ACACTCGCCGGAATCGTCTACCGGTTCCCCGAGCCCTTCCATGGGCCG     150
      |||
MaERF1_2    151  GCGTTGGGCTGTTCTACCCGGACGTTCTCTCCCTTCAACATGGATGAC     200
      |||
MbERF1_2    151  GCGTTGGGCTGTTCTACCCGGACGTTCTCTCCCTTCAACATGGATGAC     200
      |||
MaERF1_2    201  TCCGAGGAGATGCTGCTGCTCGGAATGCTCGCGGAGGCCTCCGAAAGGC     250
      |||
MbERF1_2    201  TCCGAGGAGATGCTGCTGCTCGGAATGCTCTCGGAGGCCTCCGAAAGGC     250
      |||
MaERF1_2    251  GTCGTCCTCGTCGGAGGCCTGCGACCGGAGCGTGATCCGGGCCAAGGAAG     300
      |||
MbERF1_2    251  GTCGTCCTCGTCGGAGGCCTGCGACCGGAGCGTGATCCGGGCCAAGGAAG     300
      |||
MaERF1_2    301  AAGAGGTGGATTGCGGGAGCAAGGCGGCGGATGAGCCGAAGGAGAAGTCG     350
      |||
MbERF1_2    301  AAGAAGTGGATTGCGGGAGCAAGGCGGCGGATGAGCCGAAGGAGAAGTCG     350
      |||
MaERF1_2    351  TACCGGGGGTGCGGAAGCGGCCGTGGGGGAAGTTCGCGGCGGAGATCAG     400
      |||
MbERF1_2    351  TACCGGGGGTGCGGAAGCGGCCGTGGGGGAAGTTCGCGGCGGAGATCAG     400
      |||
MaERF1_2    401  GGACTCGACGCGGCACGGGATACGGGTGTGGCTGGGGACGTTTCGACAGCG     450
      |||
MbERF1_2    401  GGACTCGACGCGGCACGGGATACGGGTGTGGCTGGGGACGTTTCGACAGCG     450
      |||

```



*In silico* Comparisons of *ERF1* Gene Between Bananas

```
#=====
#
# Aligned_sequences: 2
# 1: MaERF1_3
# 2: MbERF1_3
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 808
# Identity:   793/808 (98.1%)
# Similarity: 793/808 (98.1%)
# Gaps:       2/808 ( 0.2%)
# Score: 3893.0
#
#
#=====

MaERF1_3      1 GCCTGAGAACCACCGATCTCCACCCCAATTACGATGGATCCTTCAT      50
  |||.|||||||||||||||||||||||||||||||||||||||||
MbERF1_3      1 GCCTAAGAACCACCGATCTCCACCCCAATTACGATGGATCCTTCAT      50

MaERF1_3     51 ATCTCCAGTCCCAGAGTTACGACGAATTCTCGCCGGAAGATTCTATCGC   100
  |||.|||||||||||||||||||||||||||||||||||||||||
MbERF1_3     51 ATCTGCAGTCCCAGAGTTACGACGAATTCTCGCCGGAAGATTCTATCGC   100

MaERF1_3    101 CTCCCCTTCGACGTCAACGACAGCGACGAGATGCTCCTGTTCGACACACT   150
  |||||||||||||||||||||||||||||||||||||||||||
MbERF1_3    101 CTCCCCTTCGACGTCAACGACAGCGACGAGATGCTCCTGTTCGACACACT   150

MaERF1_3    151 GCGGGAGGCCACCCCTTGAACCCGGTCTGGCAGGGGAGGGTCGACCGA   200
  |||||||||||||||||.|||||||||||||||.||||||
MbERF1_3    151 GCGGGAGGCCACCCCTTGAACCCGGTCTGGCAGGGGAGGGCCGACCGA   200

MaERF1_3    201 CGGGCGAGCCGTGCTACCGCGGCGTCCGTAAGCGGCCGTGGGGGAAGTTC   250
  |||||||||||||||||||||||||||||||||||||||||||
MbERF1_3    201 CGGGCGAGCCGTGCTACCGCGGCGTCCGTAAGCGGCCGTGGGGGAAGTTC   250

MaERF1_3    251 GCGGCGGAGATAAGGGACTCGACGCGGGGAGGGGCGCGGGTGTGGCTGGG   300
  |||||||||||||||||||||||||||||||||||||||||||
MbERF1_3    251 GCGGCGGAGATAAGGGACTCGACGCGGGGAGGGGCGCGGGTGTGGCTGGG   300

MaERF1_3    301 GACGTTTCGACACCGCGGAGGCCCGCCCTGGCGTACGACAGGCGGCGT   350
  |||||||||||||||||||||||||||||||||||||||||||
MbERF1_3    301 GACGTTTCGACACCGCGGAGGCCCGCCCTGGCGTACGACAGGCGGCGT   350

MaERF1_3    351 TCTCCATGCGGGGGCGGCTCGCCGTGCTCAACTTCCCAGTGGAGCAGGTG   400
  |||||||||||||||||||||||||||||||||||||||||||
MbERF1_3    351 TCTCCATGCGGGGGCGGCTCGCCGTGCTCAACTTCCCAGTGGAGCAGGTG   400

MaERF1_3    401 CAGGAGTCTTGC AAGAGCTCGAATGGGATAAGGACA AACTGCTCCCCCAT   450
  |||||||||||||.|||||||||||||||.||||||
MbERF1_3    401 CAGGAGTCTTGC AAGAGCTCGAATGGGATAAGGATA AACTGCTCCCCCAT   450
```

MaERF1_3	451	CATGGCACTCAAGAAGAAGCACTCGTTAAGGAGGAGGAGGACCTGCAG	500
MbERF1_3	451	CATGGCACTCAAGAAGAAGCACTCATTAAAGGAGGAGGAGGACCTGCAG	500
MaERF1_3	501	TGAGCGGGAAGACCAAGGTGGCACAGAGCAGGATACAGAGTGCCTGGAA	550
MbERF1_3	501	TGAGCGGGAAGACCAAGGTGGCACAGAGCAGGAGACAGAGTGCCTAGAA	550
MaERF1_3	551	CTGGAGGACTTGGGCACAGATTACTTGGAGGAGTTACTGAGAGTTTCCGA	600
MbERF1_3	551	CTGGAGGACTTGGGAACAGATTACTTGGAGGAGTTACTGAGAGTTTCCGA	600
MaERF1_3	601	ACTTGCATAA-CTCAGTAAACCTGCTCCCTGCAGCTCAAATCAAACCTCCA	649
MbERF1_3	601	ACTTGCATAACCTCAGTAAACCTGCTCCCTGCAGCTAAAATCAAACCTCCA	650
MaERF1_3	650	TGGAACCTCGGATCCAGCTTTCGGTTCCTTCATCATTATTTATTCTGCTTG	699
MbERF1_3	651	TGGAACCTCGGATCCAGCTTTCGATTCCCTTCATCATTATTTATTCTGCTTG	700
MaERF1_3	700	CATCATTACTTGGTCCCCCAAATGATGTAACAGGAAAATGTATGTGTTT	749
MbERF1_3	701	CATCATTACTTGGTCCCCCAAACGATGTAACAGGAAAATGTATGTGTTT	750
MaERF1_3	750	CAGATCCGTTGAATCCATGC-AAAGATGAGCGCGATGGCTTTGCTTGCTT	798
MbERF1_3	751	CAGATCCGTTGAATCCATGCAAAAGATGAGCGCGATGGCTTTGCTTGCTT	800
MaERF1_3	799	TACACCAA 806	
MbERF1_3	801	TACACCAA 808	







*In silico* Comparisons of *ERF1* Gene Between Bananas

```

#-----
#
# Aligned_sequences: 2
# 1: MaERF1_5
# 2: MbERF1_5
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 706
# Identity:   685/706 (97.0%)
# Similarity: 685/706 (97.0%)
# Gaps:       6/706 ( 0.8%)
# Score: 3324.0
#
#-----
MaERF1_5      1  GACCTCCTTCCAAGGTTCCGAATCCTCATCGATATCCACAGCCGGATCGC      50
   |||||||||||||||||||||||||||||||||||.|.||||||||||||||
MbERF1_5      1  GACCTCCTTCCAAGGTTCCGAATCCTCATCGGTGTCCACAGCCGGATCGC      50

MaERF1_5     51  AGGAGGAATCTCTCCCTTCGACGTGAACGACGCCGGTGAGATGCTCCTG     100
   |||||||.||||||||||||||||||||||||||||||||||||||||||
MbERF1_5     51  AGGAGGAGTCTCTCCCTTCGACGTGAACGACGCCGGTGAGATGCTCCTG     100

MaERF1_5    101  TTCGACATGCTCATCGAGTCCGCCATGACCACGAAGACGTGACGGGCAA     150
   ||||||||||||||||||||||||||||||||||||||||||||||||
MbERF1_5    101  TTCGACATGCTCATCGAGTCCGCCATGACCACGAAGACGTGACGGGCAA     150

MaERF1_5    151  AGAGGCGGAGTCGAAGGGCCGACGGCGAGCGGGAAGAGCTACCGAGGGG     200
   ||||||||||||||||||||||||||||||||||||||||||||||||
MbERF1_5    151  AGAGGCGGAGTCGAAGGGCCGACGGCGAGCGGGAAGAGCTACCGAGGGG     200

MaERF1_5    201  TGCGGAGGCGGCCGTGGGGCAAGTTCGCGGCTGAGATCAGGGACTCGACG     250
   |||||||||||||||||||||||||||||||||||.||||||||||||||
MbERF1_5    201  TGCGGAGGCGGCCGTGGGGCAAGTTCGCGGCGGAGATCAGGGACTCGACG     250

MaERF1_5    251  CGGCAGGGGGTGCGGGTGTGGCTGGGCACGTTTCGACAGCGCGGAGGCCG     300
   ||||||||||||||||||||||||||||||||||||||||||||||||
MbERF1_5    251  CGGCAGGGGGTGCGGGTGTGGCTGGGCACGTTTCGACAGCGCGGAGGCCG     300

MaERF1_5    301  CGCCCTGGCTACGACCAGGCGGCGCTGTCGATGAGGGGGGCGACGGCCG     350
   |||||||||||||||||||||||||||||||||||||||||||||.
MbERF1_5    301  CGCCCTGGCTACGACCAGGCGGCGCTGTCGATGAGGGGGGCGACGGCCG     350

MaERF1_5    351  TGCTCAACTTTCGGCGGAGCGCGTGCGGGAGTCGCTGCGGGGGCTGGAG     400
   |||||||.|||||||||||||||||||||.||||||||||
MbERF1_5    351  TGCTCAACTTTCGGCGGAGCGCGTGCGGGAGTCGCTGCGGGGGCTGGAG     400

MaERF1_5    401  CTGGCGAAGGACGGGTGCTCCCCGGTGGTGGCGCTGAAGAAGAAGCACTG     450
   |||||||||||.||||||||||||||||||||||||||||||
MbERF1_5    401  CTGGCGAAGGACGGGTGCTCCCCGGTGGTGGCGCTGAAGAAGAAGCACTG     450

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MaERF1_5	451	CATGAGGAGGAGGAGGAAGAGAAAGGTGAGGGAGTCGAGTGGGGAGGAGG	500
		.	
MbERF1_5	451	CATCAGGAGGAGGAGGAAGAGAAAGGTGAGGGAGTCGAGTGG--GGAGG	497
MaERF1_5	501	GCGTAGTGGAATTAGAGGA-CTTGGGAGTGGAGTTC TTGGAGGACCTCTT	549
		.                 .	
MbERF1_5	498	GCGTAGTGGAATTAGAGGATTTTGGGAGTGGAGTTC TTGGAGGACCTCTT	547
MaERF1_5	550	GGGGCTTTTCAGGGCTTGCAGTCAAGTGCATAGTTTGGCC	599
		.                 .	
MbERF1_5	548	GGGGCTATCAGGGCTTGCAGTCAAGTGCATAGTTTGGCC	597
MaERF1_5	600	AACCTAATGATATTTTAAATATATTAATATGGATATTAAGTTGACTGTCA	649
		.	
MbERF1_5	598	AACCTTATGATATTTTAAATATATTAATATGGATATTAAGTTGACTGTCA	647
MaERF1_5	650	ATTAGATTTACTGTAATGGACACATGTGCAAGT-TTTTGTATAGTTTGT	698
		.     .	
MbERF1_5	648	ATTAGATTTACTGTAAT-GACACATGTGCAAGTGTTTTTATAGTTATGT	696
MaERF1_5	699	TAAGAT	704
MbERF1_5	697	TAAGAT	702

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#-----
#
# Aligned_sequences: 2
# 1: MaERF1_6
# 2: MbERF1_6
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 927
# Identity:      899/927 (97.0%)
# Similarity:   899/927 (97.0%)
# Gaps:         8/927 ( 0.9%)
# Score: 4401.5
#
#-----

MaERF1_6      1  AGCCGTCCTTACGATCAAGCTTAGCAGTTGCACTGATACTACTGAGACAA      50
|||||
MbERF1_6      1  AGCCGTCCTTATGATCAAGCTTAGTAGTAGCACTGATACTACTGAGACAA      50
|||||

MaERF1_6     51  GCGGAAGGCAGAGCAATCTCTGGCTTGTCTTCTCTTCGATGGATTACT     100
|.|||||
MbERF1_6     51  GAGGAAGGCAGAGCAATCCCTATCTTGTCTTCTCTTCAATGGATTACT     100
|.|||||

MaERF1_6    101  CTCTCTCCTTTCACCTCCATAAACCAGGAACACTCATCTGAGTCTCCACG     150
|||||
MbERF1_6    101  CTCTCTCCTTTCACCTCCATAAACCAGGAACACTCATCTGAGTCTCCACG     150
|||||

MaERF1_6    151  TACTCGCCCAGGTCCTCGGCAACCAGCGGCTTCGGGCTCGTCTGCCCTGA     200
|||||
MbERF1_6    151  TACTCGCCCAGGTCCTCGGCAACCAGCGGCTTCGGGCTCGTCTGCCCTGA     200
|||||

MaERF1_6    201  CAAGCCCCTTCGGTTCGACGAGAACGACTCCGAGGAGATGCTGCTGCTTA     250
|||||
MbERF1_6    201  CAAGCCCCTTCGGTTCGACGAGAACGACTCCGAGGAGATGCTGCTGCTTA     250
|||||

MaERF1_6    251  GCATGCTCGCAGAGGCCTCAGGCAAGGCGGCGTCGTCGCTCGTCCGCGGAG     300
|||||
MbERF1_6    251  GCATGCTCGCAGAGGCCTCAGGCAAGGCGGCGTCGTCGCTCGTCCGCGGAG     300
|||||

MaERF1_6    301  GTCCTTGACAGCCGAGTTACCCCGACCAAGGAAGAAGAGGTGGAATC      350
|||||
MbERF1_6    301  GTCCTTG-----ATTTTACCCCGACCAAGGAAGAAGAGGTGGAATC      342
|||||

MaERF1_6    351  GAGAAGCAAGGTGGGTCATGACACAAAGGGAGAGAAGCCCTACCGCGGGG     400
|||||
MbERF1_6    343  GAGAAGCAAGGTGGGTCATGACACAAAGGGAGAGAAGTCTACCGCGGGG     392
|||||

MaERF1_6    401  TGAGACGGCGGCCGTGGGGGAAGTTCGCCGCCGAGATAAGAGACTCAACG     450
|||||
MbERF1_6    393  TGAGACGGCGGCCGTGGGGGAAGTTCGCCGCCGAGATAAGAGACTCAACG     442
|||||

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MaERF1_6	451	CGGC	CGGGATT	CGCGT	GTGGC	TGGGA	ACGTT	CGAC	AGCG	CGGAG	GCAGC	500	
MbERF1_6	443	CGGC	CGGGATT	CGCGT	GTGGC	TGGGA	ACGTT	CGAC	AGCG	CGGAG	GCAGC	492	
MaERF1_6	501	TGCG	TGGCTT	ACGAC	CAGGC	GGCGT	TCTCG	ATGC	GGGG	ACGAC	GGCGG	550	
MbERF1_6	493	TGCG	TGGCTT	ACGAC	CAGGC	GGCGT	TCTCG	ATGC	GGGG	ACGAC	GGCGG	542	
MaERF1_6	551	TGCT	CAATTT	CCCGT	TGGAG	AGAGT	TCGGG	AGT	CGCT	GC	GGGG	CGTGAAG	600
MbERF1_6	543	TCCT	CAATTT	CCCGT	TGGAG	AGAGT	TCGGG	AGT	CGCT	GC	GGGG	CGTGAAG	592
MaERF1_6	601	TACG	AGGAG	GAGAG	ATTGGG	CTGTC	GCCCG	TGGT	GGCG	TCAAG	CGGAG	650	
MbERF1_6	593	TACG	CGGAG	GAGAG	ATTGGG	CTGTC	GCCCG	TGGT	GGCG	TCAAG	CGGAG	642	
MaERF1_6	651	GAAT	ACCCT	GAGG	AGGAAG	TCGAC	GAGCA	AGA	AAGGCC	AAAGG	CCGGG	GAGG	700
MbERF1_6	643	GAAT	ACCCT	GAGG	AGGAAG	TCGAC	GAGCA	AGA	AAGGCC	AAAGG	CCGGG	GAGG	692
MaERF1_6	701	TGAG	GACGG	CGGAG	AGTGT	GTTGG	AGTTG	GAGG	ACCTG	GGGAG	CAGAG	TAC	750
MbERF1_6	693	TGAG	GACGG	CGGAG	AGTGT	GTTGG	AGTTG	GAGG	ACCTG	GGGAG	CAGAG	TAC	742
MaERF1_6	751	TTGG	AGGAG	CTCTT	GAGCAC	CTCAGG	TTTG	CCAG	GCCGT	GTTGA	ACCGC	800	
MbERF1_6	743	TTGG	AGGAG	CTCTT	GAGCAC	CTCAGG	TTTG	CCAG	GCCGT	GTTGA	ACCGC	792	
MaERF1_6	801	AACT	CTCAAT	CCTCG	AGAC	CATGTT	CTCTG	TATAC	TTTCT	TGTTT	CCCTT	850	
MbERF1_6	793	AACT	CTCAAT	CCTCA	AGAC	CGTTCT	CTGTAT	ACTTT	CTTG	TTTCC	CTT	842	
MaERF1_6	851	TCTT	CTTTCC	TTCATT	GTTCCA	ATCCTG	CAGCAC	AAAGA	AAGCT	CT		900	
MbERF1_6	843	TCTT	CTTTCC	TTCATT	GTTCCA	ATCCTG	CAGCAC	AAAGA	AAGCT	CT		892	
MaERF1_6	901	AAGA	ATTCTA	CTTCTT	TCTCTG	TCCA					927		
MbERF1_6	893	AAGA	ATTCTA	CTTCTT	TCTCTG	TCCA					919		



MaERF1_7	451	TCGTTCAAGTATAAGAAGGAGCGGGCTTAGATCCTGTGCGACTGGACGAGC	500
MbERF1_7	449	TCGTTCAAGTATAAGAAGGAGCGGGCTTAGATCCTGTGCGACTGGACAAGC	498
MaERF1_7	501	TCGAGCACGAACAAGGAGGTGGGAGACACCCTGATTTCTCTCTCTCTCA	550
MbERF1_7	499	TCGAGCACGAACAAGGCGGTGGGAGACACCCTGATTTCTCTTTCTCTCA	548
MaERF1_7	551	TCCCTTCCTTTCTCCCCCATGGACTACTCCCTCTTCCAGTCGCTACAC	600
MbERF1_7	549	TCCCTTCCTTTCTCCCCCATGGACTACTCCCTCTTCCAGTCGCTACAC	598
MaERF1_7	601	TCGCCGGAATCTTCCACTGGCTCCGGCAGCCCTTCCCTGGACCGGCGT	650
MbERF1_7	599	TCGCCGGAATCTTCCACTGGCTCCGGCAACCCCTTCCCTGGACCGGCGT	648
MaERF1_7	651	CGGGCTGTTCTACCCGGACGTTCTGTCCCGTTCGACATGAACGACTCCG	700
MbERF1_7	649	CGGGCTGTTCTACCCGGACGTTCTGTCCCGTTCGACATGAACGACTCCG	698
MaERF1_7	701	AGGAGATGCTCCTCCTCGGAATGCTCGGGAGGCCCTCCGGTAAGGCGTCG	750
MbERF1_7	699	AGGAGATGCTCCTCCTCGGAATGCTCGGGAGGCCCTCCGGTAAGGCGTCG	748
MaERF1_7	751	TCCTCGTTAGAGGCCCTGCGAGCGCAGCCAGCCAGCCCAAGGAGGAAGA	800
MbERF1_7	749	TCCTCGTTAGAGGCCCTGCGAGCGCAGCCAGCCAGCCCAAGGAGGAAGA	798
MaERF1_7	801	GGTGGATTCGCAGAGCAAGGTGGCGGACGATCCCAAGGTGAAGTCGTACC	850
MbERF1_7	799	GGTGGATTCGCAGAGCAAGGTGGCGGACGATCCCAAGGAGAAGTCGTACC	848
MaERF1_7	851	GGGGGGTGAGAAAGCGCCGTGGGGGAAGTTCGCGGCGGAGATCCGGGAC	900
MbERF1_7	849	GGGGGGTGAGAAAGCGCCGTGGGGGAAGTTCGCGGCGGAGATCCGGGAC	898
MaERF1_7	901	TCGACGCGGCACGGCATAACGGGTGGCTGGGAACGTTTCGACAGCGCGGA	950
MbERF1_7	899	TCGACGCGGCACGGCATAACGGGTGGCTGGGAACGTTTCGACAGCGCGGA	948
MaERF1_7	951	GGCCGCGGCGCTGGCGTACGACCAGGCCGCTTCTCGATGCGGGGCTCGA	1000
MbERF1_7	949	GGCCGCGGCGCTGGCGTACGACCAGGCCGCTTCTCGATGCGGGGCTCGA	998
MaERF1_7	1001	CGGCGGTGCTCAATTTCCCGGTGGACCGGGTGC GGAGTCGCTGAACGGC	1050
MbERF1_7	999	CGGCGGTGCTCAATTTCCCGGTGGACCGGGTGC GGAGTCACTGAACGGC	1048
MaERF1_7	1051	ATGAAATGCTGGGATGAACAGGAGGAGGGGGTGTGCGCCGGTGGTGGT	1100
MbERF1_7	1049	ATGAAATGCTGGGAGGAACAGGAGGAGAAGGGGGTGTGCGCCGGTGGTGGT	1098



