Genome-wide identification and expression analysis of the β-amylase genes strongly associated with fruit development, ripening, and abiotic stress response in two banana cultivars

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Abstract β-amylase (BAM) is an important enzyme involved in conversion of starch to maltose in multiple biological processes in plants. However, there is currently insufficient information on the BAM gene family in the important fruit crop banana. This study identified 16 *BAM* genes in the banana genome. Phylogenetic analysis showed that MaBAMs were classified into four subfamilies. Most *MaBAMs* in each subfamily shared similar gene structures. Conserved motif analysis showed that all identified MaBAM proteins had the typical glyco hydro 14 domains. Comprehensive transcriptomic analysis of two banana genotypes revealed the expression patterns of *MaBAMs* in different tissues, at various stages of fruit development and ripening, and in responses to abiotic stresses. Most *MaBAM*s showed strong transcript accumulation changes during fruit development and late-stage ripening. Some *MaBAMs* showed significant changes under cold, salt, and osmotic stresses. This finding indicated that *MaBAMs* might be involved in regulating fruit development, ripening, and responses to abiotic stress. Analysis of five hormone-related and seven stress-relevant elements in the promoters of *MaBAMs* further revealed that BAMs participated in various biological processes. This systemic analysis provides new insights into the transcriptional characteristics of the *BAM* genes in banana and may serve as a basis for further functional studies of such genes.

Keywords β-amylase, banana, genome-wide identification, fruit development, abiotic stress

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1 Introduction

Starch is the main storage carbohydrate in plants, and is rapidly degraded to release the primary chemical energy and organic matter for grain germination, seedling growth, endosperm development, and response to abiotic stresses through five major enzymes, including α-amylase (AMY), β-amylase (BAM), limit dextrinase (PUL), β-glucosidase, and α-glucan phosphorylase (PHO)[1–5]. AMY initiates starch breakdown in the germinating grain[6]. BAM converts starch to β-maltose[7,8]. PUL catalyzes the hydrolysis of α-1,6 glucosidic linkages to release dextrins[9]. β-glucosidase is responsible for efficient hydrolytic activity[10]. PHO is mainly involved in the reversible cleavage of α-1,4-linked glucose polymers into α-D-glucose-1-phosphate[11]. However, several studies found that AMY, PUL, β-glucosidase, and PHO only have minor roles in transient starch breakdown in living tissues (e.g., *Arabidopsis thaliana* leaves)[12,13], while BAM is the major enzyme[14].

BAM catalyzes the hydrolysis of α-1,4 linked oligo and poly glucans and is primarily responsible for hydrolysis of starch and degradation of transient starch to generate β-maltose as the predominant sugar exported from the chloroplast at night[8,15]. BAM is a member of glycosyl hydrolase family 14. It has typical glyco hydro 14 domains (PF01373) at the N terminus and a relatively central localization, is widely distributed in higher plants and microorganism and is encoded by a multigene family[7,8,15]. So far, genome-wide analyses have identified nine BAMs in *Arabidopsis*[5], 10 in rice, 13 in corn, 11 in *Brachypodium distachyon*, 10 in sorghum, and 10 in millet[16]. Biochemical and genetic analyses have revealed that the function of *BAM* genes is related to plant

germination, growth, development, and maturation. A BAM protein from fenugreek was reported to participate in seed germination[15]. Overexpression of *BAM7* and *BAM8* in *Arabidopsis* controlled shoot growth and development through crosstalk with brassinosteroid signaling[17]. Expression of *Bmy1* gene in endosperm was involved in the development and maturation of barley grain[18]. In addition, expression analyses of the *BAM* gene family in various species indicated that its expression is altered in response to various abiotic stresses, such as cold, salt or drought[19–21]. Further studies support the suggestion that *BAM* genes play a role in plant response to abiotic stress. Mutant analysis indicated that *Arabidopsis* BAM1 protein decreases stomatal opening and improves drought tolerance, due to reduced starch breakdown in guard cells[20]. *Arabidopsis* BAM8 has been found to be involved in cold tolerance and maltose accumulation[22] while BAM proteins in barley has been identified as important regulators of salt tolerance[23]. Taken together, these studies have shown that the *BAM* gene family is involved in regulating plant germination, growth, development, maturation, and response to various stresses.

Banana (*Musa acuminata*) is not only the most popular high-starch fresh fruit (almost 20% in unripe banana fruit) but also an important staple food in some Africa and Latin American countries[24,25]. Starch degradation to sugar is very important in banana, since sugar concentration is a major component of fruit quality and economic value. Meanwhile as a large annual monocotyledonous herbaceous plant, banana production is frequently destroyed by various abiotic stresses during growth and development, such as cold, salt and drought[26–28]. Thus, investigations of key candidate genes involved in fruit development and responses to multiple stresses, based on complete genome sequences, are necessary to improve quality and enhance stresses tolerance[29,30]. However, few studies on identification and characterization of banana genes based on genome sequencing data have been reported[30]. To our knowledge, only one *BAM* gene has been reported to have been cloned in banana[31]. Considering the importance of the *BAM* gene family in plant growth and development, as well as response to abiotic stresses, we decided to conduct a genome-wide analysis of the banana genome.

In this study, we identified 16 *MaBAM* genes and analyzed their phylogenetic relationship, gene structure, protein motifs, and expression patterns in different tissues, different stages of fruit development and ripening, and the response to abiotic stresses (cold, salt, and drought) in two banana genotypes. Further, we analyzed the hormone-related and stress-relevant cis-elements in the promoters of *MaBAM* genes. This comprehensive study has increased our understanding of *BAM* genes associated with fruit development processes and abiotic stresses responses, and will provide a crucial foundation for future studies of crop improvement medicated by BAMs.

2 Materials and methods

2.1 Plant materials and treatments

In this study, two banana cultivars, BaXi Jiao (*M. acuminata* AAA group cv. Cavendish, BX) and Fen Jiao (*M.* AAB group Fenjiao, FJ), were selected to perform comparative analyses. BX is a triploid cultivar with high yield, high quality, and long-term storage, and is widely cultivated in tropical and subtropical regions in China. FJ is a triploid cultivar, but with a different genotype (AAB) compared with BX (AAA), and has the characteristics of good flavor, rapid ripening, and tolerance to abiotic stresses. It is widely cultivated in the Guangdong and Hainan Provinces of China. The two cultivars were obtained from the banana plantation at the Chinese Academy of Tropical Agricultural Sciences (Danzhou, Hainan, China). Roots, leaves, and fruits at 80 days after emergence from the pseudostem (DAF) were sampled for expression analysis in different tissues. For the banana fruit developmental process, fruits of 0, 20, and 80 DAF, which represent fruit developmental stages of budding, cutting flower, and harvest stages, were collected from both cultivars. For postharvest ripening, BX fruit were stored for 0, 8, and 14 d postharvest (DPH) and FJ 0, 3, and 6 DPH, which represented ripening stages of green, more green than yellow and full yellow according to Pua et al.[32]. Five-leaf stage young banana plants of both cultivars with uniform growth were grown in soil at 28°C, 70% RH and 200 μmol·m^{-2}·s^{-1} light intensity in 16 h light/8 h dark cycle. For salt and osmotic treatments, five-leaf stage banana plants were irrigated with 300 mmol·L^{-1} NaCl and 200 mmol·L^{-1} mannitol for 7 d. For cold treatment, banana plants were maintained at 4°C for 22 h.

2.2 Identification and phylogenetic analyses of the *MaBAM* gene family in banana

Whole banana (*M. acuminata*) protein sequences were downloaded from the banana genome database (http://banana-genome.cirad.fr)[25]. BAM amino acid sequences from *Arabidopsis* and rice were obtained from the TAIR (http://www.arabidopsis.org) and RGAP (http://rice.plant-biology.msu.edu) databases, respectively. The Hidden Markov Model (HMM) profiles of the BAM typical conserved domains (glyco hydro 14 domains; PFAM: PF01373) (http://pfam.sanger.ac.uk) were used to query the predicted BAM proteins in the banana genome databases using HMMER software (http://hmmer.org)[33]. BLAST analysis was also used to identify the predicted banana BAMs with all BAMs from *Arabidopsis* and rice as queries. Conserved domain search of the potential banana BAMs was further validated using CDD (http://www.ncbi.nlm.nih.gov/cdd) and PFAM (http://pfam.sanger.ac.uk) databases. The accession number of all identified banana

BAMs are given in Table S1 (Appendix A). Phylogenetic analysis of BAM amino acid sequences from banana, *Arabidopsis*, and rice was carried out using Clustal X 2.0 and MEGA 5.0 software with bootstrap values for 1000 replicates[34].

2.3   Protein properties and gene structure analysis

Molecular mass and isoelectric points of the MaBAM proteins were predicted using the ExPASy database (http://expasy.org). Motifs of MaBAM proteins were analyzed using MEME software (http://meme-suite.org) and annotated by InterProScan database search (http://www.ebi.ac.uk/Tools/pfa/iprscan). Structural features of the *MaBAM* genes were identified using Gene Structure Display Server (GSDS) software (http://gsds.cbi.pku.edu.cn). The sequences of promoters of *MaBAM* genes were obtained from the banana genome database (http://banana-genome.cirad.fr)[25]. The 2000 bp upstream genomic sequences of these *MaBAM* genes was truncated to confirm the transcription start site using database (http://www.fruitfly.org/seq_tools/promoter.html) and the cis-acting elements via PlantCARE software (http://bioinformatics.psb.ugent.be/webtools/plantcare/html).

2.4   Transcriptomic analysis

Total RNA from each sample was extracted using a plant RNA extraction kit (Tiangen, China) and used to construct cDNA libraries according to the protocols supplied by Illumina. The sequencing was performed with an Illumina GAII following the manufacturer's instructions with two replicates per sample. The sequencing depth was 5.34X on average. Adapter sequences in the raw sequence reads and low quality sequences were removed using FASTX-toolkit and FastQC, respectively, and clean reads were obtained and mapped to the DH-Pahang genome (*M. acuminata*, $2n = 22$, A genome)[25]. Transcriptome assemblies were constructed with Cufflinks[35]. Gene expression levels were calculated as fragments per kilobase of exon per million fragments mapped (FPKM). DEGseq was used to identify differentially expressed genes.

3   Results

3.1   Identification and phylogenetic analysis of banana BAM family members

To identify all banana BAM family members, HMM searches using the BAM typical conserved domains (glyco hydro 14 domain; PFAM: PF01373) as queries, as well as BLAST searches using *Arabidopsis* and rice BAM sequences as queries, were performed in the banana genome database. After validating the BAM domain using

the CDD and PFAM database, a total of 16 non-redundant *MaBAM* genes were identified. The *MaBAM* genes were named according to their homologous genes in *A. thaliana*. The 16 predicted MaBAM proteins ranged from 224 (MaBAM5a) to 702 (MaBAM7a) amino acid residues and the relative molecular mass varied from 25.676 (MaBAM5a) to 77.438 (MaBAM7a) kDa, with isoelectric points in the range of 5.00–9.56 (Appendix A, Table S1), suggesting their potentially different roles in regulating biological processes under different conditions.

To study the evolutionary relationships of BAM family proteins, a phylogenetic tree was constructed by aligning 9, 10, and 16 BAM proteins from *Arabidopsis*, rice, and banana using Clustal X 2.0 and MEGA 5.0 software (Fig. 1). Based on the phylogenetic tree, all MaBAM proteins were grouped into four clades, Clades I, II, III, and IV. Clades I, II, and III are large with more than four MaBAM members, whereas Clade IV contains two MaBAM proteins, similar to those in the rice[5,16]. However, in banana, the number of MaBAM3 proteins in Clade I and MaBAM5 proteins in Clade II were obviously greater than in *Arabidopsis* and rice.

3.2   Gene structure and conserved motifs analysis of banana *MaBAM* genes

The exon-intron structural evolution of the *MaBAM* genes was mapped using the GSDS software. Evolutionary analysis further confirmed the classification of the 16 banana MaBAMs into four groups (Fig. 2), which was consistent with the cluster analysis (Fig. 1). Gene structure analysis showed that the *MaBAM* genes contained four exons in Clade I, three to seven exons in Clade II, eight to nine exons in Clade III, and three exons in Clade IV. This suggests that the majority of *MaBAM* genes in the same group share similar exon-intron organization and the gene structure might reflect gene evolution.

To explore the conserved motifs and functional prediction from banana MaBAM sequences, a total of 15 conserved motifs in the MaBAMs were identified using MEME software and annotated with the InterPro database (Fig. 3). The results suggest that 11 motifs (motifs 1–7 and motifs 9–12) were annotated as glyco hydro 14 domains, which are basic characteristics of the BAM gene family. All 16 identified MaBAMs contained the typical glyco hydro 14 domain motifs. All MaBAMs in Clade I had the motifs 1–13. Most MaBAMs (except MaBAM5a) in Clade II shared motifs 1–5, 7–9, 11–13, and motif 14 at the C-terminal, whereas the MaBAMs in Clade III had motifs 1–7, 9–13, 15 and lacked motif 14 at the C-terminal. MaBAM9a and MaBAM9b in Clade IV contained motifs 2–11, 13 and lacked motifs 1, 12, and 14–15. These results suggest that most MaBAMs in the same group share conserved motif regions and further support the phylogenetic analyses of banana BAMs.
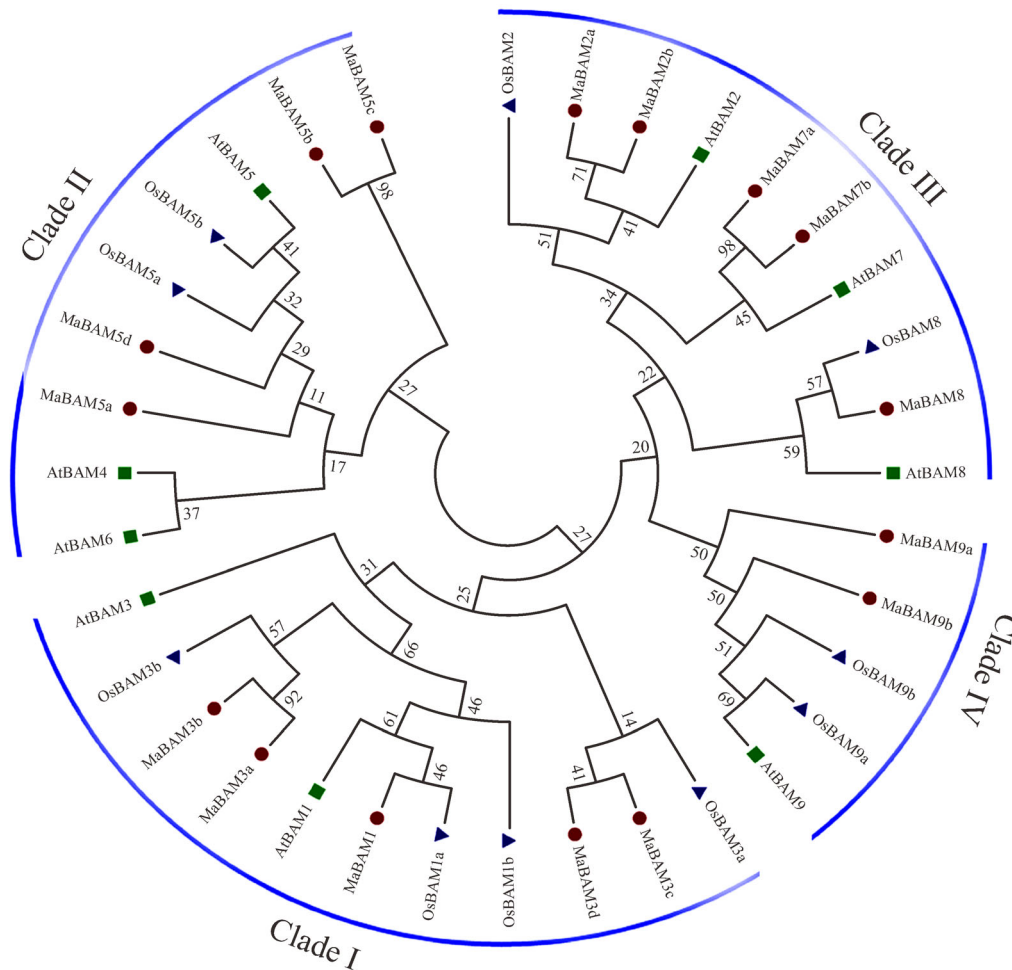
Fig. 1 Phylogenetic analysis of the BAMs from *Arabidopsis*, rice, and banana. The neighbor-joining (NJ) tree was drawn using MEGA5.0 with 1000 bootstrap. Four groups were shown as Clade I, II, III, and IV.

3.3 Expression patterns of *MaBAM* genes in different tissues of two banana cultivars

To investigate the role of *MaBAM* genes in banana growth and development, expression patterns of *MaBAM* genes in different tissues, including roots, leaves, and fruits, were tested in the two cultivars. Among the 16 *MaBAM* genes, 10 genes (excluding *MaBAM2a*, *MaBAM3a*, *MaBAM3b*, *MaBAM5c*, *MaBAM5b*, and *MaBAM7b*) showed expression in at least one tested tissue of two cultivars (Fig. 4; Appendix A, Table S2).

For BX, 9 (64%), 9 (64%), and 7 (50%) *MaBAMs* were expressed in roots, leaves and fruits, respectively, among which 3 (33%), 6 (67%), and 2 (29%) genes showed high expression levels (> 10) in roots, leaves, and fruits, respectively. Additionally, *MaBAM9b* exhibited high transcript levels (> 10) in all tissues examined.

For FJ, 8 (57%), 8 (57%), and 7 (50%) *MaBAM* genes were expressed in roots, leaves, and fruits, respectively, among which 5 (63%), 6 (75%), and 3 (43%) genes

showed high expression levels (> 10) in roots, leaves, and fruits, respectively. Furthermore, three *MaBAMs* (*MaBAM8*, *MaBAM9a*, and *MaBAM9b*) had high expression levels (> 10) in all organs tested.

Comparison of the tissue expression profiles of BX and FJ revealed that some *MaBAM* genes have similar expression patterns, including *MaBAM1*, *MaBAM3c*, *MaBAM3d*, *MaBAM7a*, and *MaBAM9b*. This indicates that these genes have similar functions in regulating tissues development in both cultivars. However, some genes exhibited differential expression patterns, e.g., *MaBAM8* had abundant transcripts (> 17.6) in roots and fruits of FJ, but low transcripts (< 5.8) in roots and fruits of BX. *MaBAM9a* showed high expression levels (> 41.5) in FJ roots, but low expression levels (< 7.8) were detected in BX roots. This indicates that some *MaBAM* members have different roles during tissue development in the two cultivars. Additionally, the *MaBAM9b* gene showed high expression levels (> 10) in all of the tested organs in both BX and FJ tissues, indicating a key role for the gene in
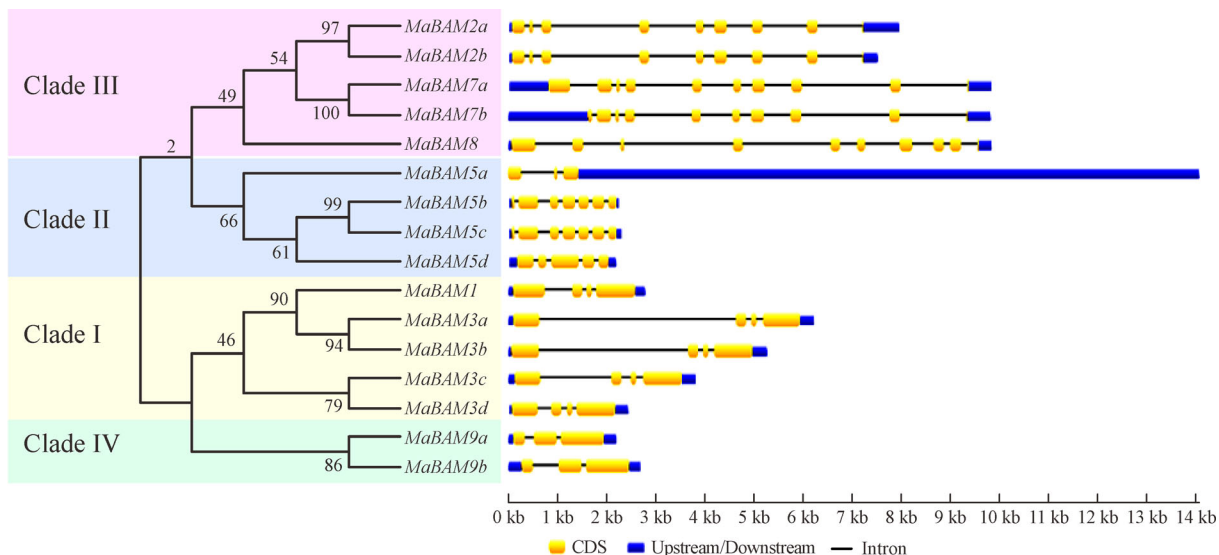
Fig. 2 Gene structure analyses of *MaBAMs*. Exon-intron structure analyses were performed by GSDS database. The blue boxes indicate upstream/downstream regions, the yellow boxes indicate exons, and the black lines indicate introns.
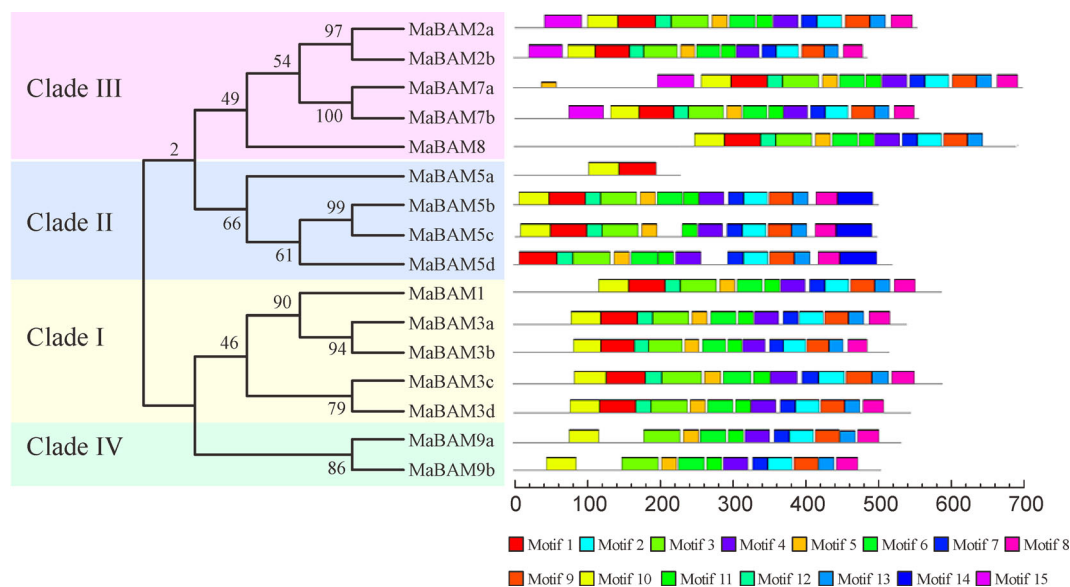

Fig. 3 Phylogenetic and motif analyses of MaBAMs. All motifs were identified by MEME database using the complete amino acid sequences of MaBAMs. The classification of MaBAMs was shown as Clade I, II, III, and IV based on the phylogenetic relationship.

regulating development of different tissues. Together, these tissue expression patterns of the *MaBAM* genes in the two cultivars may provide insights for further study of tissue development and function.

3.4 Expression profiles of *MaBAM* genes in different stages of fruit development in two banana cultivars

To investigate the transcriptional response of *MaBAM* genes in banana during fruit development, the expression patterns of the *MaBAM* genes were analyzed in fruits sampled at 0, 20 and 80 DAF (Fig. 5; Appendix A, Table S3). Among the 16 *MaBAMs*, 10 genes showed expression in different stages of banana fruit development.

For BX, 10, nine (no *MaBAM5*), and seven (no *MaBAM2a*, *MaBAM3d*, and *MaBAM5a*) *MaBAM* genes were expressed at 0, 20, and 80 DAF, with four (*MaBAM3c*, *MaBAM8*, *MaBAM9a*, and *MaBAM9b*), four (*MaBAM3c*, *MaBAM8*, *MaBAM9a*, and *MaBAM9b*), and two (*MaBAM9a* and *MaBAM9b*) genes, respectively, showing high expression levels (>10) at 0, 20, and 80 DAF. Notably, *MaBAM9a* and *MaBAM9b* had high transcript accumulation (>30) at all stages of fruit development.
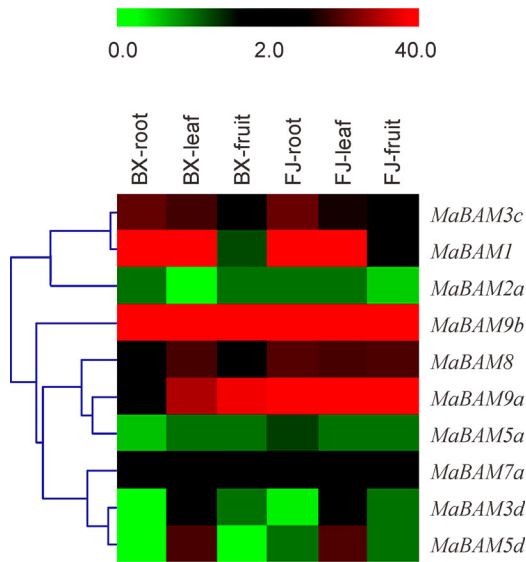
Fig. 4 Expression patterns of *MaBAMs* in roots, leaves, and fruits of two banana cultivars, BaXi Jiao (BX) and Fen Jiao (FJ). The heat map with clustering was created based on the fragments per kilobase of exon per million fragments mapped (FPKM) of *MaBAMs*. Differences in gene expression changes are shown by the color scale.
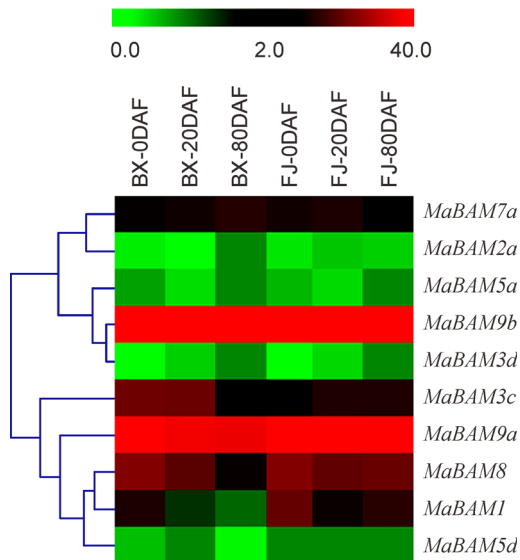

Fig. 5 Expression patterns of *MaBAMs* at different stages of fruit development in two banana cultivars, BX and FJ cultivars. The heat map with clustering was created based on the FPKM of *MaBAMs*. Differences in gene expression changes are shown by the color scale.

For FJ, nine (no *MaBAM5d*), nine (no *MaBAM5d*), and seven (no *MaBAM3d*, *MaBAM5a*, and *MaBAM5d*) *MaBAM* genes were expressed at 0, 20, and 80 DAF, among which four (*MaBAM1*, *MaBAM8*, *MaBAM9a*, and *MaBAM9b*), three (*MaBAM8*, *MaBAM9a*, and *MaBAM9b*), and three (*MaBAM8*, *MaBAM9a*, and *MaBAM9b*) genes, respectively, showed high transcript

accumulation (>10) at each stage. Notably, *MaBAM8*, *MaBAM9a*, and *MaBAM9b* displayed high transcripts levels (>15) at all stages of fruit development.

Comparison of the expression patterns of banana *MaBAM* genes at distinct phases of fruit development in both BX and FJ revealed that some *MaBAM* genes has similar high expression levels at 0 and 20 DAF in both BX and FJ, including *MaBAM8*, *MaBAM9a*, and *MaBAM9b*. This indicates that these genes have similar functions during early fruit developmental stages of both BX and FJ. However, the *MaBAM3c* had abundant transcripts (>18) at 0 DAF in BX, but low transcripts (<4) at 0 DAF in FJ. In contrast, *MaBAM1* was highly expressed (>16) at 0 DAF in FJ, but had low expression levels (<8.2) in BX. This finding indicates that some *MaBAM* members have different transcriptional responses during early fruit development in both BX and FJ. In addition, *MaBAM9a* and *MaBAM9b* displayed high transcriptional abundance (>30) at all development phases in both BX and FJ, indicating key role for these two genes in regulating banana fruit development.

3.5   Expression profiles of *MaBAM* genes in different stages of fruit ripening in two banana cultivars

To investigate the transcriptional response of *MaBAM* genes in the banana fruit ripening process, the expression patterns of the *MaBAM* genes were measured in fruits sampled at 0, 8, and 14 DPH in BX, and 0, 3, and 6 DPH in FJ (Fig. 6; Appendix A, Table S4). Among the 16 *MaBAM* genes, nine showed expression at different stages of banana fruit ripening.
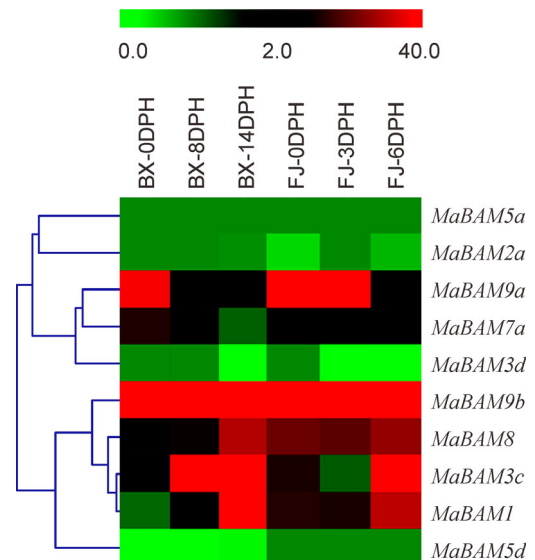

Fig. 6 Expression patterns of *MaBAMs* at different stages of fruit ripening in two banana cultivars, BX and FJ cultivars. The heat map with clustering was created based on the FPKM of *MaBAMs*. Differences in gene expression changes are shown by the color scale.

For BX, seven (no *MaBAM2a* and *MaBAM3d*), seven (no *MaBAM2a* and *MaBAM3a*), and nine *MaBAM* genes were expressed at 0, 8, and 14 DPH, respectively, of which two (*MaBAM9a* and *MaBAM9b*), two (*MaBAM3c* and *MaBAM9b*), and four (*MaBAM1*, *MaBAM3c*, *MaBAM8*, and *MaBAM9b*) genes, respectively, showed high expression levels (> 25) at each stage. Additionally, *MaBAM9b* exhibited high transcript levels (> 640) at all stages of fruit ripening.

For FJ, seven (no *MaBAM3d* and *MaBAM5d*), seven (no *MaBAM2a* and *MaBAM5d*), and eight (no *MaBAM5d*) *MaBAM* genes were expressed at 0, 3, and 6 DPH, respectively, of which three (*MaBAM8*, *MaBAM9a*, and *MaBAM9b*), three (*MaBAM8*, *MaBAM9a*, and *MaBAM9b*), and four (*MaBAM1*, *MaBAM3c*, *MaBAM8*, and *MaBAM9b*) genes, respectively, showed high transcript accumulation (> 15) at each stage. Additionally, *MaBAM8* and *MaBAM9b* exhibited high expression levels (> 15) at all stages of fruit ripening.

Comparison of the expression profiles of *MaBAMs* at different stages of fruit ripening indicated that four genes (*MaBAM1*, *MaBAM3c*, *MaBAM8*, and *MaBAM9a*) were expressed at all stages tested in both BX and FJ. Similarly, high expression (> 10) patterns were observed at late fruit ripening stages (14 DPH in BX and 6 DPH in FJ) compared with the other stages, indicating that four *MaBAM* genes had important roles in the late fruit ripening stage in both BX and FJ. However, the *MaBAM8* had abundant transcripts (> 15) at 0 and 3 DPH in FJ, but low transcripts (< 7.0) at 0 and 8 DPH in BX. The *MaBAM9a* showed high expression levels (> 40) at 3 DPH in FJ, yet low expression levels (< 4.0) were detected at 8 DPH in BX. In contrast, the *MaBAM3c* had high expression levels (> 280) at 8 DPH in BX, but low expression levels (< 1.5) at 3 DPH in FJ. This finding indicates that some *MaBAM* members had different transcriptional responses during the fruit ripening processes in BX and FJ. In addition, *MaBAM9b* displayed high transcriptional abundance (> 280) at all ripening phases in BX and FJ, indicating a key role for this gene in regulating banana fruit ripening.

3.6   Expression profiles of *MaBAM* genes in response to cold, salt, and osmotic stresses in two banana cultivars

Considerable evidence has indicated that *BAM* genes participate in plant response to various abiotic stresses, including cold, salt, and drought. To better understand the role of *MaBAM* genes in response to these 3 stresses, their expression in leaves of BX and FJ was examined under cold, salt, and osmotic treatments using data from RNA-seq assays (Fig. 7; Appendix A, Table S5). A total of 11 *MaBAM* genes showed transcriptional changes after abiotic stress in BX and FJ.

For BX, eight (no *MaBAM2a*, *MaBAM5a*, and *MaBAM5c*) *MaBAM* genes were upregulated (> 2) in response to cold, salt, and osmotic treatments, respectively,
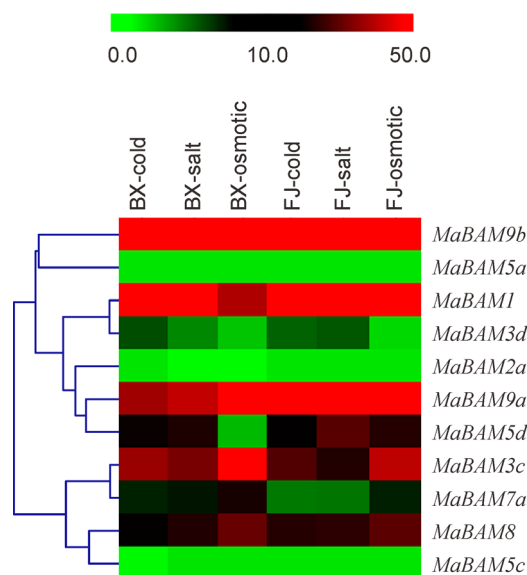

Fig. 7 Expression patterns of *MaBAMs* in response to cold, salt, and osmotic stresses in two banana cultivars, BX and FJ cultivars. The heat map with clustering was created based on the FPKM of *MaBAMs*. Differences in gene expression changes are shown by the color scale.

whereas the *MaBAM5c*, *MaBAM2a*, and *MaBAM2a* genes were downregulated (< 0.5) under cold, salt, and osmotic treatment, respectively. Additionally, five genes (*MaBAM1*, *MaBAM3c*, *MaBAM8*, *MaBAM9a*, and *MaBAM9b*) were strongly upregulated (> 10) after each of the stress treatments.

For FJ, eight (no *MaBAM2a*, *MaBAM5a*, and *MaBAM5c*), eight (no *MaBAM2a*, *MaBAM5a*, and *MaBAM5c*), and seven (no *MaBAM2a*, *MaBAM3d*, *MaBAM5a*, and *MaBAM5c*) *MaBAM* genes were upregulated in response to cold, salt, and osmotic stress, respectively, whereas the *MaBAM3d* gene was downregulated under osmotic treatment. Additionally, six genes (*MaBAM1*, *MaBAM3c*, *MaBAM5d*, *MaBAM8*, *MaBAM9a*, and *MaBAM9b*) were strongly upregulated (> 10) under all three stresses.

From these results, five genes (*MaBAM1*, *MaBAM3c*, *MaBAM8*, *MaBAM9a*, and *MaBAM9b*) were strongly induced by the three stress treatments, indicating that they have similar function in BX and FJ under cold, salt, and osmotic stress. However, two genes (*MaBAM5d* and *MaBAM7a*) showing differential expression patterns between BX and FJ under osmotic treatment. This finding indicates that some *MaBAM* members have different transcriptional responses to osmotic stress treatment in BX and FJ.

3.7   Hormone-related and stress-related cis-acting elements in the promoters of *MaBAM* genes

A promoter is a molecular switch that initiates gene

Table 1 Kinds and numbers of known hormone-related and stress-related elements in the upstream regions of *MaBAM* genes

Element	ABRE (ABA)	ARE (Anaerobic)	AuxRR (Auxin)	Box-W1 (Fungal)	CGTCA-motif (MeJA)	Circadian	ERE (Ethylene)	GARE (Gibberellin)	HSE (Heat)	LTR (Cold)	MBS (Drought)	TCA-element (Salicylic acid)	TC-rich repeats (Defense)	Total
MaBAM1	3	1	1	1	3			2				1		12
MaBAM2a		2						1	3			1	1	8
MaBAM2b		2						1	3			1	1	8
MaBAM3a			1		2	1		1			2	1	2	10
MaBAM3b		1				1					1	1	1	5
MaBAM3c	1	2	1		1	1	1		1		1			9
MaBAM3d	2	1			3			1		1	1			9
MaBAM5a	2	1			6	1		1					1	12
MaBAM5b	2	1			6	1		2						12
MaBAM5c	2	1			6	1		2						12
MaBAM5d	3	3			4	1		1					1	13
MaBAM7a		2						1	3			1	1	8
MaBAM7b		2						1	3			1	1	8
MaBAM8				1	1	1		1						4
MaBAM9a	1		1	1	3	3		1		2				12
MaBAM9b	1	2		1	2						1		1	8

expression and studies of promoter cis-acting elements are very useful for investigating genes expression regulatory mechanism and potential functions[36]. *BAM* promoters have been documented as having roles in plant development and abiotic stresses in many species[5]. However, there are no such reports in banana. We chose banana *MaBAMs* for identification of potential promoter cis-acting elements to aid future understanding of the regulation of their expression and biological function. As shown in Table 1, five hormone-related (ABA, auxin, MeJA, ethylene, and gibberellin) and seven stress-related (anaerobic, fungal, heat, cold, drought, salicylic acid, and defense) elements were identified in the promoters of these *MaBAM* genes. No less than four hormone- and stress-related elements were found in the 16 *BAM* promoters, suggesting that BAMs participate in the regulation of development, ripening, and stress response by hormone- and stress-related cis-acting elements.

4 Discussion

Despite the economic and social importance of banana, compared to other crops, research on banana has evolved slowly. To improve banana fruit quality and enhance stress tolerance, it is essential to explore the mechanisms underlying banana fruit development, ripening, and response to abiotic stresses. Plant *BAM* genes have various roles in plant growth, development and response to abiotic stresses, and are considered crucial regulators in multiple biological processes. However, limited information has been reported on the *MaBAM* gene family of banana. In this study, we performed genome-wide identification and characterization of expression of the *MaBAM* gene family during development, ripening, and response to cold, salt, and osmotic stresses in banana.

4.1 Identification and evolutionary analysis of banana *MaBAM* genes

In this study, a total of 16 *MaBAM* family genes were identified in the banana A genome (*M. acuminata*, 2n = 22). This finding indicates that the banana *MaBAM* gene family has expanded compared to *Arabidopsis*[5] and rice[16]. Based on phylogenetic analysis, these genes were classified into four subfamilies, which was consistent with the classification of those in the *Arabidopsis* and rice[5,16]. The phylogenetic results were also supported by the gene structure, which indicated that *MaBAM* genes contain three to nine exons, and each subfamily shared a similar exon-intron organization (Fig. 2), which was also found in *Arabidopsis*, rice, and barley[5,16,37]. However, the number of exons in banana *BAM* genes is greater than in barley (7)[37] and sweet potato (2)[38], implying that the banana *MaBAM* genes have changed greatly during evolution. Conserved motif analysis indicated that all MaBAMs have the typical glyco hydro 14 domains, and each subfamily shares similar motifs, further supporting the classification of MaBAMs. Together, the identification and classification of the banana *BAM* gene family was supported by

evolutionary, genetic structure, and conserved motif analyses.

4.2 Involvement of *BAM* genes in fruit development, postharvest ripening, and abiotic stress response in banana

Banana fruit yield and quality are directly affected by fruit developmental processes. Thus, it is necessary to understand the mechanisms involved in banana fruit development. The BAM family has been reported to participate in the plant development of many species such as apple[39], *Arabidopsis*[17], barley[18], and rye[40]. In apple, the amount of BAM protein was markedly greater during fruit development[39]. Two *BAM* genes in *Arabidopsis* regulate shoot development[17]. The expression of a distinct transcript, from the barley *Bmy1* gene, suggested its potential involvement in grain development[18]. In rye, expression of two *BAM* genes changed significantly during seed development[40]. However, whether BAMs participate in fruit development of banana remains unclear. In this study, we found that most banana *BAM* genes showed increased expression during fruit development. Moreover, many genes showed high transcriptional abundance (> 10) at all developmental stages of both BX and FJ (Fig. 5). Based on the evidence above, *BAM* genes are likely to be involved in fruit development in banana.

Banana postharvest ripening is crucial to improving fruit quality and extending fruit shelf life[41,42]. In this study, the number of *BAM* genes with high transcript abundance (> 15) at 14 DPH in BX and 6 DPH in FJ was greater than that at other stages (Fig. 6), suggesting that *BAM* genes are crucial in the late stages of banana fruit ripening. Previous evidence from other fruits has revealed the importance of *BAM* genes during the late stages of ripening[18,43,44]. In tomato, *BAM* gene transcript accumulation and enzyme activity were elevated during the late stages of fruit ripening and had an important role in starch depletion and ripening process[43]. The barley Bmy2 protein was increased markedly in mature grain and decreased in developing grain[18]. In mango, BAM protein was involved in starch degradation during fruit ripening[44]. Overall, these results suggest the importance of *BAM* genes in regulating fruit ripening.

Increasing evidence has suggested that *BAM* genes could transcriptionally respond to multiple abiotic stresses in various plant species[2,19–21,36]. Further biochemical and genetic evidence has demonstrated that plant BAM proteins, acting as signal regulators, positively regulate plants response to drought[20], cold[22], and salt stresses[23]. In this study, many *BAM* genes showed significant changes after cold, salt, and osmotic treatment in the two banana cultivars, suggesting their potential role in the banana abiotic stress responses. Comparison of the expression patterns of *BAMs* in BX and FJ under abiotic stress showed that *BAM* genes similar expression patterns (Fig. 7),

indicating that they function similarly in BX and FJ under the cold, salt, and osmotic treatments. Collectively, it is concluded that *BAM* genes are potentially important in contributing to abiotic stress responses in two banana cultivars.

4.3 Cis-elements analysis in the promoters of *BAM* genes

It has been reported that BAM promoters participate in multiple developmental processes and abiotic stress responses in many species. The *Arabidopsis* Ct-Bmy promoter is involved in the development of chloroplast-localized transitory leaf starch[45]. Expression of two *Arabidopsis* BAM promoters in leaves, sepals, siliques or root tips suggested their potential control of starch breakdown in different tissues[19]. In sweet potato, activity of BAM promoter was exclusively detected in the sugar metabolism process[46]. In *Poncirus trifoliata*, BAM1 contains a CBF-recognizing element and is important in cold tolerance by modulating soluble sugar levels[36]. Promoter activity analysis of *Arabidopsis* BAM1 suggested that thioredoxin-regulated BAM1 activates starch degradation in illuminated mesophyll cells under osmotic stress[5]. Additionally, gibberellic acid- and abscisic acid-responsive elements in the promoter of *AMY* genes have been identified in barley[47] and wheat[48]. In rice, the function of the AMY promoter is in the growth or development of mature leaves, stems, sheaths, roots, and seeds[49]. However, no study has reported hormone-related elements of the BAM promoter. In this study, five hormone-related and seven stress-relevant elements were analyzed in the promoters of 16 *MaBAM* genes (Table 1), indicating that BAMs participate in the regulation of multiple development process and stress responses by hormone-related and stress-related cis-acting elements. These findings have provided a solid foundation for further studies of the BAM protein-mediated development and abiotic stresses signal response in banana.

5 Conclusions

This study identified 16 *BAM* genes from the banana genome and established their classification and evolutionary relationship using phylogenetic, gene structure, and conserved protein motif analyses. Expression analyses demonstrated that banana *BAM* genes are involved in regulating fruit development, the late stage of ripening, and abiotic stress responses. Furthermore, hormone-related and stress-related cis-acting elements analyses of *MaBAM* promoters indicated that they are potentially involved in responding to developmental, ripening, and abiotic stresses signaling. These results will advance the understanding of the functional characterization of *BAM* genes in the banana developmental and ripening processes, and in responses to

abiotic and biotic stresses, and provide a solid foundation for further genetic improvement of banana quality and resistance to various stresses.

Supplementary materials The online version of this article at http://dx.doi.org/10.15302/J-FASE-2016127 contains supplementary materials (Appendix A).

Compliance with ethics guidelines Hongxia Miao, Peiguang Sun, Yulu Miao, Juhua Liu, Jianbin Zhang, Caihong Jia, Jingyi Wang, Zhuo Wang, Zhiqiang Jin, and Biyu Xu declare that they have no conflict of interest or financial conflicts to disclose.

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