

1 **The *R2R3-MYB* gene family in banana (*Musa acuminata*): genome-wide identification,**  
2 **classification and expression patterns.**

3  
4 Boas Pucker<sup>1</sup>, Ashutosh Pandey<sup>1,2</sup>, Bernd Weisshaar<sup>1</sup>, Ralf Stracke<sup>1,\*</sup>

5  
6 <sup>1</sup> Bielefeld University, Faculty of Biology, Genetics and Genomics of Plants, Sequenz 1,  
7 33615 Bielefeld, Germany

8 <sup>2</sup> National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi, Delhi  
9 110067, India

10 \* communicating author

11  
12  
13 **Abstract**

14 The *R2R3-MYB* genes comprise one of the largest transcription factor gene families in plants,  
15 playing regulatory roles in plant-specific developmental processes, defense responses and  
16 metabolite accumulation. To date MYB family genes have not yet been comprehensively  
17 identified in the major staple fruit crop banana. In this study, we present a comprehensive,  
18 genome-wide analysis of the *MYB* genes from *Musa acuminata* DH-Pahang (A genome). A  
19 total of 286 *R2R3-MYB* genes as well as genes encoding three other classes of MYB proteins  
20 containing multiple MYB repeats were identified and characterised with respect to structure  
21 and chromosomal organisation. Organ- and development-specific expression patterns were  
22 determined from RNA-seq data. For 279 *M. acuminata MYB* genes for which expression was  
23 found in at least one of the analysed samples, a variety of expression patterns were detected.

24 The *M. acuminata R2R3-MYB* genes were functionally categorised, leading to the  
25 identification of seven clades containing only *M. acuminata R2R3-MYBs*. The encoded  
26 proteins may have specialised functions that were acquired or expanded in *Musa* during  
27 genome evolution. This functional classification and expression analysis of the *MYB* gene  
28 family in banana establishes a solid foundation for future comprehensive functional analysis  
29 of MaMYBs and can be utilized in banana improvement programmes.

30  
31 Keywords: *Musa acuminata*, Zingiberales, R2R3-MYB, transcription factor, gene family

32  
33  
34 **Introduction**

*M. acuminata* MYB family

35 Banana (*Musa* spp.), including dessert and cooking types, is a staple fruit crop for a major  
36 world population, especially in developing countries. The crop is grown in more than 100  
37 countries throughout the tropics and sub-tropics, mainly in the African, Asia-Pacific, and  
38 Latin American and Caribbean regions (Frison and Sharrock 1999). Bananas provide an  
39 excellent source of energy and are rich in certain minerals and in vitamins A, C and B6.  
40 Furthermore, this perennial, monocotyledonous plant provides an important source of fibre,  
41 sugar, starch and cellulose (used for paper, textiles). Bananas have also been considered as a  
42 useful tool to deliver edible vaccines (Langridge 2006). Certain agronomic traits, such as  
43 stress and pest resistance as well as fruit quality, are thus of considerable interest. Banana  
44 improvement through breeding exercises has been challenging for various reasons. Therefore,  
45 genetic engineering-based optimisations hold great promise for crop improvement. For this  
46 purpose, candidate gene targets need to be identified. The release of a high quality banana  
47 genome sequence (D'Hont et al. 2012) provides an useful resource to understand functional  
48 genomics of important agronomic traits and to identify candidate genes to be utilized in  
49 banana improvement programmes.

50 Almost all biological processes in eukaryotic cells or organisms are influenced by  
51 transcriptional control of gene expression. Thus, the regulatory level is a good starting point  
52 for genetic engineering. Regulatory proteins are involved in transcriptional control, alone or  
53 complexed with other proteins, by activating or repressing (or both) the recruitment of RNA  
54 polymerase to promoters of specific genes (Cooper 2000). These proteins are called  
55 transcription factors. As expected from their substantial regulatory complexity, transcription  
56 factors are numerous and diverse (Riechmann et al. 2000). By binding to specific DNA  
57 sequence motifs and regulating gene expression, transcription factors control various  
58 regulatory and signaling networks involved in the development, growth and stress response in  
59 an organism.

60 One of the widest distributed transcription factor families in all eukaryotes is the MYB  
61 (myeloblastosis) protein family. In the plant kingdom, MYB proteins constitute one of the  
62 largest transcription factor families. MYB proteins are defined by a highly conserved MYB  
63 DNA-binding domain, mostly located at the N-terminus of the protein. The MYB domain  
64 generally consists of up to four imperfect amino acid sequence repeats (R) of about 50-53  
65 amino acids, each forming three alpha-helices (summarised in Dubos et al. 2010). The second  
66 and third helices of each repeat build a helix-turn-helix (HTH) structure with three regularly  
67 spaced tryptophan (or hydrophobic) residues, forming a hydrophobic core (Ogata et al. 1996).  
68 The third helix of each repeat was identified as the DNA recognition helix that makes direct

69 contact with DNA (Jia et al. 2004). During DNA contact, two MYB repeats are closely  
70 packed in the major groove, so that the two recognition helices bind cooperatively to the  
71 specific DNA recognition sequence motif. In contrast to vertebrates genomes, which only  
72 encode MYB transcription factors with three repeats, plants have different MYB domain  
73 organisations, comprising one to four repeats (Stracke et al. 2001; Dubos et al. 2010). R2R3-  
74 MYBs, which are MYB proteins with two repeats (named according to repeat numbering in  
75 vertebrate MYBs), are particularly expanded in plant genomes. Copy numbers range from 45  
76 unique *R2R3-MYBs* in *Ginkgo biloba* (Liu et al. 2017) to 360 in Mexican cotton (*Gossypium*  
77 *hirsutum*) (Salih et al. 2016). The expansion of the R2R3-MYB family was coupled with  
78 widening in the functional diversity of R2R3-MYBs, considered to regulate mainly plant-  
79 specific processes including secondary metabolism, stress responses and development (Dubos  
80 et al. 2010). As expected, R2R3-MYBs are involved in regulating several biological traits, for  
81 example wine quality, fruit color, cotton fibre length, pollinator preferences and nodulation in  
82 legumes.

83 In this study, we have used genomic resources to systematically identify members of the  
84 *M. acuminata* (A genome) *R2R3-MYB* gene family. We used knowledge from other plant  
85 species, including the model plant *A. thaliana*, leading to a functional classification of the  
86 banana *R2R3-MYB* genes based on the MYB phylogeny. Furthermore, RNA-seq data was  
87 used to analyse expression in different *M. acuminata* organs and developmental stages and to  
88 compare expression patterns of closely grouped co-orthologs. The identification and  
89 functional characterization of the *R2R3-MYB* gene family from banana will provide an insight  
90 into the regulatory aspects of different biochemical and physiological processes, as those  
91 operating during fruit ripening as well as response to various environmental stresses. Our  
92 findings offer the first step towards further investigations on the biological and molecular  
93 functions of MYB transcription factors with the selection of genes responsible for  
94 economically important traits in *Musa*, which can be utilized in banana improvement  
95 programmes.

96  
97

## 98 **Material and Methods**

### 99 **Search for MYB protein coding genes in the *M. acuminata* genome**

100 A consensus R2R3-MYB DNA binding domain sequence (Matus et al. 2008) was used as  
101 protein query in tBLASTn (Altschul et al. 1990) searches on the *M. acuminata* DH-Pahang  
102 genome sequence (version 2) (<http://banana-genome-hub.southgreen.fr/>) in an initial search

103 for MYB protein coding genes. To confirm the obtained amino acid sequences, the putative  
104 MYB sequences were manually analysed for the presence of an intact MYB domain. All  
105 *M. acuminata* MYB candidates from the initial BLAST were inspected to ensure that the  
106 putative gene models encode two or more (multiple) MYB repeats. The identified gene  
107 models were analysed to map them individually to unique loci in the genome and redundant  
108 sequences were discarded from the data set to obtain unique *MaMYB* genes. The identified  
109 *MaMYB* genes were matched to the automatically annotated genes from the Banana Genome  
110 Hub database (Droc et al. 2013). The open reading frames of the identified *MaMYB* were  
111 manually inspected and, if available, verified by mapping of RNA-seq data to the genomic  
112 sequence.

113

#### 114 **Genomic distribution of *MaMYB* genes**

115 The *MaMYB* genes were located on the corresponding chromosomes by the  
116 MapGene2chromosome web v2 (MG2C) software tool ([http://mg2c.iask.in/mg2c\\_v2.0/](http://mg2c.iask.in/mg2c_v2.0/))  
117 according to their position information of a physical map, available from the DH-Pahang  
118 genome annotation (V2).

119

#### 120 **Phylogenetic analyses**

121 Protein sequences of 133 *A. thaliana* MYBs were obtained from TAIR  
122 (<http://www.arabidopsis.org/>). We also considered other multiple MYB-repeat proteins from  
123 *A. thaliana* in the phylogenetic analysis to determine orthologs in the *M. acuminata* genome:  
124 five AtMYB3R, AtMYB4R1 and AtCDC5. Additionally, 43 well-known, functionally  
125 characterised landmark plant R2R3-MYB protein sequences were collected from GenBank at  
126 the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/>).  
127 Phylogenetic trees were constructed from ClustalΩ (Sievers et al. 2011) aligned MYB domain  
128 sequences (294 *MaMYB*s, 132 *AtMYB*s and 43 plant landmark MYBs) using MEGA7  
129 (Kumar et al. 2016) with default settings. A majority rule Maximum Likelihood (ML)  
130 consensus tree inferred from 1000 bootstrap replications was calculated. *M. acuminata* MYB  
131 proteins were classified according to their relationships with corresponding *A. thaliana* and  
132 landmark MYB proteins.

133

#### 134 **Expression analysis from RNA-seq data**

135 RNA-Seq data sets were retrieved from the Sequence Read Archive  
136 (<https://www.ncbi.nlm.nih.gov/sra>) via fastq-dump v2.9.6 (<https://github.com/ncbi/sra-tools>)

137 (Additional Table 2). STAR v2.5.1b (Dobin et al. 2013) was applied for the mapping of reads  
138 to the Pahang v2 reference genome sequence (Martin et al. 2016) using previously described  
139 parameters (Haak et al. 2018). featureCounts (Liao et al. 2014) was applied for quantification  
140 of the mapped reads per gene based on the Pahang v2 annotation. Previously developed  
141 Python scripts were deployed for the calculation of normalized expression values  
142 (<https://github.com/bpucker/bananaMYB>) (Haak et al. 2018).

143  
144

## 145 **Results and Discussion**

146 The first annotated reference genome sequence of *M. acuminata* (A genome) became  
147 available in 2012 (D'Hont et al. 2012). It was obtained from a double haploid (DH) plant of  
148 the Pahang cultivar, derived through haploid pollen and spontaneous chromosome doubling  
149 from the wild subspecies *Musa acuminata* ssp. *malaccensis* (D'Hont et al. 2012). This wild  
150 subspecies was involved in the domestication of the vast majority of cultivated bananas and  
151 its genetic signature is commonly found in dessert and cooking bananas (ProMusa,  
152 <http://www.promusa.org>). An improved version (DH-Pahang version 2) of the genome  
153 assembly and annotation was presented in 2016 comprising 450.7 Mb (86% of the estimated  
154 size) from which 89.5% are assigned to one of the 11 chromosomes, predicted to contain  
155 35,276 protein encoding genes (Martin et al. 2016). This *M. acuminata* DH-Pahang version 2  
156 genome sequence provides the platform of this study.

157

### 158 **Identification and genomic distribution of *M. acuminata* MYB genes**

159 A consensus R2R3-MYB DNA binding domain sequence (deduced from Arabidopsis, grape  
160 and sugarbeet R2R3-MYBs, Additional Table1) was used as protein query in tBLASTn  
161 searches on the DH-Pahang version 2 genome sequence to comprehensively identify MYB  
162 protein coding genes in *M. acuminata*. The resulting putative MYB sequences were proven to  
163 map to unique loci in the genome and were confirmed to contain an intact MYB domain. This  
164 ensured that the gene models contained two or more (multiple) MYB repeats. We identified a  
165 set of 286 R2R3-MYB proteins and nine multiple repeat MYB proteins distantly related to the  
166 typical R2R3-MYB proteins: six R1R2R3-MYB (MYB3R) proteins, two MYB4R proteins  
167 and one CDC5-like protein from the *M. acuminata* genome sequence (Table 1).

168

169 **Table 1: List of annotated MYB genes in the *Musa acuminata* (DH Pahang) genome**  
170 **sequence.**

*M. acuminata* MYB family

171 The genes are ordered by DH Pahang version 2 (Martin et al. 2016) pseudochromosomes,  
 172 from north to south. The annotation-version specific gene code describing the chromosomal  
 173 assignment and position on pseudochromosomes is given in the first column. "Ma00"  
 174 indicates genes located in sequences without chromosomal assignment. Functional  
 175 assignment is based on the Maximum Likelihood tree presented in Figure 2.  
 176 str.: strand, \*: paralogs, ASR: abiotic stress response, PP: phenylpropanoid, <sup>[1]</sup> Tak et al. 2017,  
 177 <sup>[2]</sup> Negi et al. 2015, <sup>[3]</sup> Fan et al. 2018, <sup>[4]</sup> Yang et al. 2015.  
 178

<i>gene ID</i>	<i>synonym</i>	<i>pseudochr. position from</i>	<i>to</i>	<i>str.</i>	<i>BHLH motif</i>	<i>coding exons</i>	<i>peptide length</i>	<i>MYB type</i>	<i>functional assignment</i>
Ma01_g00440		313923	315583	-		1	297	R2R3	stress response, hormone signaling
Ma01_g02850	MusaMYB31 <sup>[1]</sup>	1866276	1867435	+	+	2	200	R2R3	repressors PP, sinapate, lignin
Ma01_g04470		3004971	3006821	+		3	230	R2R3	ASR, flower morphogenesis, stilbene
Ma01_g10440		7520585	7526901	+		3	586	R2R3	anther development, stress response
Ma01_g10750		7722630	7723879	-		3	273	R2R3	photomorphogenesis
Ma01_g11890		8618960	8620447	+		2	234	R2R3	secondary cell wall, lignin
Ma01_g12250		8869343	8871737	-		3	334	R2R3	
Ma01_g14370		10499468	10501015	+		3	195	R2R3	defense, stress response
Ma01_g15800		11477431	11479333	+		3	197	R2R3	secondary cell wall, lignin
Ma01_g16960		12408973	12410679	+		3	293	R2R3	
Ma01_g17260		12621562	12624265	-		3	360	R2R3	
Ma01_g17450		12782326	12783615	+		3	270	R2R3	defense, stress response
Ma01_g18470		13704376	13705557	-		3	330	R2R3	suberin
Ma01_g19610	MYB32 <sup>[2]</sup>	15270989	15272451	-	+	2	241	R2R3	repressors PP, sinapate, lignin
Ma01_g19960		15935559	15937046	-		1	282	R2R3	stress response, hormone signaling
Ma01_g21340		20977295	20978433	-		3	196	R2R3	stamen development
Ma02_g00280		2701936	2703155	+		3	281	R2R3	defense, stress response
Ma02_g00290		4086418	4089233	+		3	320	R2R3	flavonols, phlobaphene
Ma02_g03780		15236704	15238133	+		3	307	R2R3	mucilage, lignin, stomatal closure
Ma02_g04860		16268774	16270113	+		3	325	R2R3	
Ma02_g05880		17024271	17029698	-		7	489	3R	cell cycle control
Ma02_g06190		17224931	17226388	+		3	301	R2R3	axillary meristem, root growth
Ma02_g06670		17595310	17597055	+		3	317	R2R3	suberin
Ma02_g09720	MYB46 <sup>[2]</sup>	19581503	19582589	+		2	297	R2R3	secondary wall, lignin
Ma02_g09870		19644772	19647000	-		3	381	R2R3	
Ma02_g13370		21804465	21806215	+		3	308	R2R3	photomorphogenesis
Ma02_g15770		23338151	23340844	-		3	444	R2R3	anther development, stress response
Ma02_g16570		23869343	23870392	+		3	297	R2R3	root development
Ma02_g17950	MYB48 <sup>[2]</sup>	24668074	24669369	-		3	205	R2R3	
Ma02_g19650		25862200	25864721	+	+	3	258	R2R3	
Ma02_g19770	MYB63 <sup>[2]</sup>	25962423	25963930	-		3	303	R2R3	PP, lignin

*M. acuminata* MYB family

Ma02_g20270	26305416	26308011	+		3	365	R2R3	
Ma02_g21230	26925164	26926280	+		2	244	R2R3	general flavonoid, trichome
Ma02_g21760	27303352	27318151	-		11	1076	3R	cell cycle control
Ma02_g22540	27850957	27852253	+		3	175	R2R3	repressors PP, sinapate, lignin
Ma02_g23870	28706468	28707735	+		3	253	R2R3	defense, stress response
Ma02_g24520	29095023	29096192	-		3	292	R2R3	defense, stress response
Ma03_g01260	944639	946045	+		3	124	R2R3	defense, stress response
Ma03_g06410	4438151	4439467	-		3	287	R2R3	root development
Ma03_g07620	5357785	5358916	-		3	205	R2R3	
Ma03_g07840 *	5566056	5567272	-	+	3	282	R2R3	proanthocyanidins
Ma03_g07850 *	5573615	5574872	+	+	3	238	R2R3	proanthocyanidins
Ma03_g08300	5984884	5986166	-		3	287	R2R3	defense, stress response
Ma03_g08930	6570843	6573022	+		3	360	R2R3	embryogenesis, seed maturation
Ma03_g09310	6861251	6862614	+		3	289	R2R3	defense, stress response
Ma03_g09340	6900527	6902243	+		3	319	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma03_g09840	7294462	7296176	+		3	320	R2R3	anther-, tapetum development
Ma03_g11910	9246252	9248138	+		3	360	R2R3	
Ma03_g12480	9622470	9625578	-		4	175	R2R3	defense, stress response
Ma03_g12720	9782618	9784261	-		1	290	R2R3	stress response, hormone signaling
Ma03_g14020	11197595	11199013	-		3	313	R2R3	suberin
Ma03_g18410	23945756	23946927	+		2	168	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma03_g19810	25073643	25074748	+		2	259	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma03_g20390	25556441	25558664	-		3	382	R2R3	embryogenesis, seed maturation
Ma03_g21920	26789930	26791072	+	+	3	254	R2R3	flavonoid repressor
Ma03_g21970	26827699	26828820	+		3	303	R2R3	
Ma03_g23170	27799296	27800913	-	+	3	217	R2R3	repressors PP, sinapate, lignin
Ma03_g25780	29742482	29744122	+		3	344	R2R3	mucilage, lignin, stomatal closure
Ma03_g28720	31825467	31827667	+	+	3	274	R2R3	proanthocyanidins
Ma03_g29070	32109705	32110872	-		3	325	R2R3	
Ma03_g29510	32402433	32403599	-		1	288	R2R3	stress response, hormone signaling
Ma03_g29770	32613488	32614916	+		3	307	R2R3	
Ma04_g00460	405117	406515	+		3	265	R2R3	defense, stress response
Ma04_g01010	896344	897266	-		3	226	R2R3	
Ma04_g05460	4086523	4089539	+		4	290	R2R3	photomorphogenesis
Ma04_g06410	4737137	4738569	+		3	306	R2R3	
Ma04_g09430	6706206	6707827	+		1	302	R2R3	stress response, hormone signaling
Ma04_g11930	8517172	8518919	+		3	246	R2R3	photomorphogenesis
Ma04_g12940	9788762	9790485	+		1	266	R2R3	stress response, hormone signaling

*M. acuminata* MYB family

Ma04_g13260		10044518	10045599	+		3	308	R2R3	
Ma04_g16770		16592801	16594124	-		3	348	R2R3	suberin
Ma04_g18740		20887851	20889334	+		3	146	R2R3	ASR, flower morphogenesis, stilbene
Ma04_g19500		22140557	22142266	+		3	347	R2R3	suberin
Ma04_g20120		22833446	22834622	+		3	322	R2R3	anther development, stress response
Ma04_g22200		24575736	24576706	-		2	195	R2R3	repressors PP, sinapate, lignin
Ma04_g22930		25119938	25121516	-		3	254	R2R3	axillary meristem, root growth
Ma04_g23220		25400377	25401463	-		3	243	R2R3	PP, lignin
Ma04_g24670		26638956	26640374	+		3	243	R2R3	
Ma04_g26220		27753147	27754781	+		3	283	R2R3	
Ma04_g26550	MYB85 <sup>[2]</sup>	27973129	27974181	-		2	249	R2R3	secondary cell wall, lignin
Ma04_g26660		28040878	28042092	-		3	128	R2R3	defense, stress response
Ma04_g26810		28140431	28141878	-		1	269	R2R3	stress response, hormone signaling
Ma04_g28300		29374641	29376644	-	+	3	232	R2R3	
Ma04_g28510		29563724	29565208	+		3	219	R2R3	ASR, flower morphogenesis, stilbene
Ma04_g30160		30889022	30891484	-		3	368	R2R3	anther development, stress response
Ma04_g31800		32024735	32025860	+		3	279	R2R3	axillary meristem, root growth
Ma04_g31880		32082474	32084373	-		3	142	R2R3	photomorphogenesis
Ma04_g32240		32306362	32307006	+		2	192	R2R3	repressors PP, sinapate, lignin
Ma04_g33920	MYB72 <sup>[2]</sup>	33328174	33329487	+		3	300	R2R3	PP, lignin
Ma04_g34300		33574944	33576372	+		3	382	R2R3	anther development, stress response
Ma04_g34660		33734710	33735657	+		3	226	R2R3	axillary meristem, root growth
Ma04_g35350		34164151	34165124	-		3	281	R2R3	PP, lignin
Ma04_g35730		34354754	34362279	+		4	204	R2R3	PP, lignin
Ma04_g35890		34456195	34457741	-		3	279	R2R3	photomorphogenesis
Ma04_g38740		36138963	36140410	+		3	279	R2R3	axillary meristem, root growth
Ma05_g01100		651244	652185	+		3	206	R2R3	repressors PP, sinapate, lignin
Ma05_g01880		1152994	1154327	-		3	281	R2R3	axillary meristem, root growth
Ma05_g03340		2404252	2405715	+		3	303	R2R3	PP, lignin
Ma05_g03690		2718181	2719844	-		3	240	R2R3	ASR, flower morphogenesis, stilbene
Ma05_g05670		4316913	4318043	-		3	246	R2R3	secondary cell wall, lignin
Ma05_g06310		4706038	4707394	-	+	2	375	R2R3	general flavonoid, trichome
Ma05_g07140		5206984	5207790	-		1	269	R2R3	stress tolerance
Ma05_g07450		5428289	5429962	-		3	295	R2R3	defense, stress response
Ma05_g08960		6598602	6600986	+		3	192	R2R3	
Ma05_g10430		7526267	7527710	+		3	279	R2R3	axillary meristem, root growth
Ma05_g12030		8749406	8751293	+		3	255	R2R3	flower meristem identity
Ma05_g14510		10595097	10596227	-		3	229	R2R3	ASR, flower morphogenesis, stilbene



*M. acuminata* MYB family

Ma05_g17720		21202263	21203407	-	3	234	R2R3	
Ma05_g18420		23864457	23865647	+	3	241	R2R3	repressors PP, sinapate, lignin
Ma05_g18710		24607776	24609429	-	3	288	R2R3	defense, stress response
Ma05_g19630		28141902	28143134	-	3	295	R2R3	root development
Ma05_g20320		31975488	31976981	+	3	307	R2R3	suberin
Ma05_g20740		32405856	32407834	+	3	375	R2R3	
Ma05_g20940		32642123	32643636	+	3	333	R2R3	
Ma05_g23480		35547417	35548389	+	3	182	R2R3	defense, stress response
Ma05_g23640		35794588	35797244	-	3	321	R2R3	flavonols, phlobaphene
Ma05_g24200		36482627	36483579	+	3	207	R2R3	axillary meristem, root growth
Ma05_g24840		36981509	36982629	+	3	323	R2R3	anther-, tapetum development
Ma05_g25150		37166044	37167618	+	3	316	R2R3	axillary meristem, root growth
Ma05_g25490		37422954	37423941	-	3	226	R2R3	secondary cell wall, lignin
Ma05_g25630		37499737	37501266	-	3	290	R2R3	trichome branching, petal morphogenesis
Ma05_g25680		37532195	37533810	+	3	302	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma05_g28370		39401272	39403071	- +	3	210	R2R3	repressors PP, sinapate, lignin
Ma05_g30120		40637050	40638724	+	3	252	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma05_g30720		40993150	40994479	+	3	237	R2R3	cell cycle regulation
Ma05_g31160		41198403	41202707	-	2	397	R2R3	stress tolerance
Ma05_g31440		41356205	41357951	+	1	344	R2R3	leaf-, shoot-, germ morphogenesis
Ma06_g00910		745494	747844	+	4	394	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma06_g03570		2604323	2605964	+	3	333	R2R3	axillary meristem, root growth
Ma06_g04210		3057847	3059452	- +	3	361	R2R3	proanthocyanidins
Ma06_g04240		3073024	3074684	-	3	345	R2R3	trichome branching, petal morphogenesis
Ma06_g04270		3094694	3096881	+	3	354	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma06_g04370		3152384	3153807	+	3	250	R2R3	defense, stress response
Ma06_g05680		4227417	4228614	+	3	305	R2R3	root development
Ma06_g05960		4396733	4397886	- +	3	275	R2R3	anthocyanins
Ma06_g06660		4800702	4808542	-	7	517	3R	cell cycle control
Ma06_g08100		5748553	5749986	+	3	276	R2R3	embryogenesis, seed maturation
Ma06_g08440		5976662	5977609	-	3	243	R2R3	
Ma06_g08910		6239004	6240845	+	3	199	R2R3	repressors PP, sinapate, lignin
Ma06_g11140	MaMYB3 <sup>[3]</sup>	7827947	7829034	+	3	200	R2R3	starch degradation, flavonoid repressor
Ma06_g11270		7905151	7906898	+	3	308	R2R3	defense, stress response
Ma06_g12110		8407734	8411775	-	12	469	R2R3	guard cell division, root gravitropism
Ma06_g12160		8449752	8451062	-	3	256	R2R3	defense, stress response
Ma06_g14470		9916014	9917324	- +	3	286	R2R3	repressors PP, sinapate, lignin
Ma06_g16350		11053372	11054799	+	2	290	R2R3	general flavonoid, trichome

*M. acuminata* MYB family

Ma06_g16920	11470718	11471708	+	3	220	R2R3	cell cycle regulation
Ma06_g17440	11851409	11852643	-	3	275	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma06_g19030	13020068	13021561	+	2	263	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma06_g27210	29243106	29244308	-	3	237	R2R3	repressors PP, sinapate, lignin
Ma06_g29060	30553182	30554508	+	3	195	R2R3	stamen development
Ma06_g31020	32236972	32238507	+	3	351	R2R3	defense, stress response
Ma06_g32530	33447706	33449189	+	3	315	R2R3	axillary meristem, root growth
Ma06_g33100	33858221	33872663	+	12	838	4R	SNAP complex
Ma06_g33190	33914323	33915299	+	3	244	R2R3	
Ma06_g33430 *	34072591	34074225	+	3	399	R2R3	axillary meristem, root growth
Ma06_g33440 *	34079035	34083709	+	2	335	R2R3	axillary meristem, root growth
Ma06_g33920	34371133	34372942	+	3	346	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma06_g35430	35258465	35260108	+	1	225	R2R3	stress tolerance
Ma06_g35620	35399156	35400631	+	4	400	R2R3	embryogenesis, seed maturation
Ma06_g37660	36662931	36664139	+	3	299	R2R3	anther-, trichome development
Ma06_g38880	37506759	37508514	-	3	311	R2R3	anther-, trichome development
Ma07_g00270	249167	250371	+	2	326	R2R3	
Ma07_g02470	1968475	1973718	-	3	600	R2R3	anther development, stress response
Ma07_g05660	4115493	4116032	+	1	272	R2R3	defense, stress response
Ma07_g05780	4199983	4201888	+	2	251	R2R3	secondary cell wall, lignin
Ma07_g08110	6059901	6061028	+	3	256	R2R3	axillary meristem, root growth
Ma07_g10330	7698294	7710182	+	7	568	3R	cell cycle control
Ma07_g10340	7710102	7712664	-	1	332	R2R3	leaf-, shoot-, germ morphogenesis
Ma07_g11110	8262980	8264078	-	3	1286	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma07_g12330	9212596	9214045	+	3	272	R2R3	secondary cell wall, lignin
Ma07_g13590	10215552	10217192	- +	2	307	R2R3	general flavonoid, trichome
Ma07_g17600	20758827	20760547	+	3	343	R2R3	
Ma07_g19350	27376391	27377767	-	3	270	R2R3	defense, stress response
Ma07_g19470	27474531	27475523	+	3	241	R2R3	cell cycle regulation
Ma07_g19700	27655370	27656957	-	3	470	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma07_g19720	27682847	27684927	+	3	357	R2R3	trichome branching, petal morphogenesis
Ma07_g19880 *	27793461	27794847	+ +	3	264	R2R3	proanthocyanidins
Ma07_g19890 *	27798123	27799369	- +	3	269	R2R3	proanthocyanidins
Ma07_g20020	27925622	27926695	+	4	270	R2R3	secondary cell wall, lignin
Ma07_g20990	28973981	28975550	-	3	341	R2R3	axillary meristem, root growth
Ma07_g22540	30450165	30451931	-	3	269	R2R3	defense, stress response
Ma07_g23060	30808440	30823676	+	4	551	R2R3	anther development, stress response
Ma07_g23180	30931923	30933477	+	3	289	R2R3	defense, stress response

*M. acuminata* MYB family

Ma07_g23230 *		30951474	30953174	+	3	274	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma07_g23240 *		30953852	30959687	+	5	1124	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma07_g26530		33294249	33295626	-	3	292	R2R3	
Ma08_g01300		1210739	1212318	-	3	263	R2R3	defense, stress response
Ma08_g01760		1466320	1467246	+	1	249	R2R3	stress tolerance
Ma08_g02100		1707379	1708993	+	3	421	R2R3	embryogenesis, seed maturation
Ma08_g02450		1904208	1906264	+	3	241	R2R3	
Ma08_g03420		2489605	2490995	+	3	319	R2R3	anther-, trichome development
Ma08_g10260		7475666	7478250	-	3	371	R2R3	flavonols, phlobaphene
Ma08_g10600		7758560	7759645	-	3	256	R2R3	defense, stress response
Ma08_g11120		8204459	8205747	+	3	278	R2R3	photomorphogenesis
Ma08_g12510		9469637	9470606	+	3	231	R2R3	PP, lignin
Ma08_g13070		10389004	10390770	-	3	313	R2R3	axillary meristem, root growth
Ma08_g14720		14652239	14654273	+	3	370	R2R3	
Ma08_g15820		16034463	16037767	- +	3	260	R2R3	
Ma08_g15960		16625932	16636910	-	3	572	R2R3	
Ma08_g16760		20724394	20725390	- +	3	194	R2R3	flavonoid repressor
Ma08_g17860		27301469	27302427	-	3	249	R2R3	defense, stress response
Ma08_g18540		32070622	32071809	-	4	276	R2R3	defense, stress response
Ma08_g23390		36792752	36794121	+	3	290	R2R3	proanthocyanidins
Ma08_g25570		38350574	38352398	+	3	307	R2R3	axillary meristem, root growth
Ma08_g25960	MYBS3 <sup>[4]</sup>	38629094	38631076	+	1	296	R2R3	stress response, hormone signaling
Ma08_g26720		39209905	39211361	-	3	541	R2R3	
Ma08_g30360		41652638	41653604	+	3	269	R2R3	defense, stress response
Ma08_g31720	MYB83 <sup>[2]</sup>	42548860	42549971	+	2	279	R2R3	secondary wall, lignin
Ma08_g32760		43364665	43366044	+	3	200	R2R3	repressors PP, sinapate, lignin
Ma08_g34230		44310373	44311394	- +	2	203	R2R3	repressors PP, sinapate, lignin
Ma08_g34710		44706406	44707610	+	3	245	R2R3	
Ma09_g03310		2240424	2242599	-	1	342	R2R3	leaf-, shoot-, germ morphogenesis
Ma09_g03740		2480875	2484428	+	2	322	R2R3	stress tolerance
Ma09_g04930		3159017	3160626	-	3	302	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma09_g06730		4303577	4304528	+	3	204	R2R3	stamen development
Ma09_g08140		5353765	5354763	+	2	256	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma09_g08260		5451761	5453265	- +	3	272	R2R3	repressors PP, sinapate, lignin
Ma09_g09400		6192674	6193933	-	3	263	R2R3	
Ma09_g09720		6397365	6410516	-	13	798	4R	SNAP complex
Ma09_g10800		7342785	7344298	-	3	327	R2R3	anther-, trichome development
Ma09_g11770		8000781	8002111	-	3	262	R2R3	defense, stress response

*M. acuminata* MYB family

Ma09_g13170		8908572	8910010	-	3	310	R2R3	axillary meristem, root growth
Ma09_g14260		9744056	9745210	-	3	187	R2R3	root development
Ma09_g15050		10356702	10357805	-	3	248	R2R3	secondary cell wall, lignin
Ma09_g15130		10456782	10469060	+	5	211	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma09_g15440		10764111	10765364	-	3	257	R2R3	proanthocyanidins
Ma09_g15940		11297144	11298342	-	3	260	R2R3	defense, stress response
Ma09_g16940		12437966	12439652	+	3	279	R2R3	defense, stress response
Ma09_g16980		12476466	12478547	+	3	304	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma09_g20280		28997934	28999432	+	3	273	R2R3	axillary meristem, root growth
Ma09_g22730		34631674	34632781	-	3	315	R2R3	
Ma09_g23100		35024297	35025651	+	1	288	R2R3	stress response, hormone signaling
Ma09_g23770		35511400	35518711	+	5	1120	2R	CDC5
Ma09_g24640	MYB31 <sup>[2]</sup>	36291635	36292658	-	2	173	R2R3	repressors PP, sinapate, lignin
Ma09_g25010		36615281	36616548	+	3	301	R2R3	defense, stress response
Ma09_g25590		36999254	37000706	-	3	315	R2R3	anther-, trichome development
Ma09_g27990		38852455	38853644	- +	3	260	R2R3	anthocyanins
Ma09_g28970		39592459	39593487	+	1	343	R2R3	leaf-, shoot-, germ morphogenesis
Ma09_g29010		39617312	39618287	-	3	214	R2R3	
Ma09_g29660	MYB52 <sup>[2]</sup>	40061201	40062117	-	3	210	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma10_g01730		5102443	5104125	-	3	286	R2R3	defense, stress response
Ma10_g01750		5158925	5160589	+	3	288	R2R3	defense, stress response
Ma10_g04420		15095580	15096722	-	3	284	R2R3	
Ma10_g04920		15562002	15563726	-	3	321	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma10_g05260		15989100	15989831	+	1	243	R2R3	stress tolerance
Ma10_g05680		17016063	17017845	+	3	499	R2R3	embryogenesis, seed maturation
Ma10_g06140		17599985	17600776	+	3	212	R2R3	
Ma10_g09100		23305076	23306673	-	3	297	R2R3	anther-, trichome development
Ma10_g09370		23565723	23566809	-	3	268	R2R3	axillary meristem, root growth
Ma10_g10820		24568694	24569501	+	3	176	R2R3	repressors PP, sinapate, lignin
Ma10_g11100		24705798	24706830	-	2	260	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma10_g13000		25941801	25943526	+	3	304	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma10_g13640		26378268	26379297	-	3	236	R2R3	cell cycle regulation
Ma10_g14150		26690021	26691729	- +	2	298	R2R3	general flavonoid, trichome
Ma10_g14950		27210210	27223227	+	11	869	3R	cell cycle control
Ma10_g16050		27917256	27918773	+ +	3	217	R2R3	repressors PP, sinapate, lignin
Ma10_g17650		28964249	28965577	+ +	3	278	R2R3	anthocyanins
Ma10_g18840		29620788	29624706	+	12	467	R2R3	guard cell division, root gravitropism
Ma10_g19130		29806295	29807639	-	3	297	R2R3	root development

*M. acuminata* MYB family

Ma10_g19820	30233785	30235049	-	3	175	R2R3	defense, stress response
Ma10_g19970	30311359	30312430	- +	3	206	R2R3	flavonoid repressor
Ma10_g24510	33076381	33078033	-	3	305	R2R3	root development
Ma10_g25660	33694709	33695874	+	3	319	R2R3	root development
Ma10_g26540	34187286	34196507	+	7	566	3R	cell cycle control
Ma10_g26660	34247740	34248867	+	2	226	R2R3	
Ma10_g29230	35877570	35878873	-	3	315	R2R3	defense, stress response
Ma10_g29290	35933157	35937337	-	3	386	R2R3	anther development, stress response
Ma10_g29660	36170110	36171732	-	3	289	R2R3	trichome branching, petal morphogenesis
Ma10_g29900	36334717	36336097	+	3	285	R2R3	secondary cell wall, lignin
Ma11_g00330	235199	236300	-	3	235	R2R3	
Ma11_g00350	255253	257212	+	3	375	R2R3	
Ma11_g02310	1659706	1660645	-	3	213	R2R3	PP, lignin
Ma11_g03860	2954679	2956968	-	3	294	R2R3	flower meristem identity
Ma11_g04680	3650617	3651708	+	3	264	R2R3	defense, stress response
Ma11_g06880	5504819	5505826	-	2	167	R2R3	repressors PP, sinapate, lignin
Ma11_g07330	5826847	5827933	+	3	261	R2R3	axillary meristem, root growth
Ma11_g07530	6014287	6015836	-	3	319	R2R3	suberin
Ma11_g08730	6941295	6943798	-	3	275	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma11_g10680	10271323	10272648	-	3	275	R2R3	defense, stress response
Ma11_g10710	10304401	10305535	+	3	275	R2R3	
Ma11_g11300	12744754	12745994	+	3	251	R2R3	defense, stress response
Ma11_g11940	15469297	15470702	-	3	315	R2R3	defense, stress response
Ma11_g14670	20385599	20386631	-	3	262	R2R3	defense, stress response
Ma11_g15740	21412142	21413141	-	3	230	R2R3	PP, lignin
Ma11_g16150	21712282	21713968	-	3	233	R2R3	
Ma11_g16430	21944276	21945346	+	3	287	R2R3	PP, lignin
Ma11_g19220	24164773	24166212	-	3	344	R2R3	defense, stress response
Ma11_g21160	25411481	25412732	-	3	303	R2R3	
Ma11_g21730	25754193	25755462	-	3	326	R2R3	suberin
Ma11_g21820	25812612	25817014	- +	3	235	R2R3	
Ma11_g23010	26544673	26545555	-	2	241	R2R3	cell cycle regulation
Ma11_g23420	26767577	26769029	+	3	183	R2R3	cell wall, lignin, seed oil, axillary meristem
Ma00_g01590	9744102	9745946	+	1	283	R2R3	stress response, hormone signaling
Ma00_g04340	36355056	36356431	+	2	283	R2R3	secondary wall, lignin
Ma00_g04960	43098831	43099818	-	3	267	R2R3	defense, stress response

*M. acuminata* MYB family

180 The number of R2R3-MYB genes is one of the highest among the species that have been  
181 studied to date, ranging from 45 in *Ginkgo biloba* (Liu et al. 2017) over 157 in *Zea mays* (Du  
182 et al. 2012a) and 249 in *Brassica napus* (Hajiebrahimi et al. 2017) to 360 in *Gossypium*  
183 *hirsutum* (Salih et al. 2016). This is probably due to three whole-genome duplications  
184 ( $\gamma$  100 Myr ago and  $\alpha$ ,  $\beta$  65 Myr ago) that occurred during *Musa* genome evolution (D'Hont et  
185 al. 2012; Wu et al. 2016). The number of atypical multiple repeat MYB genes identified in  
186 *M. acuminata* is in the same range as those reported for most other plant species, up to six  
187 MYB3R and up to two MYB4R and CDC5-like genes.

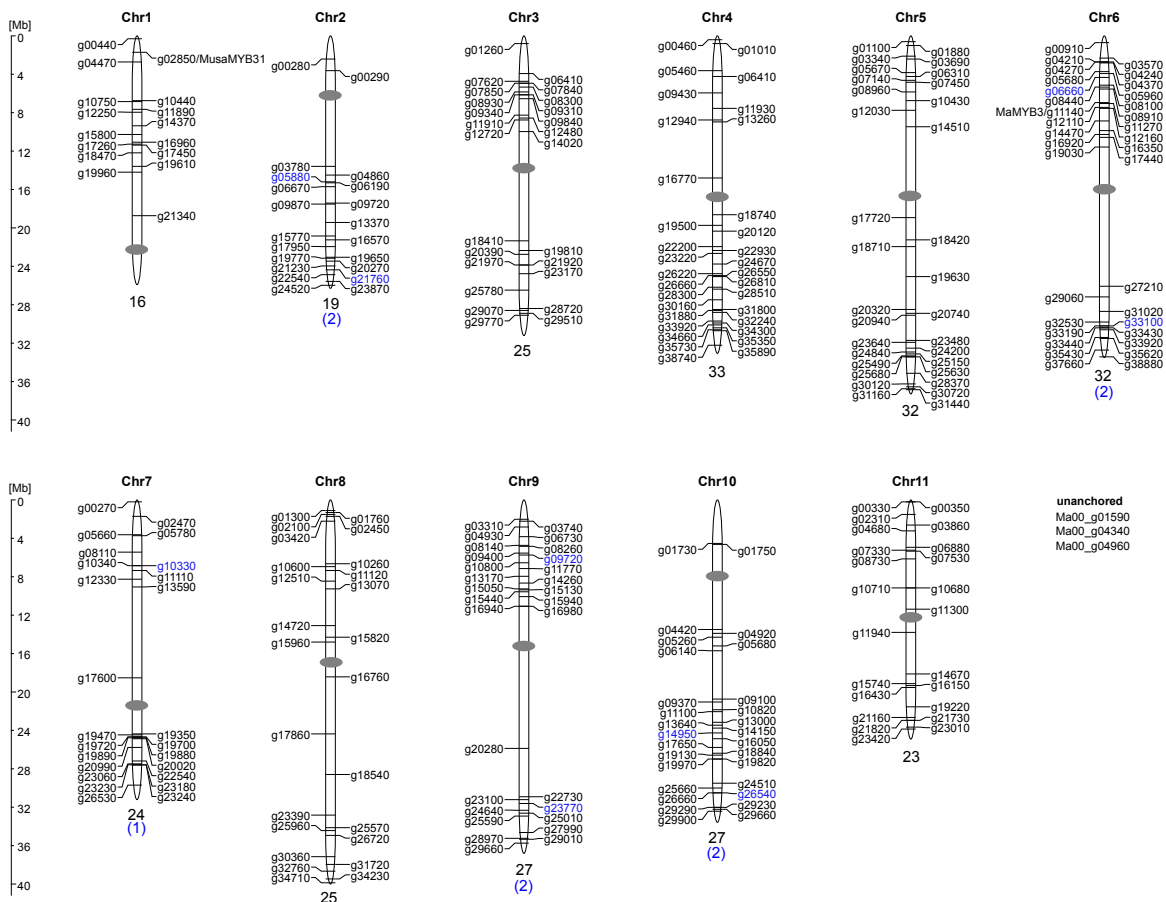
188 The 286 MaR2R3-MYB genes identified constitute approximately 0.81 % of the 35,276  
189 predicted protein-coding *M. acuminata* genes and 9.1 % of the 3,155 putative *M. acuminata*  
190 transcription factor genes (Martin et al. 2016). These were subjected to further analyses. The  
191 identified MaR2R3-MYB genes were named following the nomenclature of the locus tags  
192 provided in the DH-Pahang version 2 genome annotation (Table 1). A keyword search in the  
193 NCBI database (<http://www.ncbi.nlm.nih.gov/>) revealed no evidence for *M. acuminata* MYB  
194 genes not present in Table 1.

195 Six publications dealing with *M. acuminata* MYB genes were identified: one study describes  
196 the elevated expression of nine MaMYB genes in transgenic banana plants overexpressing the  
197 NAC domain transcription factor MusaVND1 (vascular related NAC domain) indicating a  
198 role of these MaMYBs in the regulation of secondary wall deposition (Negi et al. 2015).  
199 MYBS3 (Ma08\_25960) was found to be differentially expressed between cold-sensitive and  
200 cold-tolerant bananas (Yang et al. 2015). Another publication described the *M. acuminata*  
201 R2R3-MYB gene Ma05\_03690 being upregulated in the early response to the endoparasitic  
202 root-knot nematode *Meloidogyne incognita* in roots (Castañeda et al. 2017). MusaMYB31  
203 (Ma01\_02850) was identified as a negative regulator of lignin biosynthesis and the general  
204 phenylpropanoid biosynthesis pathway (Tak et al. 2017). MaMYB3 (Ma06\_11140) was found  
205 to repress starch degradation in fruit ripening (Fan et al. 2018) and MaMYB4 (Ma01\_19610)  
206 was recently described to control fatty acid desaturation (Song et al. 2019).

207 On the basis of the DH-Pahang version 2 annotation, 292 of the 295 MaMYB genes could be  
208 assigned to the eleven chromosomes. The chromosomal distribution of MaMYB genes on the  
209 pseudochromosomes is shown in Figure 1 and revealed that *M. acuminata* MYB genes are  
210 distributed across all chromosomes.

211 Gene structure analysis revealed that most MaR2R3-MYB genes (226 of 286; 79 %) follow the  
212 previously reported rule of having two introns and three exons, and display the highly  
213 conserved splicing arrangement that has also been reported for other plant species (Du et al.

214 2012a; Du et al. 2012b; Stracke et al. 2014). A total of 29 (10.1 %) *MaR2R3-MYB* genes have  
 215 two exons and 19 (6.6 %) were one exon genes. Eight (2.8 %) *MaR2R3-MYB* genes have four  
 216 exons, and two of each with five and twelve exons, respectively. The complex exon-intron  
 217 structure of Ma06\_12110 and Ma10\_18840 is conserved in their *A. thaliana* orthologs  
 218 AtMYB88 and AtMYB124/FOUR LIPS (FLP) containing ten and eleven exons, respectively.  
 219 This supports their close evolutionary relationship, but also suggests the conservation of this  
 220 intron pattern in evolution since the monocot-dicot split 140-150 Myr ago (Chaw et al. 2004).  
 221



222  
 223 **Figure 1: Distribution of *MaMYB* genes on the eleven *M. acuminata* chromosomes.**  
 224 Chromosomes are drawn to scale. The positions of centromeres (grey ovals) are roughly  
 225 estimated from repeat distribution data. The chromosomal positions of the *MaMYB* genes  
 226 (given in DH Pahang version 2 annotation ID) are indicated. *R2R3-MYB* genes are given in  
 227 black letters, *MYB* genes with more than two MYB repeats are given in blue letters. The  
 228 number of *MYB* genes on each chromosome is given below the respective chromosome.  
 229

230  
 231 **Phylogenetic analysis of the *M. acuminata* MYB family**

232 With the aim to explore the putative function of the predicted *M. acuminata* MYBs, we  
 233 assigned them to plant MYB proteins with known function. For this, we chose primarily data

*M. acuminata* MYB family

234 from *A. thaliana*, which is the source of most functional MYB characterisations. From  
235 comparable studies, MYB function appears conserved across MYB clades, suggesting that  
236 closely related MYBs recognise similar/same target genes and possess cooperative,  
237 overlapping or redundant functions.

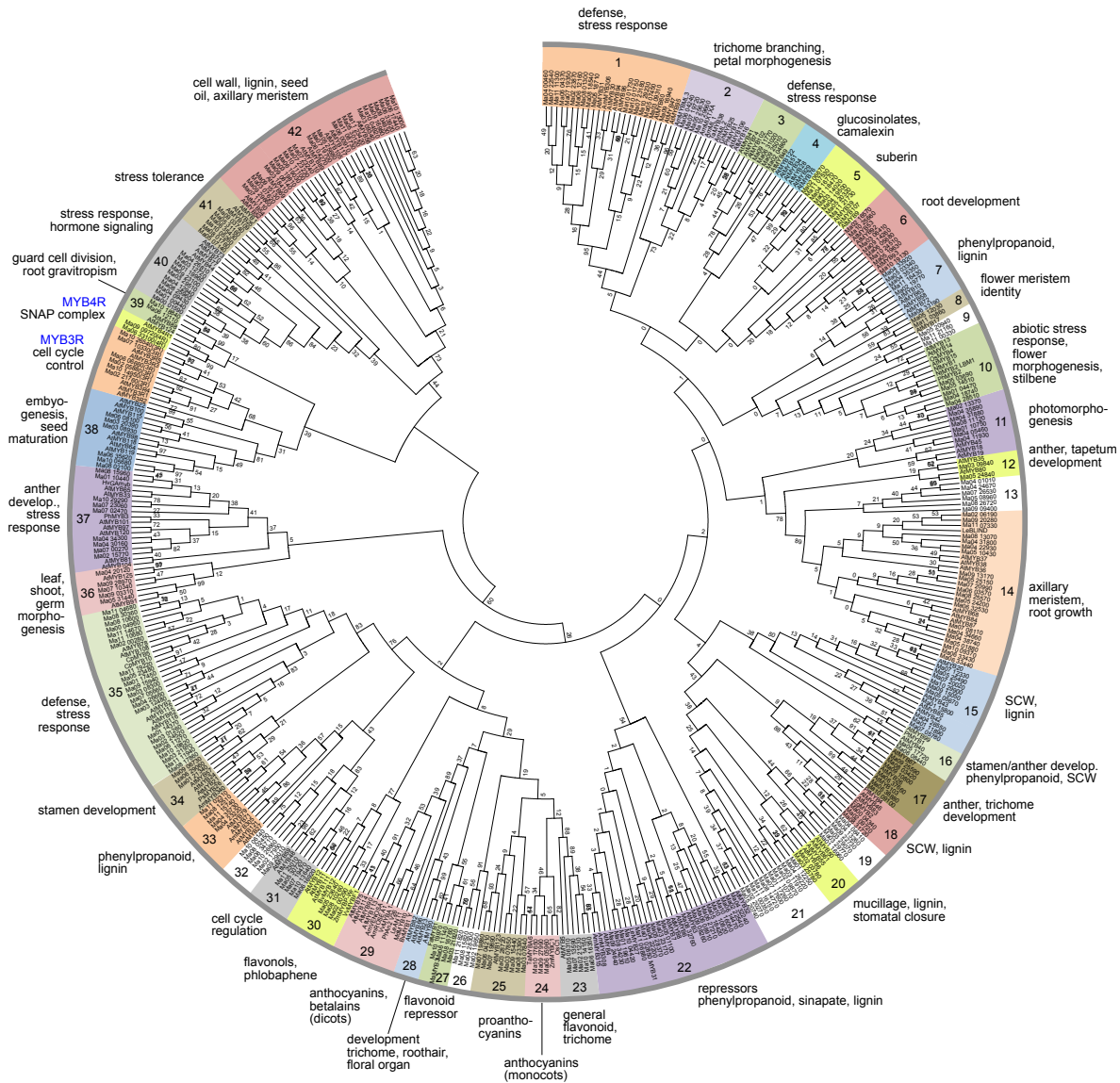
238 To unravel the relationships, we constructed a phylogenetic tree with 469 MYB domain  
239 amino acid sequences of MYB proteins. We used 294 MaMYBs (omitting the CDC5-like  
240 MaMYB), the complete *A. thaliana* MYB family (132 members, including 126 R2R3-MYB,  
241 five MYB3R and one MYB4R) and 43 functionally well characterised landmark R2R3-  
242 MYBs from other plant species. The phylogenetic tree topology allowed us to classify the  
243 analysed MYBs into one MYB3R clade, one MYB4R clade and 42 R2R3-MYB protein  
244 clades (Figure 2).

245 Most R2R3-MYB clades (31 of 42) include variable numbers of MYB proteins from  
246 Arabidopsis and banana, indicating that the appearance of most *MYB* genes in these two  
247 species predates the monocot-dicot split as observed in other studies (Du et al. 2015). Several  
248 of these clades also contain landmark MYBs from other plant species. The two clades 4 and  
249 28 only contain Arabidopsis R2R3-MYB members, while seven clades (9, 13, 19, 21, 26, 29  
250 and 32, displayed with white background in Figure 2) only contain banana R2R3-MYB  
251 members. Additionally, two clades, 24 with monocot anthocyanin biosynthesis regulators and  
252 27 with the strawberry landmark flavonoid biosynthesis repressor FaMYB1 (Aharoni et al.  
253 2001), do not contain any *A. thaliana* MYB (Figure 2).

254



*M. acuminata* MYB family



255

256 **Figure 2: Phylogenetic Maximum Likelihood (ML) tree** (consensus tree inferred from  
 257 1000 bootstraps) with 466 MYB domain amino acid sequences of MYB proteins from *Musa*  
 258 *acuminata* (Ma), *Arabidopsis thaliana* (At) and landmark MYBs from other plant species  
 259 built with MEGA7. Subgroups are labeled with different colors and functional annotations are  
 260 given. The numbers at the branches give bootstrap support values from 1000 replications.  
 261 SCW, secondary cell wall

262

263 The lineage specificity of some MYB clades could indicate that these clades may have been  
 264 lost or gained in a single order or species during plant evolution, as indicated by other studies  
 265 (Baum 2008; Zhong et al. 2015; Mehta et al. 2016). For example, clade 28 lacked  
 266 *M. acuminata* orthologs, but includes the *A. thaliana* R2R3-MYBs AtMYB0/GLABRA1 and  
 267 AtMYB66/WEREWOLF, which have been identified as being involved in the formation of  
 268 trichomes and root hairs from epidermal cells (Oppenheimer et al. 1991; Lee and Schiefelbein  
 269 1999). Similar observations have been made in maize (monocot) and sugarbeet (eudicot,  
 270 caryophyllales) (Du et al. 2012b; Stracke et al. 2014), both not containing clade 28 orthologs,

*M. acuminata* MYB family

271 while grape (eudicot, rosid) and poplar (eudicot, rosid) do (Matus et al. 2008; Wilkins et al.  
272 2009). It has been hypothesized that GLABRA1-like *MYB* genes have been acquired in rosids  
273 after the rosid-asterids split (Brockington et al. 2013). The absence of MaMYBs in clade 28 is  
274 consistent with this hypothesis, since monocots branched off before the separation of asterids  
275 and rosids in eudicots. Clade 4 also lacks banana R2R3-MYBs. This clade contains the  
276 glucosinolate biosynthesis regulators AtMYB28, AtMYB34 and AtMYB51 (Sonderby et al.  
277 2007; Gigolashvili et al. 2007). The absence of MaMYBs in this clade is concordant with the  
278 fact that glucosinolates are only present in the Brassicaceae family. This clade is thought to  
279 have originated from a duplication event before the divergence of Arabidopsis from Brassica  
280 (Yanhui et al. 2006).

281 The seven clades containing only MaMYBs were manually inspected by applying BLAST  
282 searches at the NCBI protein database in order to identify high homology to functionally  
283 characterized landmark plant R2R3-MYBs. In no case could landmark MYB be identified.  
284 Consequently, these clades could be described as a lineage-specific expansion in  
285 *M. acuminata*, reflecting a species-, genus- or order-specific evolutionary change. These  
286 MaMYB proteins may have specialised functions that were acquired or expanded in  
287 *M. acuminata* during genome evolution. Further research will be needed to decipher the  
288 biological roles of these *MaMYB* genes.

289 R2R3-MYBs may interact with basic helix-turn-helix (bHLH)-type transcription factors,  
290 together with WD-repeat (WDR) proteins, forming a trimeric MBW complex. These R2R3-  
291 MYBs are defined by a bHLH-binding consensus motif [D/E]Lx2[R/K]x3Lx6Lx3R  
292 (Zimmermann et al. 2004) found in all bHLH-interacting R2R3-MYBs. A search in the  
293 MaMYB proteins for the mentioned bHLH-interaction motif identified 30 MaMYBs  
294 containing this motif (Table 1) and thus putatively interacting with bHLH proteins. 36 of  
295 these 40 MaMYBs were all functionally assigned to clades containing (potentially) known  
296 bHLH-interacting R2R3-MYBs: nine in clade 22 (repressors phenylpropanoid, sinapate-,  
297 lignin regulators), six in clade 25 (proanthocyanin regulators), four in clade 23 (general  
298 flavonoid, trichome regulators), four in clade 27 (flavonoid repressors) and three in clade 24  
299 (monocot anthocyanin regulators). Four MaMYBs, Ma02\_19650, Ma04\_28300, Ma08\_15820  
300 and Ma11\_21820 were all found in the *M. acuminata*-specific clade 26. These MaMYBs may  
301 interact with bHLH-type transcription factors. The close relation to flavonoid biosynthesis  
302 regulators suggests that they may act similarly to R2R3-MYB proanthocyanin regulators.

303 Further research is needed to determine if they regulate a *Musa*-specific biosynthesis pathway.

304

305 **Expression profiles for *M. acuminata* MYB genes in different organs and developmental**  
 306 **stages**

307 Since it is not unusual for large transcription factor families in higher organisms to have  
 308 redundant functions, a particular transcription factor needs to be studied and characterised in  
 309 the context of the whole family. In this regard, the gene expression pattern can provide a hint  
 310 to gene function. In this study we used publicly-available RNA-seq reads from the Sequence  
 311 Read Archive (Additional Table 2) to analyse the expression of the 294 *MaMYB* genes in  
 312 different organs and developmental stages: embryonic cell suspension, seedling, root and  
 313 young, adult and old leaf, pulp stages S1-S4 and peel stages S1-S4. Filtered RNA-seq reads  
 314 were aligned to the genome reference sequence and the number of mapped reads per  
 315 annotated transcript were quantified and compared across the analysed samples to calculate  
 316 normalised RNA-seq read values which are given in Table 2.

317  
 318 **Table 2: The expression profiles of *MaR2R3-MYB* genes in different organs and**  
 319 **developmental stages based on RNA-seq data.** The intensity of green background is  
 320 correlated to expression level given in FPKM.  
 321

gene	embryogenic	seedling	root	leaf	young_leaf	adult_leaf	old_leaf	pulp	pulpS1	pulpS2	pulpS3	pulpS4	peel	peelS1	peelS2	peelS3	peelS4
Ma00_g01590	50	35	6	151	77	175	348	6	5	5	8	5	8	2	19	10	2
Ma00_g04340	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Ma00_g04960	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ma01_g00440	181	49	38	129	53	175	276	32	31	58	34	7	30	9	63	35	11
Ma01_g02850	9	2	14	27	21	31	38	20	24	23	23	10	11	7	9	17	12
Ma01_g04470	1	0	12	4	1	6	2	9	13	9	7	8	6	6	3	7	7
Ma01_g10440	3	16	4	5	7	5	5	4	5	5	3	3	3	2	3	3	4
Ma01_g10750	0	0	2	2	0	0	4	1	1	1	1	2	4	5	4	4	5
Ma01_g11890	0	0	3	3	10	1	0	0	0	0	0	0	0	0	0	0	0
Ma01_g12250	1	0	4	5	17	2	0	3	4	3	2	2	4	4	4	3	3
Ma01_g14370	0	17	11	0	0	0	0	5	7	7	8	0	8	3	7	18	4
Ma01_g15800	1	0	4	1	0	0	0	4	2	5	7	3	6	8	5	2	9
Ma01_g16960	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Ma01_g17260	1	0	11	1	0	0	0	3	3	3	2	3	4	5	3	4	5
Ma01_g17450	0	0	6	2	0	1	6	5	13	2	2	1	1	1	2	2	0
Ma01_g18470	0	0	0	0	0	0	0	0	1	0	0	0	1	2	1	0	0
Ma01_g19610	13	0	6	39	56	40	57	29	78	22	15	2	12	7	36	2	5
Ma01_g19960	35	1	6	97	94	148	145	5	7	6	5	4	19	5	58	10	4
Ma01_g21340	0	0	0	0	0	0	0	2	8	0	0	0	0	0	0	0	0
Ma02_g00280	1	1	21	4	1	1	5	6	8	10	4	2	5	5	4	5	7
Ma02_g00290	0	0	3	9	5	26	1	8	25	0	6	2	3	2	3	2	5
Ma02_g03780	0	0	29	18	26	0	0	13	14	9	18	12	30	39	31	17	33
Ma02_g04860	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Ma02_g05880	4	2	2	3	2	5	3	2	4	2	2	1	2	1	1	1	3
Ma02_g06190	0	0	19	8	0	0	0	16	30	12	13	11	28	42	18	18	32
Ma02_g06670	1	1	4	1	0	0	0	4	3	7	4	4	5	6	2	6	5
Ma02_g09720	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Ma02_g09870	29	1	23	37	99	33	11	19	15	28	27	6	11	11	10	9	13
Ma02_g13370	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ma02_g15770	6	0	1	0	0	1	0	1	2	0	1	0	1	1	1	0	1







*M. acuminata* MYB family

Ma10_g01750	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Ma10_g04420	0	0	1	4	10	2	2	1	2	1	1	0	1	1	3	0
Ma10_g04920	3	0	0	1	3	2	0	0	1	0	0	0	0	0	1	0
Ma10_g05260	0	0	0	1	1	1	3	0	0	0	0	0	0	0	0	0
Ma10_g05680	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Ma10_g06140	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Ma10_g09100	0	0	0	1	4	0	0	0	0	0	0	0	0	0	0	0
Ma10_g09370	0	0	4	3	1	0	5	2	2	3	3	1	3	3	2	4
Ma10_g10820	0	0	2	0	0	0	0	4	14	1	0	1	1	2	1	1
Ma10_g11100	0	1	6	2	0	0	6	0	0	0	0	0	2	0	0	7
Ma10_g13000	3	0	5	10	2	21	16	1	1	1	0	2	1	1	1	1
Ma10_g13640	2	1	40	31	30	34	12	22	25	19	20	25	35	48	21	28
Ma10_g14150	0	1	1	2	2	3	4	1	3	1	0	1	1	0	1	1
Ma10_g14950	0	0	3	2	0	0	0	4	8	1	2	3	4	4	2	3
Ma10_g16050	5	1	10	28	74	23	13	9	14	16	4	3	7	4	16	3
Ma10_g17650	0	30	0	0	0	0	0	4	13	1	0	0	2	2	4	1
Ma10_g18840	3	1	5	2	1	2	2	4	6	3	6	2	3	5	2	2
Ma10_g19130	0	0	2	0	0	0	0	1	0	0	1	1	1	0	1	0
Ma10_g19820	1	0	2	6	0	0	22	0	0	0	0	0	5	0	12	9
Ma10_g19970	1	0	0	2	6	3	1	1	3	1	0	0	0	0	0	0
Ma10_g24510	9	4	2	7	16	6	4	3	4	2	4	2	1	1	1	1
Ma10_g25660	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Ma10_g26540	5	7	6	4	3	4	4	6	8	4	4	6	4	5	2	3
Ma10_g26660	2	0	6	3	1	2	2	10	14	11	8	5	4	3	3	5
Ma10_g29230	6	0	1	4	6	9	3	0	1	1	0	0	1	0	2	0
Ma10_g29290	4	1	1	2	2	2	2	1	1	0	1	1	1	1	0	0
Ma10_g29660	2	0	1	15	60	0	0	1	3	0	0	0	0	0	0	0
Ma10_g29900	5	1	9	10	7	11	11	8	8	7	9	8	9	11	10	10
Ma11_g00330	0	0	4	0	0	0	0	0	1	0	0	1	0	0	0	0
Ma11_g00350	0	0	6	5	13	1	0	3	5	2	2	2	5	7	5	3
Ma11_g02310	0	0	2	1	0	0	0	2	1	1	4	0	2	2	1	1
Ma11_g03860	0	0	2	13	47	1	0	5	14	4	1	0	2	1	5	1
Ma11_g04680	20	2	0	32	6	57	64	0	1	1	0	0	4	0	7	10
Ma11_g06880	22	4	15	13	7	19	12	31	41	39	30	14	7	6	4	9
Ma11_g07330	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ma11_g07530	0	0	5	2	1	1	1	6	3	14	4	2	3	5	1	4
Ma11_g08730	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0
Ma11_g10680	1	2	5	2	1	0	3	12	21	20	6	3	6	7	5	8
Ma11_g10710	0	1	2	1	4	0	0	2	5	2	1	2	3	1	6	2
Ma11_g11300	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Ma11_g11940	0	0	5	0	0	0	0	0	1	0	0	0	0	0	0	0
Ma11_g14670	0	1	2	0	0	0	0	0	0	0	0	0	2	0	2	5
Ma11_g15740	4	0	2	5	7	4	6	6	9	4	6	5	2	1	3	2
Ma11_g16150	1	2	3	10	37	2	1	4	7	4	6	1	3	3	3	2
Ma11_g16430	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ma11_g19220	1	0	1	0	0	0	0	0	1	1	0	0	2	0	0	7
Ma11_g21160	1	0	9	3	0	1	12	2	4	0	4	0	0	1	0	0
Ma11_g21730	0	0	2	1	0	1	0	2	2	3	0	1	2	4	0	2
Ma11_g21820	0	0	0	2	2	4	0	0	0	0	0	0	0	0	1	0
Ma11_g23010	0	0	6	1	0	0	0	3	3	5	2	2	2	3	1	2
Ma11_g23420	1	0	2	0	0	0	0	1	2	0	0	0	0	0	0	0

322

323

324 Our expression analyses revealed that *M. acuminata* MYBs have diverse expression patterns  
 325 in different organs. Many of the *MaMYBs* exhibited low transcript abundance levels with  
 326 expression in only one or a few organs. This is consistent with other transcription factor genes,  
 327 typically found to be expressed in this manner due to functional specificity and diversity. The  
 328 highest number of expressed *MaMYB* genes (222; 75.5%) is observed in roots, followed by  
 329 pulp (210; 71.4%), leaf (204; 69.4%), peel (197; 67%) and embryonic cell suspension (130;  
 330 44.2%). The fewest *MaMYB* genes are expressed in seedlings (99; 33.7%) in the considered  
 331 dataset. 41 *MaMYB* genes (14%) were expressed in all samples analysed (albeit with varying

332 expression levels), which suggested that these MaMYBs play regulatory roles at multiple  
333 developmental stages in multiple tissues. 14 *MaMYB* genes (4.8%) lacked expression  
334 information in any of the analysed samples, possibly indicating that these genes are expressed  
335 in other organs (e.g. pseudostem, flower, bract), specific cells, at specific developmental  
336 stages, under special conditions or are pseudogenes. 279 MaMYBs (94.8%) are expressed in  
337 at least one analysed sample, although the transcript abundance of some genes was very low.  
338 Some *MaMYB* genes were expressed in all analysed RNA-seq samples at similar levels (e.g.  
339 R2R3-MYBs *Ma06\_33440* and *Ma11\_06880*) while others show variance in transcript  
340 abundance with low (no) levels in one or several organs and high levels in others (or vice  
341 versa). For example, *Ma02\_16570*, *Ma03\_09310*, *Ma07\_11110*, *Ma10\_01730*, *Ma10\_05260*  
342 and *Ma10\_09100* show organ-specific expression, as their transcripts were exclusively  
343 detected in leaves, which hints to leaf-specific functionality. *Ma03\_07840* and *Ma07\_19470*  
344 were found to be predominantly expressed in pulp, showing expression also in other analysed  
345 organs, but not in the seedling. Overall, this results suggests that the corresponding MaMYB  
346 regulators are limited to distinct organs, tissues, cells or conditions.  
347 Some paralogous *MaMYB* genes clustered in the genome (Table 1) showed different  
348 expression profiles, while other clustered paralogous *MaMYB* genes did not. For example, the  
349 clustered R2R3-MYB genes *Ma06\_33430* and *Ma06\_33440* (both in the axillary  
350 meristem/root growth cluster 14) are both expressed in pulps, peels and roots, but  
351 *Ma06\_33440* is also highly expressed in leaves and embryonic cells, while *Ma06\_33430* is not.  
352 The expression pattern of *Ma03\_07840* and *Ma03\_07850* (both in the proanthocyanin-related  
353 cluster 25) is, in contrast, very similar, with low expression in root, leaf, pulp and peel, but no  
354 expression in embryonic cells and roots. These results could point to functional redundancy of  
355 the genes *Ma03\_07840* and *Ma03\_07850*, while *Ma06\_33430* and *Ma06\_33440* could be  
356 (partly) involved in distinct aspects of growth processes.

357

358

### 359 **Conclusions**

360 The present genome-wide identification, chromosomal organisation, functional classification  
361 and expression analyses of *M. acuminata* MYB genes provide a first step towards cloning and  
362 functional dissection to decode the role of MYB genes in this economically interesting species.

363

364

### 365 **Authors' contributions**



366 AP and RS conceived and designed research. BP and RS conducted experiments. BP analyzed  
367 and visualized transcriptome data. RS and BW interpreted the data. RS wrote the manuscript.  
368 All authors read and approved the final manuscript.

369

### 370 **Acknowledgements**

371 We are grateful to Melanie Kuhlmann for excellent technical assistance. The research stay of  
372 Ashutosh Pandey at the Weisshaar lab was made possible by the Alexander von Humboldt  
373 Foundation (AvH). We acknowledge support for the Article Processing Charge by the  
374 Deutsche Forschungsgemeinschaft and the Open Access Publication Fund of Bielefeld  
375 University. We thank Nathanael Walker-Hale for language editing.

376

### 377 **References**

- 378 Aharoni A, De Vos CHR, Wein M, Sun Z, Greco R, Kroon A, Mol JNM, O'Connell AP  
379 (2001) The strawberry FaMYB1 transcription factor suppresses anthocyanin and  
380 flavonol accumulation in transgenic tobacco. *The Plant Journal* 28 (3):319-332
- 381 Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search  
382 tool. *Journal of Molecular Biology* 215 (3):403-410
- 383 Baum D (2008) Reading a Phylogenetic Tree: The Meaning of Monophyletic Groups. *Nature*  
384 *Education* 1 (1):190
- 385 Brockington SF, Alvarez-Fernandez R, Landis JB, Alcorn K, Walker RH, Thomas MM,  
386 Hileman LC, Glover BJ (2013) Evolutionary analysis of the MIXTA gene family  
387 highlights potential targets for the study of cellular differentiation. *Molecular Biology*  
388 *and Evolution* 30 (3):526-540
- 389 Castañeda NEN, Alves GSC, Almeida RM, Amorim EP, Fortes Ferreira C, Togawa RC,  
390 Costa MMDC, Grynberg P, Santos JRP, Cares JE, Miller RNG (2017) Gene  
391 expression analysis in *Musa acuminata* during compatible interactions with  
392 *Meloidogyne incognita*. *Annals of Botany* 119 (5):915-930
- 393 Chaw SM, Chang CC, Chen HL, Li WH (2004) Dating the monocot-dicot divergence and the  
394 origin of core eudicots using whole chloroplast genomes. *Journal of Molecular*  
395 *Evolution* 58 (4):424-441
- 396 Cooper GM (2000) Regulation of transcription in eukaryotes. In: *The cell: A molecular*  
397 *approach*. Sinauer Associates, Sunderland, MA,
- 398 D'Hont A, Denoeud F, Aury JM, Baurens FC, Carreel F, Garsmeur O, Noel B, Bocs S, Droc  
399 G, Rouard M, Da Silva C, Jabbari K, Cardi C, Poulain J, Souquet M, Labadie K,  
400 Jourda C, Lenggellé J, Rodier-Goud M, Alberti A, Bernard M, Correa M,  
401 Ayyampalayam S, Mckain MR, Leebens-Mack J, Burgess D, Freeling M, Mbéguié-A-  
402 Mbéguié D, Chabannes M, Wicker T, Panaud O, Barbosa J, Hribova E, Heslop-  
403 Harrison P, Habas R, Rivallan R, Francois P, Poirion C, Kilian A, Burthia D, Jenny C,  
404 Bakry F, Brown S, Guignon V, Kema G, Dita M, Waalwijk C, Joseph S, Dievart A,  
405 Jaillon O, Leclercq J, Argout X, Lyons E, Almeida A, Jeridi M, Dolezel J, Roux N,  
406 Risterucci AM, Weissenbach J, Ruiz M, Glaszmann JC, Quétier F, Yahiaoui N,  
407 Wincker P (2012) The banana (*Musa acuminata*) genome and the evolution of  
408 monocotyledonous plants. *Nature* 488 (7410):213-217
- 409 Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M,  
410 Gingeras TR (2013) STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29

- 411 (1):15-21
- 412 Droc G, Larivière D, Guignon V, Yahiaoui N, This D, Garsmeur O, Dereeper A, Hamelin C,  
413 Argout X, Dufayard JF, Lengelle J, Baurens FC, Cenci A, Pitollat B, D'Hont A, Ruiz  
414 M, Rouard M, Bocs S (2013) The banana genome hub. Database (Oxford) 2013  
415 (bat035)
- 416 Du H, Feng BR, Yang SS, Huang YB, Tang YX (2012a) The R2R3-MYB transcription factor  
417 gene family in maize. PLoS One 7 (6):e37463.
- 418 Du H, Liang Z, Zhao S, Nan MG, Phan Tran LS, Lu K, Huang YB, Li JN (2015) The  
419 Evolutionary History of R2R3-MYB Proteins Across 50 Eukaryotes: New Insights  
420 Into Subfamily Classification and Expansion. Scientific Reports 5 (11037)
- 421 Du H, Yang SS, Liang Z, Feng BR, Liu L, Huang YB, Tang YX (2012b) Genome-wide  
422 analysis of the MYB transcription factor superfamily in soybean. BMC Plant Biology  
423 12 (106):106
- 424 Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C, Lepiniec L (2010) MYB  
425 transcription factors in Arabidopsis. Trends in Plant Science 15:573-581
- 426 Fan ZQ, Ba LJ, Shan W, Yun-Yi X, Wang-Jin L, Jian-Fei K, Jian-Ye C (2018) A banana  
427 R2R3-MYB transcription factor MaMYB3 is involved in fruit ripening through  
428 modulation of starch degradation by repressing starch degradation-related genes and  
429 MabHLH6. The Plant Journal 96(1): 1191-1205
- 430 Frison EA, Sharrock SL (1999) The economic, nutritional and social importance of bananas  
431 in the world. In: Picq Cea (ed) Bananas and Food Security. INIBAP, Montpellier,  
432 France, pp 21-35
- 433 Gigolashvili T, Berger B, Mock HP, Muller C, Weisshaar B, Flugge UI (2007) The  
434 transcription factor HIG1/MYB51 regulates indolic glucosinolate biosynthesis in  
435 Arabidopsis thaliana. The Plant Journal 50 (5):886-901
- 436 Haak M, Vinke S, Keller W, Droste J, Rückert C, Kalinowski J, Pucker B (2018) High  
437 Quality de Novo Transcriptome Assembly of Croton tiglium. Frontiers in Molecular  
438 Biosciences 5:62
- 439 Hajiebrahimi A, Owji H, Hemmati S (2017) Genome-wide identification, functional  
440 prediction, and evolutionary analysis of the R2R3-MYB superfamily in Brassica  
441 napus. Genome 60 (10):797-814
- 442 Jia L, Clegg MT, Jiang T (2004) Evolutionary dynamics of the DNA-binding domains in  
443 putative R2R3-MYB genes identified from rice subspecies indica and japonica  
444 genomes. Plant Physiology 134 (2):575-585
- 445 Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis  
446 Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33 (7):1870-1874
- 447 Langridge WHR (2006) Edible Vaccines. Scientific American Sp 16:46-53
- 448 Lee MM, Schiefelbein J (1999) WEREWOLF, a MYB-related protein in Arabidopsis, is a  
449 position-dependent regulator of epidermal cell patterning. Cell 24 (5):473-483
- 450 Liao Y, Smyth GK, Shi W (2014) featureCounts: an efficient general purpose program for  
451 assigning sequence reads to genomic features. Bioinformatics 30 (7):923-930
- 452 Liu X, Yu W, Zhang X, Wang G, Cao F, Cheng H (2017) Identification and expression  
453 analysis under abiotic stress of the R2R3-MYB genes in Ginkgo biloba L. Physical  
454 and Molecular Biology of Plants 23 (3):503-516
- 455 Martin G, Baurens FC, Droc G, Rouard M, Cenci A, Kilian A, Hastie A, Doležel J, Aury JM,  
456 Alberti A, Carreel F, D'Hont A (2016) Improvement of the banana "Musa acuminata"  
457 reference sequence using NGS data and semi-automated bioinformatics methods.  
458 BMC Genetics 17 (243)
- 459 Matus JT, Aquea F, Arce-Johnson P (2008) Analysis of the grape MYB R2R3 subfamily  
460 reveals expanded wine quality-related clades and conserved gene structure  
461 organization across Vitis and Arabidopsis genomes. BMC Plant Biology 8 (83)

*M. acuminata* MYB family

- 462 Mehta RS, Bryant D, Rosenberg NA (2016) The probability of monophyly of a sample of  
463 gene lineages on a species tree. *Proceedings of the National Academy of Sciences U S*  
464 *A* 113 (29):8002-8009
- 465 Negi S, Tak H, Ganapathi TR (2015) Cloning and functional characterization of MusaVND1  
466 using transgenic banana plants. *Transgenic Research* 24 (3):571-585
- 467 Ogata K, Kanei-Ishii C, Sasaki M, Hatanaka H, Nagadoi A, Enari M, Nakamura H,  
468 Nishimura Y, Ishii S, Sarai A (1996) The cavity in the hydrophobic core of Myb  
469 DNA-binding domain is reserved for DNA recognition and trans-activation. *Nature*  
470 *Structural Biology* 3 (2):178-187
- 471 Oppenheimer DG, Herman PL, Sivakumaran S, Esch J, Marks MD (1991) A *myb* gene  
472 required for leaf trichome differentiation in *Arabidopsis* is expressed in stipules. *Cell*  
473 67:483-493
- 474 Riechmann JL, Heard J, Martin G, Reuber L, Jiang CZ, Keddie J, Adam L, Pineda O,  
475 Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D,  
476 Sherman BK, Yu CL (2000) *Arabidopsis* transcription factors: Genome-wide  
477 comparative analysis among eukaryotes. *Science* 290 (5499):2105-2110
- 478 Salih H, Gong W, He S, Sun G, Sun J, Du X (2016) Genome-wide characterization and  
479 expression analysis of MYB transcription factors in *Gossypium hirsutum*. *BMC*  
480 *Genetics* 17 (1):129
- 481 Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H,  
482 Remmert M, Söding J, Thompson JD, Higgins DG (2011) Fast, scalable generation of  
483 high-quality protein multiple sequence alignments using Clustal Omega. *Molecular*  
484 *Systems Biology* 7:539
- 485 Sonderby IE, Hansen BG, Bjarnholt N, Ticconi C, Halkier BA, Kliebenstein DJ (2007) A  
486 systems biology approach identifies a R2R3 MYB gene subfamily with distinct and  
487 overlapping functions in regulation of aliphatic glucosinolates. *PLoS One* 2  
488 (12):e1322
- 489 Song C, Yang Y, Yang T, Ba L, Zhang H, Han Y, Xiao Y, Shan W, Kuang J, Chen J, Lu W  
490 (2019) MaMYB4 recruits histone deacetylase MaHDA2 and modulates the expression  
491 of omega-3 fatty acid desaturase genes during cold stress response in banana fruit.  
492 *Plant and Cell Physiology* 60 (11):2410-2422
- 493 Stracke R, Holtgräwe D, Schneider J, Pucker B, Rosleff Sörensen T, Weisshaar B (2014)  
494 Genome-wide identification and characterisation of R2R3-MYB genes in sugar beet  
495 (*Beta vulgaris*). *BMC Plant Biology* 14 (249)
- 496 Stracke R, Werber M, Weisshaar B (2001) The *R2R3-MYB* gene family in *Arabidopsis*  
497 *thaliana*. *Current Opinion in Plant Biology* 4:447-456
- 498 Tak H, Negi S, Ganapathi TR (2017) Overexpression of MusaMYB31, a R2R3 type MYB  
499 transcription factor gene indicate its role as a negative regulator of lignin biosynthesis  
500 in banana. *PLoS ONE* 12 (2):e0172695
- 501 Wilkins O, Nahal H, Foong J, Provart NJ, Campbell MM (2009) Expansion and  
502 diversification of the *Populus* R2R3-MYB family of transcription factors. *Plant*  
503 *Physiology* 149 (2):981-993
- 504 Wu W, Yang YL, He WM, Rouard M, Li WM, Xu M, Roux N, Ge XJ (2016) Whole genome  
505 sequencing of a banana wild relative *Musa itinerans* provides insights into lineage-  
506 specific diversification of the *Musa* genus. *Scientific Reports* 6 (31586):srep31586
- 507 Yang QS, Gao J, He WD, Dou TX, Ding LJ, Wu JH, Li CY, Peng XX, Zhang S, Yi GJ  
508 (2015) Comparative transcriptomics analysis reveals difference of key gene  
509 expression between banana and plantain in response to cold stress. *BMC Genomics*  
510 16:446
- 511 Yanhui C, Xiaoyuan Y, Kun H, Meihua L, Jigang L, Zhaofeng G, Zhiqiang L, Yunfei Z,  
512 Xiaoxiao W, Xiaoming Q, Yunping S, Li Z, Xiaohui D, Jingchu L, Xing-Wang D,

*M. acuminata* MYB family

- 513 Zhangliang C, Hongya G, Li-Jia Q (2006) The MYB transcription factor superfamily  
514 of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB  
515 family. *Plant Molecular Biology* 60 (1):107-124
- 516 Zhong Y, Yin H, Sargent DJ, Malnoy M, Cheng ZM (2015) Species-specific duplications  
517 driving the recent expansion of NBS-LRR genes in five Rosaceae species. *BMC*  
518 *Genomics* 16:77
- 519 Zimmermann IM, Heim MA, Weisshaar B, Uhrig JF (2004) Comprehensive identification of  
520 *Arabidopsis thaliana* MYB transcription factors interacting with R/B-like bHLH  
521 proteins. *The Plant Journal* 40:22-34
- 522