Three R2R3-MYB transcription factors from banana (*Musa* spp.) activate structural anthocyanin biosynthesis genes as part of an MBW complex

Mareike Busche[1], Boas Pucker[2], Bernd Weisshaar[1], Ralf Stracke[1]*

[1] Genetics and Genomics of Plants, Faculty of Biology, Bielefeld University, 33615 Bielefeld, Germany

[2] Institute of Plant Biology & Braunschweig Integrated Centre of Systems Biology (BRICS), TU Braunschweig, 38106 Braunschweig, Germany

*Corresponding author:

ralf.stracke@uni-bielefeld.de

Abstract

Bananas are among the most popular fruits in the world and provide food security and employment opportunities in several developing countries. An increased anthocyanin content could enhance the health promoting properties of banana fruits. The biosynthesis of anthocyanins is largely regulated at the transcriptional level. However, little is known about transcriptional activation of anthocyanin biosynthesis in banana. We analysed the regulatory activity of three *Musa*MYBs predicted to transcriptionally regulate anthocyanin biosynthesis in banana. Co-transfection experiments in *A. thaliana* protoplasts showed that *Musa*MYB-α, *Musa*MYB-β and *Musa*MYB-γ function as components of a transcription factor complex with a bHLH and WD40 protein, called MBW complex, resulting in the activation of the *anthocyanin synthase* and *dihydroflavonol 4-reductase* promoters from *A. thaliana*. The activation potential of *Musa*MYB-α, *Musa*MYB-β and *Musa*MYB-γ increased when combined with the monocot bHLH *Zm*R instead of the dicot *At*EGL3. This work paves the path towards decoding the MBW complex-mediated transcriptional activation of anthocyanin biosynthesis in banana. Moreover, it facilitates research towards an elevated anthocyanin content in banana fruits.

Keywords: *Musa acuminata*, banana, specialised metabolites, transcription factor, flavonoid biosynthesis, R2R3-MYB

32 **Introduction**

33 Bananas (*Musa*) are monocotyledonous, perennial plants which are grown in many tropical
34 and subtropical countries. They are one of the most important food crops, particularly in the
35 developing world (1). While the sweet fruits of dessert bananas are popular in Europe and
36 Northern America, plantains or cooking bananas are commonly eaten as a staple food in Africa
37 and Latin America where they provide food security, as well as employment opportunities (2).
38 Furthermore, banana fruits are rich in several health promoting minerals and beneficial
39 phytochemicals such as vitamins and flavonoids (3).

40 Flavonoids are a major group of plant specialised metabolites which share a basic structure of
41 two aromatic C6-rings connected by a heterocyclic ring (4). Reorganisations and modifications
42 e.g. oxidation, glycosylation, acylation, and methylation of the carbon skeleton create a
43 versatile group comprising more than 9,000 different flavonoid derivatives (5). Consequently,
44 flavonoids do not only contribute to the nutritional value of fruits, but also play important roles
45 in manifold processes. While the group of red coloured anthocyanin pigments attracts animals
46 for pollination and dispersal of seeds by colouring flowers and fruits, other flavonoids protect
47 plants against UV-B irradiation or increase plant fertility (6-10). Flavonoids from many species
48 have been reported to have anti-pathogenic properties, this includes flavonoids from *Dianthus*
49 *caryophyllus* which exhibit antifungal activity against the plant's major pest *Fusarium*
50 *oxysporum* f.sp. *dianthi* (11, 12). The tropical race 4 (TR4) of the banana Fusarium wilt
51 (commonly known as 'panama disease') is caused by another *Fusarium* subspecies called
52 *Fusarium oxysporum* f. sp. *cubense* (Foc) and threatens the global banana production (13).
53 Transcriptome analysis of susceptible and resistant banana cultivars infected by Foc TR4
54 revealed an increased transcription of flavonoid biosynthesis related genes in the resistant
55 cultivar, suggesting an involvement of flavonoids in the defence against Foc TR4 (14).

56 Flavonoid biosynthesis is one of the best characterised pathways of the secondary metabolism
57 and has been extensively studied in many plant species (15). In banana, several flavonoid
58 biosynthesis related enzymes have been identified and characterised (16, 17). Regulation of
59 structural genes on a transcriptional level allows a specific response to environmental

influences as well as development and organ specific expression (summarised in18). MYB transcription factors are common transcriptional regulators of flavonoid biosynthesis. While some MYBs act independently, others interact with basic helix-loop-helix (bHLH) and WD40 proteins to form a protein complex called MBW complex (19). MYB proteins are present in all eukaryotes and characterised by highly conserved DNA-binding domains (20). These MYB domains consist of up to three imperfect amino acid repeat sequences, based on which they are classified. R2R3-MYBs are the most abundant class of plant MYBs and reveal versatile functions in plant-specific processes (21). Besides core- and specialised metabolism they are also involved in cell fate and -identity definition, developmental processes and the response to biotic and abiotic stresses (21). Well-known R2R3-MYBs which act as activators of anthocyanin biosynthesis include C1 (COLOURED ALEURONE1) from *Zea mays* (*Z. mays*), as well as PAP1 (PRODUCTION OF ANTHOCYANIN PIGMENT1/MYB75) and PAP2 (MYB90) from *Arabidopsis thaliana* (*A. thaliana*) (22, 23). They act as part of an MBW complex and control the promoters of the anthocyanin biosynthesis related structural genes as for example *ANTHOCYANIDIN SYNTHASE* (*ANS*) and *DIHYDROFLAVONOL 4-REDUCTASE* (*DFR*) (24-27).

In banana (*Musa acuminata, M. acuminata*), 285 R2R3-MYB proteins, including several putative regulators of flavonoid biosynthesis, have been identified in a genome-wide study (28). Furthermore, MYB31, MYB4 and MYBPR1 - MYBPR4 have been identified as negative regulators of flavonoid biosynthesis in banana (29, 30). Despite the recent identification of two proanthocyanidin biosynthesis activating R2R3-MYBs (31), little functional data is available on positive regulators (activators) of flavonoid and in particular anthocyanin biosynthesis in *M. acuminata*.

Here, we describe the regulatory properties of three *Musa*MYBs, named *Musa*MYB-α, *Musa*MYB-β and *Musa*MYB-γ, with a possible role in the regulation of anthocyanin biosynthesis. Regulatory activity was investigated by *in planta* complementation experiments of the anthocyanin deficient *A. thaliana* regulatory *pap1/pap2* mutant and co-transfection experiments in *A. thaliana* protoplasts. Our results show that *Musa*MYB-α, *Musa*MYB-β and

88 *Musa*MYB-γ are able to activate the promoters of *AtANS* and *AtDFR* as part of an MBW
89 complex. Moreover, we show that the activation potential of *Musa*MYB-α, *Musa*MYB-β and
90 *Musa*MYB-γ is increased when combined with the monocot bHLH *Zm*R instead of the dicot
91 bHLH protein ENHANCER OF GLABRA 3 (*At*EGL3).
92
93 **Methods**
94
95 **Cladistic analysis**
96 The approximately maximum-likelihood trees of plant MYB domain sequences and bHLH
97 protein sequences were constructed as described previously (32): Multiple protein sequence
98 alignments were created using MAFFT v7 (33) with default settings and cleaned with pxclsq
99 (34). The tree was constructed with FastTree v2.1.10 using the WAG+CAT model (35) and
100 visualised with interactive tree of life (36). GenBank IDs of protein sequences used for the
101 construction of the tree are given in Supplementary Table S2.
102
103 **Plant material**
104 Banana plants (*M. acuminata* (AAA Group) 'Grand Naine') for RNA extraction were grown in
105 the field in Lucknow, India. *Musa* gene annotation identifiers refer to the study of Martin *et al.*
106 (37). *A. thaliana* Columbia-0 (Col-0, NASC ID N1092) and Nössen-0 (Nö-0, NASC ID N3081)
107 accessions were used as wildtype controls corresponding to the *pap1/pap2* (*pap1*: Nö-0
108 background, PST16228; *pap2*: Col-0 background, SALK093731; (24)) double mutant, used for
109 complementation assays. Hypocotyl-derived, cultured *A. thaliana* At7 cells (38) for co-
110 transfection experiments were grown in MS medium (39) and maintained at 26 °C in the dark
111 on a rotary shaker (105 rpm). Cells were subcultured weekly and cultures for protoplast
112 isolation were inoculated 5 days prior to harvest.
113
114 **cDNA synthesis and molecular cloning**

115 RNA from Grand Naine peel was isolated according to a protocol from Asif *et al*. (40). cDNA
116 synthesis was performed from 1 µg total RNA using the ProtoScript First Strand cDNA
117 Synthesis Kit (New England Biolabs, NEB). Full length coding sequences (CDSs) were
118 amplified using Q5® High-Fidelity DNA polymerase (NEB) and gene-specific primers
119 (Supplementary Table S1). To generate Gateway Entry plasmids, PCR products were
120 recombined into pDONR™/Zeo (Invitrogen) with BP clonase (Invitrogen) according to the
121 manufacturer's protocol. Full length CDSs were introduced into the binary 2x35S
122 overexpression vector pLEELA (41) and the 2x35S overexpression vector pBTdest (42) using
123 Gateway LR reaction (Invitrogen). All plasmids were verified by Sanger sequencing.
124

125 ***Agrobacterium tumefaciens*-mediated transformation of *A. thaliana***

126 *Agrobacterium tumefaciens* (GV3101::pMP90RK (43)) mediated gene transfer was carried out
127 using the floral dip method (44). T-DNA integration from pLEELA-based plasmids into the
128 *A. thaliana* genome was screened by BASTA selection and PCR-based genotyping (refer to
129 Supplementary Figure 1 and Supplementary Table 1 for details).
130

131 **Determination of anthocyanin content**

132 *A. thaliana* seedlings were grown with 16 h of light illumination per day on 0.5x MS agar plates
133 with 4 % sucrose to induce anthocyanin production. Anthocyanin content in a bulk of six-day-
134 old seedlings was quantified as described by Mehrtens *et al.* (45). All samples were measured
135 in three independent biological replicates. Error bars indicate standard deviation of the average
136 (mean) anthocyanin content.
137

138 **Co-transfection experiments in *A. thaliana* protoplasts**

139 Cell culture, protoplast isolation, PEG-mediated DNA transfer into protoplasts and
140 determination of standardised GUS activity were performed as described by Hartmann *et al*.
141 (46). As an adaption we used 1.3 % (w/v) Cellulase Onozuka RS (Duchefa) and 0.8 % (w/v)
142 Macerozyme R-10 (Duchefa) in 240 mM $CaCl_2$ as the enzyme solution for protoplast

143 preparation and 30 µg of plasmid DNA for the PEG-mediated DNA transfer into protoplasts.
144 This comprised 10 µg of the promoter-GUS reporter construct, 0.5 µg of each
145 2x35S:transcription factor effector construct, 5 µg of the standardisation plasmid (pBT8 UBI-
146 LUCm3), and an inactive luciferase expression vector (pBTΔLUC) to fill up to the amount
147 of 30 µg of plasmid DNA. GUS-fused promoters, 520 bp *proAtDFR* and 1.95 kb *proAtANS,* as
148 well as expression constructs for *At*PAP1 (*At*MYB75), *At*EGL3 (*At*bHLH2), *At*TTG1 and *Zm*C1
149 were available from previous studies (24, 25, 42). A *Zm*R entry clone (47) was kindly provided
150 by Martin Hülskamp. Statistical analysis was performed using the Mann-Whitney *U* test (48).
151

152 **Results and Discussion**

153 To analyse the regulatory properties of three *Musa*MYBs which have been previously assigned
154 with a possible role in positive regulation of anthocyanin biosynthesis (Ma06_g05960 or
155 *MusaMYB-α*, Ma09_g27990 or *MusaMYB-β*, Ma10_g17650 or *MusaMYB-γ*), we tried to
156 amplify the corresponding coding sequences (CDSs) on a template collection containing cDNA
157 from different banana samples. The CDSs of all three *MusaMYB*s were successfully amplified
158 on cDNA derived from peel tissue.
159 In a first approach, we performed a complementation assay with the regulatory *A. thaliana*
160 *pap1/pap2* double mutant which cannot produce anthocyanins in the seedling (Figure 1).
161 Seedlings were grown on anthocyanin synthesis-inducing media to analyse the ability of
162 2x35S-driven *MusaMYB*s to complement the *pap1/pap2* anthocyanin deficiency. While wild-
163 type seedlings accumulated high amounts of red anthocyanin pigments, *pap1/pap2* seedlings
164 did not. Even though *MusaMYB-α*, *MusaMYB-β* or *MusaMYB-γ* were successfully expressed
165 in the transgenic seedlings (Supplementary Figure S1), the anthocyanin level in *pap1/pap2*
166 plants expressing *MusaMYB-α*, *MusaMYB-β* or *MusaMYB-γ* did not differ from the double
167 mutant. Accordingly, *MusaMYB-α*, *MusaMYB-β* and *MusaMYB-γ* do not seem to be able to
168 complement the mutant phenotype and thus to regulate anthocyanin biosynthesis in *A. thaliana*
169 in combination with the *bHLH* and *WD40* genes expressed in *A. thaliana* seedlings.

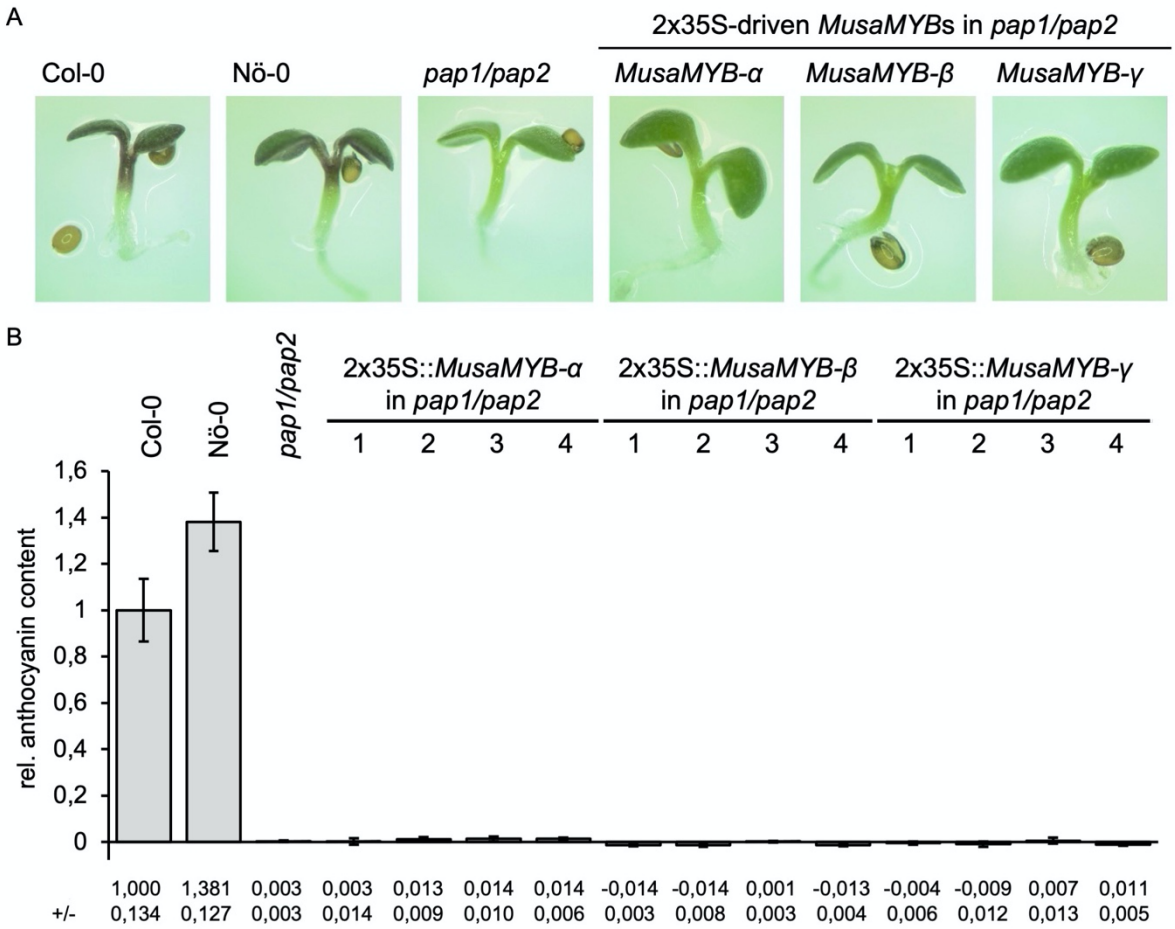


Figure 1: *MusaMYB-α, MusaMYB-β* and *MusaMYB-γ* cannot complement the anthocyanin deficient phenotype of *A. thaliana pap1/pap2* mutant seedlings. (A) Representative pictures of anthocyanin accumulation in 6-day-old *MusaMYB*-expressing *pap1/pap2* seedlings. A representative plant per construct is displayed. (B) Photometrical measurement of the sucrose induced anthocyanin content in *pap1/pap2* seedlings expressing 2x35S-driven *MusaMYB*s. Col-0, Nö-0 (wildtypes) and *pap1/pap2* were used as controls. Error bars indicate the standard deviation of three biological replicates. The different numbers represent individual, independent, transgenic lines.

Lloyd *et al.* (26) analysed the *Z. mays* anthocyanin regulator C1 by generation of transgenic *A. thaliana* overexpression lines. In their study, Lloyd *et al.* generated three independent *ZmC1*-expressing *A. thaliana* lines that did not show an increased anthocyanin content compared to wildtype. Similar results were obtained in transgenic tobacco. However, further experiments suggested that *Zm*C1 needs to interact with the maize bHLH *Zm*R to activate anthocyanin biosynthesis in the heterologous *A. thaliana* system (26). Phylogenetic analysis (49) showed that the anthocyanin biosynthesis regulating R2R3-MYBs from several monocots such as *Z. mays* and *Oryza sativa* (*O. sativa*) are part of a different phylogenetic clade than

187 the anthocyanin regulators from several dicots, such as *A. thaliana* or *Vitis vinifera* and further
188 monocots, including *Allium cepa* and *Lilium hybrida*. Experiments in snapdragon also showed
189 that the monocot *Ac*MYB1 can activate anthocyanin production in dicots (49). The phylogenetic
190 differences between anthocyanin biosynthesis activating R2R3-MYBs and the dependency of
191 *Zm*C1 on *Zm*R in dicots hint at an explanation for our observations. For example, the
192 *Musa*MYBs could depend on their endogenous or at least monocot bHLH for effective
193 activation of structural anthocyanin biosynthesis genes. To investigate the phylogenetic
194 differences between anthocyanin biosynthesis regulating R2R3-MYBs from different plant
195 species, an approximately maximum-likelihood tree was built (Figure 2A). The resulting tree,
196 which also contained R2R3-MYBs which activate other branches of flavonoid biosynthesis,
197 revealed two distinct clades of anthocyanin-related R2R3-MYBs (highlighted in red).
198 *Musa*MYB-α, *Musa*MYB-β and *Musa*MYB-γ form a clade with MYB10 from *Triticum aestivum*
199 and exhibit a close evolutionary relationship with *Zm*C1, *Os*C1 and anthocyanin regulating
200 MYBs from further monocots. In contrast, *At*PAP1 and *At*PAP2 fall into a second clade of
201 anthocyanin-related MYBs. Furthermore, differences between anthocyanin biosynthesis
202 regulating bHLHs were analysed in a second approximately maximum-likelihood tree (Figure
203 2B). The tree showed that monocot bHLH proteins involved in the regulation of anthocyanin
204 biosynthesis form a separate clade (highlighted in red). Both, *Zm*R and several putative
205 anthocyanin biosynthesis regulating bHLHs from banana fall into this clade. These
206 phylogenetic analyses show that anthocyanin biosynthesis regulating R2R3-MYB and bHLH
207 proteins from several monocot species seem to be distinct from other R2R3-MYB and bHLH
208 proteins regulating anthocyanin biosynthesis, for example from *A. thaliana*. These differences
209 could imply a dependency of *Musa*MYB-α, *Musa*MYB-β and *Musa*MYB-γ on a banana or other
210 monocot bHLH.
211 To follow up this idea, we further investigated the regulatory properties of the three *Musa*MYBs
212 performing co-transfection assays in At7 protoplasts with different bHLH proteins from
213 *A. thaliana* (*At*EGL3) or *Z. mays* (*Zm*R) and the *A. thaliana* WD40 protein TRANSPARENT
214 TESTA GLABRA 1 (*At*TTG1, Figure 3). Thereby, their potential to activate the promoters of
215

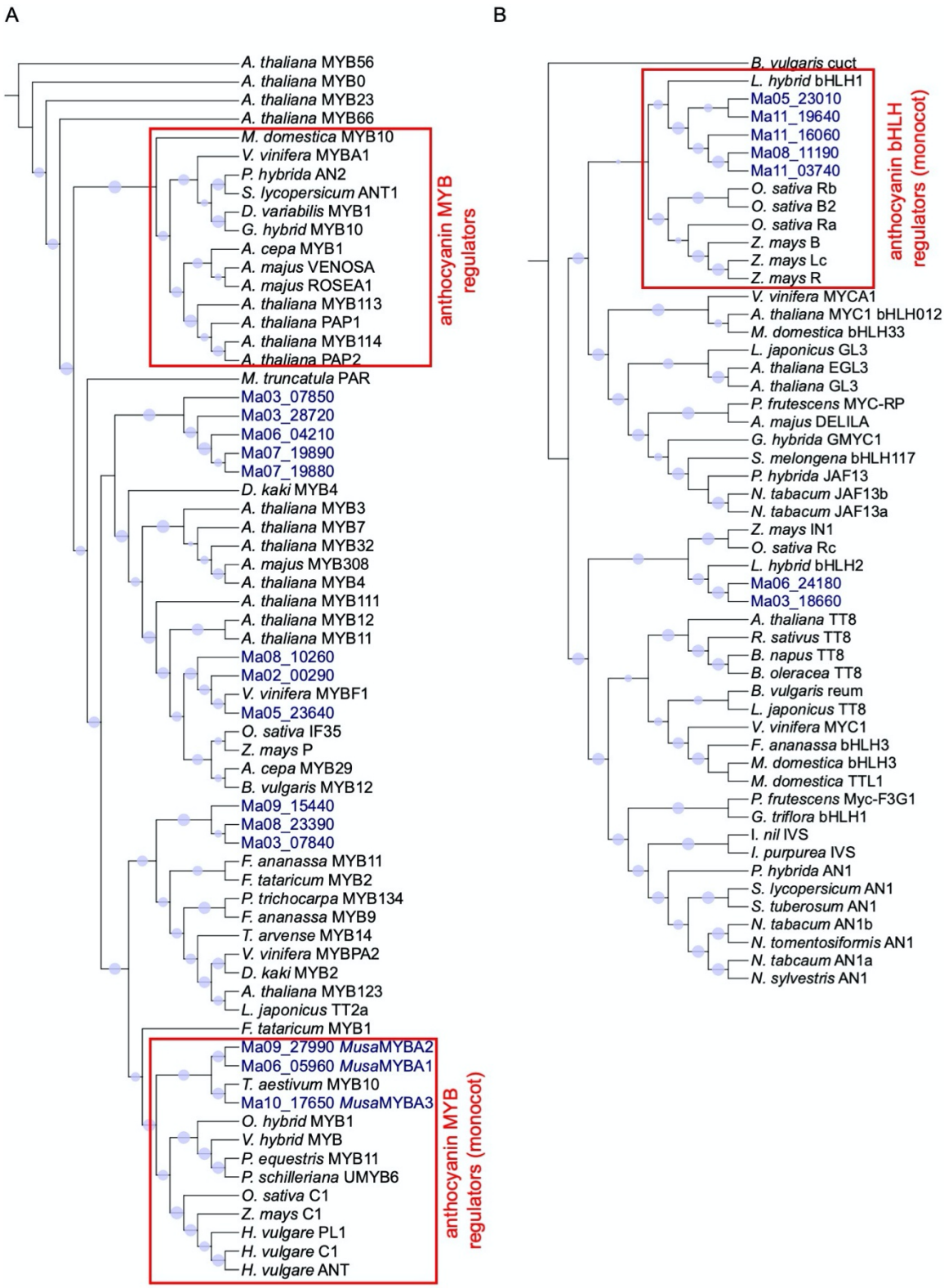


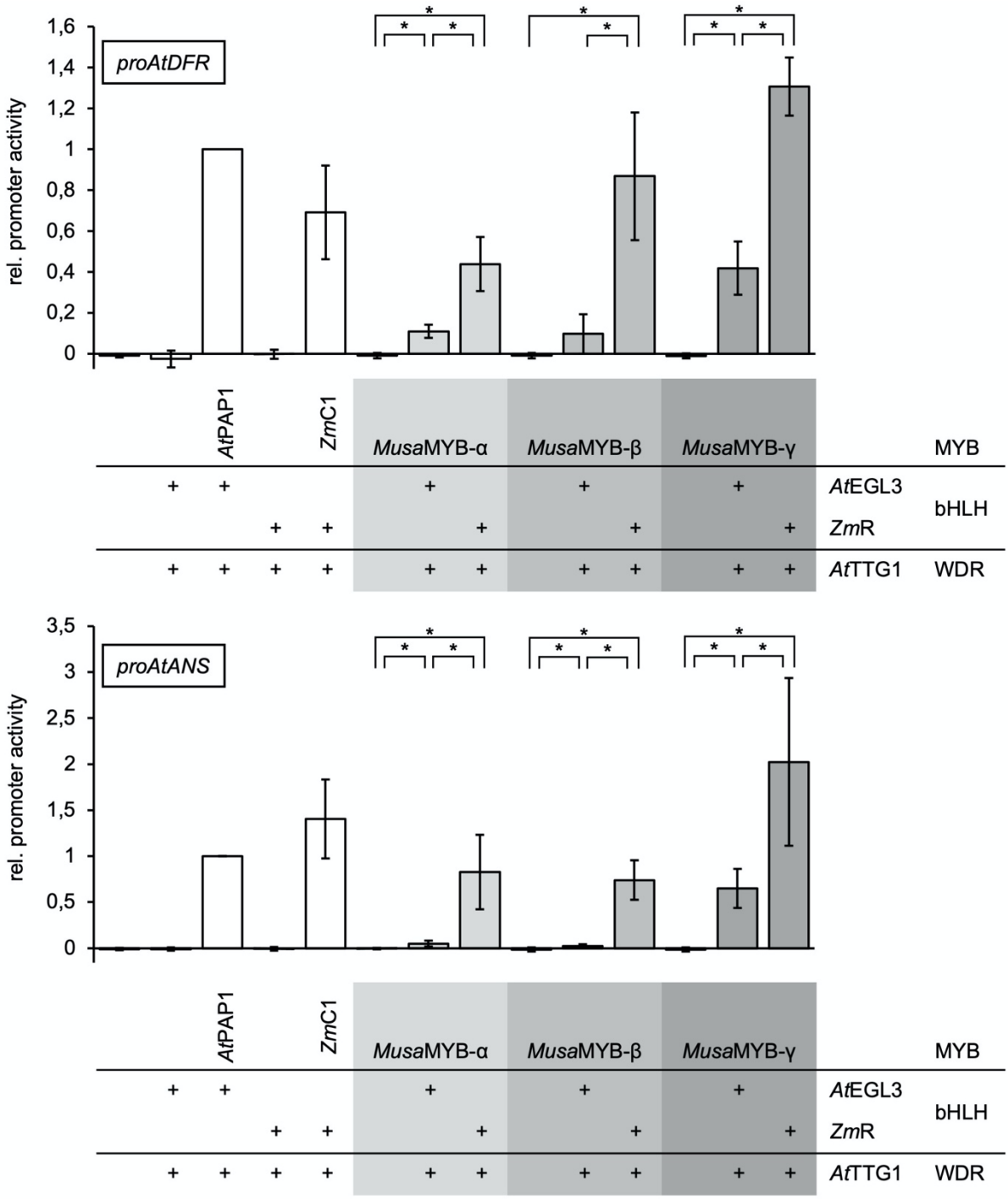
Figure 2: Rooted approximately maximum-likelihood trees of MYB (A) and bHLH (B) transcription factors. Circle sizes represent bootstrap values. Banana proteins are highlighted in blue.

AtANS and *AtDFR*, which are important structural genes of anthocyanin biosynthesis (summarised in 50), was analysed. While none of the *Musa*MYBs was able to independently

222 activate *proAtANS* or *proAtDFR*, which both contain conserved *cis*-regulatory elements, a
223 slight activation of *proAtDFR* was detectable when *Musa*MYB-α or *Musa*MYB-γ were
224 combined with *At*EGL3 and *At*TTG1. In combination with *At*EGL3 and *At*TTG1, *Musa*MYB-γ
225 showed the strongest activation of *proAtDFR* and was also able to activate *proAtANS*. When
226 combined with *Zm*R and *At*TTG1, the three MYBs *Musa*MYB-α, *Musa*MYB-β, and *Musa*MYB-γ
227 exhibited a significant activation potential on *proAtDFR* and *proAtANS*. All three *Musa*MYBs
228 showed a higher activation potential in an MBW complex with *Zm*R and *At*TTG1 than in
229 combination with *At*EGL3 and *At*TTG1.
230 These results display that *Musa*MYB-α, *Musa*MYB-β and *Musa*MYB-γ are able to activate
231 *proAtANS* and *proAtDFR* as part of an MBW complex and *Musa*MYB-γ shows the strongest
232 activation potential. Furthermore, *Musa*MYB-α, *Musa*MYB-β and *Musa*MYB-γ show a higher
233 activation potential when combined with the monocot bHLH *Zm*R instead of the dicot bHLH
234 *At*EGL3.
235 The previously mentioned phylogenetic differences could be an explanation for the observed
236 higher activation potentials of the analysed *Musa*MYBs in combination with *Zm*R and *At*TTG1
237 instead of *At*EGL3 and *At*TTG1. It is conceivable that these differences impede the interaction
238 between the tested *Musa*MYBs and the dicot bHLH *At*EGL3. As *Musa*MYB-γ shows the
239 highest activation potential and is also able to activate *proAtANS* in combination with *At*EGL3
240 or *Zm*R, differences between the three *Musa*MYBs that affect the interaction with the *At*EGL3
241 are likely and should be further studied. Interestingly, *Musa*MYB-γ did not complement the
242 anthocyanin deficient phenotype of *A. thaliana pap1/pap2* mutant seedlings, but was able to
243 activate *proAtANS* and *proAtDFR* when combined with *At*EGL3 in co-transfection experiments
244 in *A. thaliana* protoplasts. Even though previous studies showed *AtEGL3* expression in
245 *A. thaliana* seedlings (19), it is possible that the level was too low to activate anthocyanin
246 biosynthesis in combination with *Musa*MYB-γ. Moreover, the At7 cell line used for co-
247 transfection experiments was established more than 25 years ago. This long time of
248 propagation in suspension cell culture caused a variety of genomic and transcriptomic changes

249 (51). These changes could also explain differences between the analysis in seedlings and cell

250 culture.



251

252 **Figure 3: *Musa*MYB-α, *Musa*MYB-β and *Musa*MYB-γ can activate *proAtDFR* and**
253 ***proAtANS* as part of an MBW complex**. The ability of *Musa*MYBs to activate *proAtDFR-GUS*
254 and *proAtANS-GUS* reporter constructs in combination with different bHLH proteins (*At*EGL3,
255 *Zm*R) and a WDR (*At*TTG1) was analysed by co-transfection in *A. thaliana* At7 protoplasts.
256 The relative promoter activity refers to the measured GUS reporter enzyme activity. Promoter
257 activity is given relative to the values obtained for the *A. thaliana* MBW complex (*At*PAP1,
258 *At*EGL3, *At*TTG1). Error bars indicate the standard deviation of five independent biological
259 replicates. Statistical significance is indicated by asterisks which mark p-values < 0.05.

260 The expression profiles of *MusaMYB-α*, *MusaMYB-β* and *MusaMYB-γ* have previously been
261 analysed in embryogenic cell suspension, seedling, root and different developmental stages
262 of leaf, pulp and peel (28). The expression of *MusaMYB-α* was strongest in pulp
263 (developmental stage S2-S3), *MusaMYB-β* expression was relatively low in all samples and
264 *MusaMYB-γ* showed highest expression in seedlings and early developmental stages of pulp
265 (S1). This gene expression pattern could give a hint at organ specific *MusaMYB-α*,
266 *MusaMYB-β* and *MusaMYB-γ* activity in banana. Since flavonoid biosynthesis is largely
267 regulated at the transcriptional level, it would be particularly interesting to analyse the
268 expression levels of *MusaMYB-α*, *MusaMYB-β,* and *MusaMYB-γ* in anthocyanin rich organs
269 such as bract or pseudostem to relate anthocyanin content to *MusaMYB* transcript abundance.
270 Deng *et al*. (30) performed an expression analysis of flavonoid biosynthesis related genes
271 using leaves of banana plants overexpressing the anthocyanin repressor *MusaMYB4*. They
272 found that the expression of *MusaMYB-α* and *MusaMYB-γ* is decreased along with *MusaDFR*
273 and *MusaANS* in plants overexpressing *Musa*MYB4, compared to wildtype. This data supports
274 a proposed functionality of *Musa*MYB-α and *Musa*MYB-γ as transcriptional activators of
275 anthocyanin biosynthesis in banana, as *MusaMYB4* could cause a feedback regulation of the
276 positive regulators of anthocyanin biosynthesis, of *MusaMYB-α* and *MusaMYB-γ*.
277 Very recently, Rajput *et al*. (31) showed that *Musa*MYB-γ, which they refer to as MaMYBPA2,
278 can activate the banana *ANS, ANR* and LAR promoters. Moreover, they showed that
279 *Musa*MYB-γ/MaMYBPA2 can partially rescue the proanthocyanin deficiency of the *A. thaliana*
280 *tt2-1* mutant. The reported ability of *Musa*MYB-γ/MaMYBPA2 to activate *proMusaANS*
281 supports the proposed role of *Musa*MYB-γ/MaMYBPA2 in the regulation of anthocyanin
282 biosynthesis. However, the partial complementation of the proanthocyanin deficient phenotype
283 of the *A. thaliana tt2-1* R2R3-MYB mutant, as well as the ability to activate the banana *ANR*
284 and *LAR* promoters, shows an additional role in the regulation of proanthocyanidin
285 biosynthesis. Such dual roles in the regulation of flavonoid biosynthesis have previously been
286 suggested for R2R3-MYB transcription factors from grape and apple (52, 53). In several
287 species, including *A. thaliana, Z. mays* and *Petunia hybrida*, anthocyanin biosynthesis is

288 regulated by an MBW complex (summarised in 54). Our co-transfection assays showed that
289 *Musa*MYB-α, *Musa*MYB-β and *Musa*MYB-γ are able to activate *proAtANS* and *proAtDFR* as
290 part of an MBW complex with *Zm*R and *At*TTG1 *in planta*. As presented in a previous study by
291 Pucker *et al.* (28), it is known that *Musa*MYB-α, *Musa*MYB-β, and *Musa*MYB-γ all contain a
292 bHLH-binding consensus motif (25). Thus, the regulation of anthocyanin biosynthesis by
293 *Musa*MYB-α, *Musa*MYB-β, and *Musa*MYB-γ likely depends on bHLH and WD40 proteins.
294 Based on our tree (Figure 2B), the bHLH encoding genes Ma05_g23010, Ma11_g19640,
295 Ma11_g16060, Ma08_g11190 and Ma11_g03740 could be suitable candidates for further
296 studies on the transcriptional activation of anthocyanin biosynthesis by MBW complexes.
297 Moreover, future analyses should additionally include *Musa*TTG1 to identify a whole functional
298 MBW complex in banana and to find out if the use of endogenous WD40 protein further
299 improves the activation potential of the MBW complex.
300 Resumed, the presented results show that *Musa*MYB-α, *Musa*MYB-β, and *Musa*MYB-γ have
301 the capacity to transcriptionally activate structural anthocyanin biosynthesis genes in an MBW
302 complex with a suitable bHLH partner. This is a step towards decoding the MBW complex-
303 mediated transcriptional activation of flavonoid biosynthesis in banana. Moreover, it is a basis
304 for further research towards increased anthocyanin production in banana which could promote
305 fruit quality and disease resistance.
306

307 **Acknowledgements**

308 We are grateful to Melanie Kuhlmann and Janina Grabowski for their excellent technical
309 assistance and to Andrea Voigt for her competent help in the greenhouse. We thank Martin
310 Hülskamp for providing the *Zm*R entry clone plasmid and Ashutosh Pandey for providing the
311 banana cDNAs.
312

313 **Author Contributions**

314 MB, BP and RS planned the experiments. MB performed the experiments and analysed the
315 data. RS and BW supervised the project. MB wrote the initial draft. RS and BP revised the
316 manuscript.
317
318 **Abbreviations**

319 ANS	anthocyanidin synthase
320 *A. thaliana*	*Arabidopsis thaliana*
321 bHLH	basic helix-loop-helix
322 C1	COLOURED ALEURONE1
323 DFR	dihydroflavonol 4-reductase
324 EGL3	ENHANCER OF GLABRA 3
325 Foc	*Fusarium oxysporum* f. sp. *cubense*
326 *M. acuminata*	*Musa acuminata*
327 *O. sativa*	*Oryza sativa*
328 PAP	production of anthocyanin pigment
329 TR4	tropical race 4
330 TTG1	TRANSPARENT TESTA GLABRA 1
331 *Z. mays*	*Zea mays*

332
333 **Supplementary information**
334 Figure S1: Expression analysis of *MusaMYB-α, MusaMYB-β and MusaMYB-γ* in *A. thaliana*
335 *pap1/pap2* seedlings.
336 Table S1: Oligonucleotide primers used in this work.
337 Table S2: IDs of protein sequences used for the construction of the phylogenetic tree
338
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