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Title

AtMYB92 enhances fatty acid synthesis and suberin deposition in leaves of *Nicotiana benthamiana*

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Running head

AtMYB92 enhances fatty acid and suberin synthesis

Summary

Acyl lipids are important constituents of the plant cell. Depending on the cell type, requirements in acyl lipids vary greatly, implying a tight regulation of fatty acid and lipid metabolism. The discovery of the WRINKLED transcription factors, members of the AP2/EREBP family, has emphasized the importance of transcriptional regulations for adapting the rate of acyl chain production to cell requirements. Here we describe the identification of another activator of the fatty acid biosynthetic pathway, the Arabidopsis MYB92 transcription factor. This MYB, like all the members of the subgroups S10 and S24 of MYB transcription factors, can directly activate the promoter of *BCCP2* that encodes a component of the fatty acid biosynthetic pathway. Two adjacent MYB *cis*-regulatory elements are essential for the binding and activation of the *BCCP2* promoter by MYB92. Overexpression of *MYB92* or *WRI1* in *Nicotiana benthamiana* induces the expression of fatty acid biosynthetic genes but results in the accumulation of different types of acyl lipids. In the presence of WRI1, triacylglycerol biosynthetic enzymes coded by constitutively expressed genes efficiently channel the excess fatty acids toward reserve lipid accumulation. In contrast, MYB92 activates both fatty acid and suberin biosynthetic genes; hence the remarkable increase in suberin monomers measured in leaves expressing *MYB92*. These results provide additional insight into the molecular mechanisms that control the biosynthesis of an important cell wall-associated acylglycerol polymer playing critical roles in plants.

Keywords

MYB92, suberin, fatty acid, transcription factor, Arabidopsis, *Nicotiana benthamiana*

Significance statement

The identification of transcriptional regulators of the fatty acid biosynthetic pathway is essential to elucidate how plant cells adapt the rate of acyl chain production to varying cell requirements. The Arabidopsis MYB92 transcription factor is an activator of both fatty acid and polyester biosynthetic genes, and ectopic expression of *MYB92* in leaves of *Nicotiana benthamiana* yields remarkable increase in suberin deposition.

INTRODUCTION

In plant cells, the biosynthesis of fatty acids (FAs) occurs in plastids (Harwood, 1996). The building block used for FA production, acetyl-CoA, is generated from end products of the glycolysis by the pyruvate dehydrogenase complex. Then, malonyl-CoA is formed from acetyl-CoA by heteromeric acetyl-CoA carboxylase (ACCase). The malonyl group of malonyl-CoA is subsequently transferred to an acyl carrier protein (ACP). Production of saturated acyl chains is catalyzed by the type II FA synthase, which uses acetyl-CoA as a starting unit while the two-carbon units required for chain elongation are provided by malonyl-ACP. Thioesterases ultimately hydrolyze 16- or 18-carbon acyl chains, releasing FAs. Acyl lipids are derived from FAs and fulfill different roles in plant cells. As in other organisms, they are the basic structural components of the cell membranes (Mamode Cassim *et al.*, 2019). Triacylglycerols, triesters of FAs and glycerol, then represent a major form of carbon and energy storage in most oleaginous species (Baud, 2018). In addition, cuticular lipids (suberin, cutin, cuticular waxes) constitute lipophilic cell-wall associated barriers that prevent water loss, entry of pathogenic microorganisms, and organ adherence (Kunst and Samuels, 2009; Delude *et al.*, 2016). Finally, a minor fraction of acyl lipids and their derivatives (e.g. jasmonate) participate in signaling pathways (Wasternak, 2007).

It was estimated that mesophyll cells contain 5-10% acyl lipids by dry weight, mostly in the form of membrane lipids (Ohlrogge and Browse, 1995). In contrast, in the seeds and in the fruit mesocarp of certain oleaginous species, cells can accumulate up to 90% triacylglycerols by dry weight (Bourgis *et al.*, 2011). The rate of acyl chain production thus varies considerably from one cell type to another, allowing the balancing of carbon supply and demand for acyl chains to meet their specific requirements (Ohlrogge and Jaworski, 1997). The question of the nature of the molecular mechanisms underpinning the regulation of FA metabolism follows logically. Post-translational regulations targeting enzymes of the pathway such as heteromeric ACCase have been documented (Feria Bourrellier *et al.*, 2010; Andre *et al.*, 2012; Salie *et al.*, 2016). They allow subtle and rapid modifications of the rate of FA biosynthesis in response to varying environmental or physiological conditions (Salie and Thelen, 2016; Troncoso-Ponce *et al.*, 2016). Aside from these regulations, extensive transcriptomic analyses have revealed a strong correlation between

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3 transcripts levels of genes encoding core FA biosynthetic enzymes and the rate of
4 acyl chain production (Girke *et al.*, 2000; Troncoso-Ponce *et al.*, 2011). These
5 studies have emphasized the key role played by transcriptional regulations in the
6 developmental control of FA biosynthesis, in oleaginous seeds and fruits in particular.
7 They have also outlined the coexpression cluster made of FA biosynthetic genes
8 (Baud and Lepiniec, 2009; Peng and Weselake, 2011; Guerin *et al.*, 2016).
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13 *Arabidopsis thaliana* WRINKLED1 (WRI1), the first transcriptional activator of FA
14 biosynthesis identified in plants, is a member of the APETALA2-ethylene-responsive
15 element binding protein (AP2-EREBP) family of transcription factors (TFs) (Cernac
16 and Bening, 2004). Upon binding to AW *cis*-regulatory elements (CnTnG[n]₇CG)
17 conserved among proximal upstream regions of several late glycolytic and FA
18 biosynthetic genes (Maeo *et al.*, 2009), WRI1 recruits the Mediator complex and, in
19 turn, the Pol II complex so as to initiate their transcription (Kim *et al.*, 2016). WRI1
20 TFs and AW *cis*-regulatory elements have been described in a range of plant
21 species, where their function appears to be well conserved (Shen *et al.*, 2010;
22 Marchive *et al.*, 2014). The genomes of land plants usually code for several related
23 WRI1-like TFs that define a family of transcriptional regulators capable of triggering
24 sustained rates of acyl chain synthesis. Contrasting expression patterns of the genes
25 encoding these TFs usually contribute to specify their functions in planta (To *et al.*,
26 2012; Dussert *et al.*, 2013).
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The observation that the *Arabidopsis wri1 wri3 wri4* triple knockout mutants
maintain a basal level of FA biosynthetic gene expression suggests that the
transcriptional machinery governing FA biosynthesis may include additional
regulatory proteins. What is more, putative *cis*-regulatory elements distinct from the
AW-box are over-represented in the promoter regions of FA biosynthetic genes
(Peng and Weselake, 2011) and some of these were shown to be essential for
transcriptional activation of the corresponding genes (Baud *et al.*, 2009b). This
further suggests that TFs not related to the WRIs and belonging to redundant
regulatory pathways play a meaningful role in the transcriptional activation of FA
biosynthesis. Besides, two members of the DNA-binding-with-One-Finger (DOF)
family of TFs have been proposed to activate the transcription of some FA
biosynthetic genes in soybean (Wang *et al.*, 2007).

To isolate other regulators involved in the transcriptional regulation of the FA
biosynthetic pathway, complementary screening procedures were undertaken. In this

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3 article, we report the identification and functional characterization of the Arabidopsis
4 MYB92 (At5g10280) TF and of close homologs of the MYB family. Yeast one-hybrid
5 experiments and electrophoretic mobility shift assays show that MYB92 is able to
6 interact with the promoter of *BIOTIN CARBOXYL CARRIER PROTEIN2 (BCCP2)*, a
7 gene coding for a subunit of heteromeric ACCase. The binding of MYB92 to
8 *ProBCCP2* requires two adjacent MYB *cis*-elements and is independent from the
9 binding of WRI1 to the same promoter. It is then demonstrated that MYB92 activates
10 in planta the *BCCP2* promoter, as well as other promoters of FA biosynthetic genes.
11 By overexpressing *MYB92* in leaves of *Nicotiana benthamiana*, we further
12 demonstrate its ability to enhance expression of not only FA but also aliphatic suberin
13 biosynthetic genes, hence the remarkable deposition of suberin measured in this
14 system.
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29 RESULTS

30 Isolation of transcription factors binding *ProBCCP2*

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32 A yeast one-hybrid screening procedure was implemented so as to isolate
33 transcriptional regulators of the FA biosynthetic pathway. We employed as bait a
34 180-bp DNA fragment located immediately upstream of the translational start codon
35 of the Arabidopsis *BCCP2* gene and referred to as *ProBCCP2*. *BCCP2*, whose
36 expression profile is representative of FA biosynthetic genes (Baud and Lepiniec,
37 2009), codes for a subunit of heteromeric ACCase. Functional analyses previously
38 established that the 180-bp DNA fragment used, which is part of the 5' UTR of
39 *BCCP2*, contained information required for the proper regulation of *BCCP2*
40 expression in planta (Baud *et al.*, 2009b). The REGIA library, an ordered cDNA
41 expression library for a set of Arabidopsis TFs (Paz-Ares, 2002), was screened by
42 mating with a yeast strain containing the *ProBCCP2:HIS3* reporter construct. Among
43 the 926 open reading frames of the library, two candidates were isolated. As
44 previously reported, one of the candidates encoded WRI3, a member of the AP2-
45 EREBP family (To *et al.*, 2012). The second encoded MYB92, the function of which
46 was further investigated.
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MYB92 interacts with *ProBCCP2* through adjacent MYB elements

Confirmation of the interaction between MYB92 and the *BCCP2* promoter was firstly performed using a yeast one-hybrid approach. Yeast strains that presented the *HIS3* reporter gene under the control of a *BANYULS* (*BAN*, used as a negative control) or *BCCP2* promoter were constructed and transformed with different plasmids allowing the expression of MYB92 or WRI1 (positive control) as GAL4 activating domain (AD) fusions. A transformation of the strain presenting the *HIS3* reporter gene under the control of *BAN* promoter gave no positive interaction results, showing that MYB92 and WRI1 were not able to interact with this promoter (Figure 1A). On the contrary, the expression of MYB92 or WRI1 in the strain presenting the *HIS3* reporter gene under the control of the *BCCP2* promoter resulted in the specific growth of the strain on medium lacking histidine, demonstrating the interaction between the TFs and this promoter sequence. To compare the strength of these interactions, quantitative β -galactosidase assays were performed in yeast containing the *lacZ* reporter gene cloned downstream of *ProBCCP2*. Both MYB92 and WRI1 could activate the reporter gene and the reporter activity measured in the presence of WRI1 was higher (Figure 1B).

The binding of MYB92 to the *BCCP2* promoter sequence was further examined in vitro by electrophoretic mobility shift assay (EMSA). Purified recombinant MYB92 was produced and incubated with a 79-bp DNA fragment from the *BCCP2* promoter containing three putative MYB-core *cis*-elements (according to AthaMap; Steffens *et al.*, 2004) plus an AW-box (Maeo *et al.*, 2009) (Figure 2a). Binding was determined using biotin-labeled DNA probes. Addition of MYB92 to the DNA fragment yielded gel retardation (Figure 1c). The signal intensity increased with the concentration of the protein in the assay. The binding seemed specific since addition of recombinant LEAFY COTYLEDON2 (LEC2) used as a negative control did not result in the apparition of shifted bands. This specificity was confirmed by competition experiments with increasing amounts of unlabeled oligonucleotides that suppressed the binding of MYB92 to the labeled probe. Addition of WRI1 also led the formation of a shifted band of lower molecular weight (Figure 1d). The super-shifted band observed upon addition of both MYB92 and WRI1 suggested that the two TFs could bind simultaneously to the *BCCP2* promoter (Figure 1d).

The 79-bp *BCCP2* promoter fragment used for EMSA was divided into five partially overlapping fragments containing one to three of the putative MYB-core *cis*-

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3 element each. MYB92 was able to bind only the fragments harboring both MYB
4 elements 1 and 2 (Figure 2b). Thanks to nucleotide substitutions targeting one or
5 several MYB-core *cis*-elements, mutagenized versions of the 79-bp *BCCP2* promoter
6 sequence were then prepared. Mutations of the MYB boxes 1 or 2 impaired MYB92
7 binding, whereas the TF was still able to bind the *BCCP2* promoter containing a
8 mutagenized version of MYB box 3 (Figure 2b). To precisely examine the specificity
9 of the MYB92 binding sequence, we prepared various mutant forms of the sites
10 surrounding the MYB-core elements 1 and 2 with single-base substitutions.
11 Considering the high number of promoter versions to be assayed in this experiment,
12 we decided to test them as unlabeled oligonucleotides in a competition assay.
13 Mutations in five out of the seven nucleotides composing the MYB element 1 and in
14 four out of the seven nucleotides composing the MYB element 2 resulted in a loss of
15 MYB92 binding, whereas mutations outside of the motif showed no effect on MYB92
16 binding (Figure 2c). Altogether, these results established the importance of two
17 adjacent MYB-core elements in the promoter sequence of *BCCP2* for MYB92
18 binding.
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32 **MYB92 is a transcriptional regulator able to activate expression of the *uidA*** 33 **reporter gene cloned downstream of *ProBCCP2***

34 To demonstrate that MYB92 is a functional TF, we first investigated the *in vivo*
35 subcellular localization of the protein with mGFP6, a derivative of GFP. This
36 derivative was fused to *MYB92* cDNA and placed under the control of the CaMV
37 dual35S promoter for high and ubiquitous expression. The resulting construct was
38 transfected into leaves of *N. benthamiana* stably transformed with a cassette coding
39 for RFP fused to histone 2B, used as a nuclear marker (Martin *et al.*, 2009). Confocal
40 imaging of transfected cells showed a co-localization of the GFP and RFP signals,
41 establishing the nuclear targeting of the MYB92-GFP fusion (Figure 3a).
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50 In order to determine whether MYB92 possesses transcriptional activity, the
51 coding region of the TF was cloned in frame with the GAL4 DNA binding domain
52 (DBD). The construct obtained was introduced into a yeast strain (AH109) carrying
53 the *ADE2* and *HIS3* reporter genes under the control of heterologous GAL4-
54 responsive upstream activating sequences and promoter elements. The expression
55 of the two reporters could be activated in the presence of MYB92 fused to GAL4-
56 DBD, thus establishing the transcriptional activity of the TF (Figure 3b).
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3 To test the ability of MYB92 to directly activate *ProBCCP2*, we used a
4 *ProBCCP2:uidA* reporter construct in transactivation assays, in *N. benthamiana*. The
5 promoter of the *2-OXOGLUTARATE-DEPENDENT DIOXYGENASE (ODD)* gene
6 cloned upstream of the *uidA* reporter gene was used as a negative control. Reporter
7 constructs were infiltrated alone or in combination with a vector allowing the
8 expression of *MYB92*, *WRI1* (positive control; Baud *et al.*, 2009b), or *MYB118*
9 (negative control; Barthole *et al.*, 2014) in young leaves of *N. benthamiana*. MYB92,
10 like WRI1, was able to activate the *ProBCCP2:uidA* reporter construct, showing a
11 strong increase in GUS activity compared with the reporter alone (Figure 3c).
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19 Transient activation assays were repeated with mutagenized versions of the
20 *BCCP2* promoter (Figure 3d). Mutations in the MYB-core *cis*-elements did not affect
21 the ability of WRI1 to activate the promoter. On the contrary, MYB92 was no longer
22 able to activate mutagenized versions of the *BCCP2* promoter comprising a
23 mutagenized version of MYB box 1 or 2, confirming the importance of these two
24 adjacent *cis*-regulatory elements for transcriptional activation of *ProBCCP2* by the
25 MYB TF.
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32 **All the members of the subgroups S10 and S24 of MYB transcription factors** 33 **activate *ProBCCP2:uidA* in transactivation assays**

34 MYB92 belongs to subgroup S24 of MYB TFs (Dubos *et al.*, 2010) (Figure 4a). Since
35 several members of this and related S10 subgroups were absent from the REGIA
36 library, the six full-length cDNAs (*MYB93*, *MYB92*, *MYB53*, *MYB39*, *MYB107*, and
37 *MYB9*) were systematically cloned in the pDEST22 vector allowing expression of
38 GAL4-AD-MYB fusion proteins in yeast cells carrying the *HIS3* reporter gene under
39 the control of the *BCCP2* promoter. The corresponding fusion proteins were all able
40 to induce yeast growth on selective medium, demonstrating their interaction with
41 *ProBCCP2* (Figure 4b). Quantitative β -galactosidase assays performed in yeast cells
42 containing the *lacZ* reporter gene cloned downstream of *ProBCCP2* confirmed these
43 interactions. In these quantitative assays, the reporter activity measured in the
44 presence of MYB118 (Barthole *et al.*, 2014), a member of subgroup S25, was similar
45 to the control, demonstrating a lack of interaction with *ProBCCP2* and establishing
46 the specificity of the interactions observed with the six MYBs composing subgroups
47 S10 and S24 (Figure 4c). Additional experiments carried out with yeast strain AH109
48 established that all six TFs possessed transcriptional activity (Figure 4d). Finally,
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3 transactivation assays in *N. benthamiana* established their ability to activate the
4 *ProBCCP2:uidA* reporter construct, whereas MYB118 (used as a negative control)
5 was not (Figure 4e).
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10 **MYB92 activates expression of the *uidA* reporter gene cloned downstream of** 11 **promoters of various lipogenic genes**

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13 As a first approach to evaluate whether MYB92 could activate expression of other
14 lipogenic genes, we considered two Arabidopsis genes whose expression profiles
15 closely resembled that of *BCCP2* according to Expression Angler 2016
16 (developmental map; <http://bar.utoronto.ca/ExpressionAngler/>) (Austin *et al.*, 2016)
17 (Figure 5a). These genes coded for the acyl carrier protein 1 (ACP1; At3g05020) and
18 the lipoamide dehydrogenase 1 (LPD1; At3g16950), two components of the FA
19 biosynthetic network. A close examination of proximal upstream regions of these
20 genes allowed identifying AW-boxes and adjacent MYB-core elements that were
21 reminiscent of the structure of *ProBCCP2* (Figure S1). Reporter constructs containing
22 600 bp and 515 bp, respectively, of upstream sequences from these genes fused to
23 GUS were used in transactivation assays in *N. benthamiana* leaves. MYB92 was
24 able to specifically activate *ProACP1:uidA* and *ProLPD1:uidA* reporter constructs,
25 showing an important increase in GUS activity compared with the reporters alone or
26 the reporters co-transfected with MYB118 (negative control) (Figure 5b).
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39 **Transient expression of *MYB92* in leaves of *N. benthamiana* affects lipid** 40 **homeostasis and yields a 50-fold increase in suberin deposition**

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42 A previous report has described the transcriptional transitions occurring in *N.*
43 *benthamiana* leaves upon induction of FA synthesis by WRI1 homologs from diverse
44 species (Grimberg *et al.*, 2015). A similar experiment was set up to evaluate the
45 impact of a transient expression of *MYB92*, *WRI1* (positive control), and *MYB118*
46 (negative control) on FA metabolism in *N. benthamiana* leaves. Expression vectors
47 obtained by placing the corresponding cDNAs under the control of the CaMV
48 dual35S promoter were used. Efficient overexpression of the transgenes was
49 checked by RT-qPCR five days after leaf agro-infiltration (Figure 6). Then,
50 expression levels of several *N. benthamiana* lipogenic genes were quantified. All the
51 glycolytic and FA biosynthetic genes assayed appeared to be up-regulated in leaves
52 expressing *WRI1* or *MYB92* with respect to controls. These included the enolase-
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3 encoding *ENO1*, the 3-ketoacyl-acyl carrier protein synthase I-encoding *KAS1*, the
4 enoyl-acyl carrier protein reductase-encoding *MOD1*, the lipoamide dehydrogenase-
5 encoding *LPD1*, the acyl carrier protein-encoding *ACP5*, and the biotin carboxyl
6 carrier protein subunit-encoding *BCCP2*. For all these genes, increases in transcript
7 levels measured in leaves expressing *WRI1* were two to five times higher than the
8 increases observed in leaves expressing *MYB92*. This induction of lipogenic genes
9 was specific since the relative mRNA levels of genes participating in other cellular
10 processes like the xyloglucan endotransglucosylase/hydrolase protein 9-encoding
11 *NbXTH*, the dicarboxylate transporter-encoding *NbATTD1*, or the beta-carbonic
12 anhydrase-encoding *NbBCA1* did not display the same pattern (Figure 6).

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15 To evaluate the impact of these transcriptional transitions on lipid homeostasis in
16 transformed leaves, total lipids were extracted from a first set of samples and
17 subjected to thin layer chromatography so as to separate polar lipids from
18 triacylglycerols. The two lipid fractions were analyzed separately by gas
19 chromatography (GC) (Figure 7a and Figure S2). In parallel, a second set of leaf
20 samples was subjected to extensive delipidation, and the amount and composition of
21 residual bound lipids were analyzed by GC-mass spectrometry (Figure 7b). Whereas
22 expression of *MYB118* in leaves of *N. benthamiana* did not affect lipid homeostasis,
23 *WRI1* induced triacylglycerol accumulation, as previously reported (Vanhercke *et al.*,
24 2013; Grimberg *et al.*, 2015). In contrast, expression of *MYB92* yielded a 50-fold
25 increase in the production of surface lipid polyesters. Analysis of the leaf polyester
26 composition revealed aliphatic monomers typical of suberin. Particularly diagnostic of
27 suberin aliphatics were the 223- and 152-fold increases in dicarboxylic C16:0 and
28 C18:1 FA content, respectively, the 130- and 110-fold increases in C22:0 and ω -
29 hydroxy C22:0 FA content, respectively, accompanied by a 17-fold increase in ferulic
30 acid content. The important accumulation of saturated and mono-unsaturated
31 alcohols and the large proportion of very long-chain monomers ($C \geq 20$) were also
32 strongly indicative of suberin deposition. Importantly, enhancement of polyester
33 production in leaves of *N. benthamiana* by *MYB92* had only limited effects, in any, on
34 polar lipid and TAG contents (Figure 7a and Figure S2), suggesting an efficient
35 channeling of the excess FAs produced in the polyester biosynthesis pathway.

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58 **Overexpression of *MYB92* in leaves of *N. benthamiana* increases the**
59 **abundance of suberin biosynthetic gene transcripts**
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3 These observations prompted us to analyze the expression of several *N.*
4 *benthamiana* genes homologous to Arabidopsis genes encoding well-characterized
5 actors of the polyester biosynthetic pathway (Figure 8). In leaves expressing *MYB92*,
6 the 1,075- and 75-fold increases in transcript abundance of the ferulate transferase-
7 encoding *ASFT* (Molina *et al.*, 2009) and FA elongase-encoding *KCS1* (Todd *et al.*,
8 1999) were fully consistent with the accumulation of ferulic acid and monomers with a
9 chain length of > 20 carbons, respectively (Figure 7). *CYPB86B1* transcripts were
10 elevated by more than 400-fold, in agreement with the remarkable increase in ω -
11 hydroxy and dicarboxylic FAs. Cytochrome P450 CYP86B1 is responsible for the ω -
12 hydroxylation of very long-chain saturated FAs (Compagnon *et al.* 2009). Induction of
13 the glycerol-3-phosphate dehydrogenase-encoding *GPDHc1* probably contributed to
14 the production of glycerol backbones. The 120-fold induction of *GPAT5* transcript
15 abundance was probably critical for the proper assembly of suberin considering the
16 importance of glycerol-3-phosphate acyltransferase 5 in the synthesis of *sn*-2
17 monoacylglycerol intermediates (Beisson *et al.*, 2007; Yang *et al.*, 2010). In
18 comparison with the strong induction of *GPAT5*, the moderate 18-fold increase in
19 transcript abundance of *GPAT4* measured in leaves expressing *MYB92* might be
20 related to a limited involvement of this acyltransferase in suberin assembly: GPAT4
21 would also participate in cutin biosynthesis (Kosma *et al.*, 2014). Together, these
22 data indicate that overexpression of *MYB92* in leaves of *N. benthamiana* also leads
23 to significant increases in the accumulation of suberin-related biosynthetic gene
24 transcripts. The ability of this TF to activate a wide array of genes involved in late
25 glycolysis, FA and polyester biosynthesis appears as a rare example of
26 transcriptional control able to trigger a whole biosynthetic process, from primary
27 metabolism to the final assembly of a complex polymer made of specialized
28 metabolites.
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50 **Effects of stable ectopic expression of *MYB92* in leaves of Arabidopsis**

51 To test the effect of *MYB92* overexpression in the native system, stable
52 *Pro35Sdual:MYB92* lines were generated. Three independent transformants with a
53 single insertion locus were characterized. Homozygous lines were grown under
54 controlled conditions and vegetative growth was not affected in these lines. *MYB92*
55 mRNA level was quantified in rosette leaves of the transgenic lines by RT-qPCR,
56 confirming efficient overexpression of *MYB92* (from 1,900 to 2,500-fold compared to
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3 expression in the wild type) (Figure 9a). RT-qPCR analyses then established that
4 *BCCP2* and two genes coding for enzymes of the polyester biosynthesis pathway,
5 *ASFT* and *CYP86B1*, were significantly up-regulated in the transgenic lines.
6
7 However, induction levels measured in the native system for *ASFT* and *CYP86B1*
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9 were one order of magnitude lower than the ones observed in *N. benthamiana*. To
10
11 evaluate the impact of these transcriptional modifications on leaf polyester
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13 biosynthesis, delipidated leaf samples were subjected to GC-mass spectrometry
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15 analyses (Figure 9b and Figure S3). Consistent with the upregulation of *CYP86B1A*
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17 in transgenic leaves, a limited though significant increase in dicarboxylic FA content
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19 could be observed. Beyond these limited compositional changes, polyester
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21 biosynthesis was not reoriented toward massive suberin production as in leaves of *N.*
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23 *benthamiana* though. The contrasted responses observed in these two expression
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25 systems invite us to examine further the post-transcriptional regulations affecting
26
27 MYB92 activity in Arabidopsis.
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31 DISCUSSION

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33 Regulatory mechanisms modulating the rate of FA biosynthesis and integrating this
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35 biochemical process into a developmental framework have long remained ignored in
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37 plants. Identification of the WRI1 TF was a first, significant step towards the
38
39 elucidation of the regulation of acyl chain production (Cernac and Benning, 2004).
40
41 This discovery has emphasized the key role played by transcriptional regulations in
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43 the developmental activation of the FA biosynthetic pathway (Baud *et al.*, 2009b;
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45 Maeo *et al.*, 2009). It was then established that the regulatory mechanism involving
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47 WRI1 is well conserved among plant species and that plant genomes usually encode
48
49 several WRI-like TFs with partially redundant functions (Pouvreau *et al.*, 2011;
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51 Dussert *et al.*, 2013; Tang *et al.*, 2018). In genetic backgrounds where these WRI
52
53 regulators are absent, several developmental defects have been reported. In the
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55 *Arabidopsis wri1 wri3 wri4* triple mutant for instance, a severe depletion in storage
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57 lipid biosynthesis yields wrinkled seeds while defects in cutin deposition in floral
58
59 tissues increase the susceptibility of flowers to organ fusions, leading to semisterility
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(To *et al.*, 2012). Yet, mutants deprived of WRI TFs are not lethal and vegetative
development of these plants seems unmodified under controlled growth conditions,
suggesting that FA biosynthesis is not drastically compromised during a large part of

1
2
3 the plant life cycle. What is more, the expression of WRIs' target genes is not
4 completely abolished in *wri* mutant backgrounds. Together, these observations
5 suggested that the transcriptional machinery regulating FA biosynthesis might involve
6 additional TFs partially redundant with the WRIs. Accordingly, different types of
7 putative *cis*-acting regulatory elements were shown to be over-represented in FA
8 biosynthesis genes (Peng and Weselake, 2011). Aside from the AW-box motif
9 interacting with the WRIs, binding sites for DOF, GATA, and MYB TFs were thus
10 predicted. The functional importance of one of these elements was further confirmed
11 in planta by approaches of promoter dissection coupled with site-directed
12 mutagenesis (Baud *et al.*, 2009b).

21
22 The yeast one-hybrid screening presented in this report allowed the identification of
23 MYB92, a TF able to bind the promoter of *BCCP2*. Complementary analyses carried
24 out in yeast and in planta have then established that the six TFs comprising
25 subgroups S10 (MYB9, MYB107, and MYB30) and S24 (MYB92, MYB53, and
26 MYB93), according to the classification proposed by Dubos *et al.* (2010) for the MYB
27 family, could activate this promoter. These TFs share a highly conserved DNA
28 binding domain called the MYB domain and consisting of up to four amino acid
29 sequence repeats (R) of ~52 residues. MYB TFs from subgroups S10 and S24
30 belong to the R2R3-MYB class, which gathers most of the plant MYBs (126 out of
31 196 in *Arabidopsis*) (Dubos *et al.*, 2010).

32
33 Together, transactivation of the *BCCP2* promoter in leaves of *N. benthamiana* and
34 in vitro binding of MYB92 to the corresponding nucleotidic sequence strongly suggest
35 that MYB92 directly binds to this promoter in planta. If many R2R3-MYBs usually
36 recognize DNA motifs enriched in adenosine and cytosine residues (called AC
37 elements), the range of their binding sites remains poorly described in planta (Prouse
38 and Campbell, 2012). Our data suggest that the GTTTGGT and GTTAGTT elements
39 can be part of the in vivo MYB92 binding site(s). The reverse complement of the first
40 element identified (ACCAAAC) is also found in the *cis*-element bound by MYB61 as
41 defined by cyclic amplification and selection of targets (Prouse and Campbell, 2013).
42 The sequence is also very similar to the secondary wall MYB-responsive element
43 (SMRE) bound by MYB46 and MYB83 (ACC[A/T]A[A/C][T/C]) and identified by
44 promoter deletion and EMSA (Zhong and Ye, 2012). The second element is found in
45 the consensus sequence of MBSII (aaaAGTTAGTTA), a MYB DNA binding site of
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3 MYB.Ph3 from *Petunia hybrida* identified by EMSA (Solano *et al.*, 1995). This
4 sequence also fits the position weight matrices representing top-scoring 8-mers for
5 MYB46, MYB52, MYB55, MYB59, and MYB11 as defined by protein-binding
6 microarray approaches (Franco-Zorrilla *et al.*, 2014). If the two elements bound by
7 MYB92 are not identical, site-directed mutagenesis experiments have pointed out
8 common nucleotides within these two elements that are critical for MYB92 binding
9 (Figure 2c). Extensive characterization of DNA-binding specificities of plant TFs have
10 revealed that an unexpectedly elevated number of TFs, including members of the
11 MYB family, can recognize secondary *cis*-acting regulatory elements with similar or
12 slightly lower affinities to their primary ones (Franco-Zorrilla *et al.*, 2014). Secondary
13 motifs usually represent sequence variants of the primary elements. A more detailed
14 structure-function study using *in vitro* and *in vivo* techniques like Biacore, SELEX,
15 protein-binding microarray or Ch-IP will be necessary to comprehensively
16 characterize the MYB92 DNA binding matrix(ces).

17
18 The data obtained both *in vitro* and *in planta* emphasize the importance of the
19 concomitant presence of two adjacent MYB *cis*-elements for the binding and
20 transcriptional activation of the promoter of *BCCP2* by MYB92. It is possible that
21 MYB92 proteins interact with the promoter as dimers. If further studies are now
22 required to test this hypothesis, examples of MYB dimerization have already been
23 reported for MYB123 (TT2), involved in the production of condensed tannins (Baudry
24 *et al.*, 2004), MYB21 and MYB24, which regulate jasmonate-mediated stamen
25 development (Huang *et al.*, 2017), or BpMYB46, involved in the response to abiotic
26 stresses in *Betula platyphylla* (Wang *et al.*, 2019). According to the supershifted
27 bands observed in EMSA experiments (Figure 1d), the 15 nucleotides separating the
28 MYB *cis*-elements bound by MYB92 from the AW motif recognized by WR11 allow
29 the simultaneous presence of the two types of TFs on the promoter of *BCCP2*. The
30 results obtained within the frame of this study show that they can bind and activate
31 the promoter of *BCCP2* independently from each other and that they do not activate
32 the promoters of FA biosynthetic genes with the same efficiency. Both in yeast one-
33 hybrid assays and in transient activation assays carried out in leaves of *N.*
34 *benthamiana*, expression of *WR11* yielded a stronger activation of these target
35 promoters. Considering that *wri1 myb92* mutant seeds exhibit the same phenotype
36 as *wri1* seeds (Figure S4c), it seems unlikely that MYB92 and WR11 have redundant
37 functions in the oil-accumulating tissues of the seed.

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5 The qRT-PCR approaches carried out in leaves of *N. benthamiana* and *Arabidopsis*
6 overexpressing *MYB92* have unraveled a second subset of target genes specifically
7 activated by the TF. This subset comprises genes characteristic of the suberin
8 aliphatic polyester biosynthesis pathway like *ASFT*, *CYP86B1*, or *GPAT5*.
9 Interestingly, increases in the corresponding transcript levels upon *MYB92*
10 expression were at least one order of magnitude higher than the increases in the
11 accumulation of FA biosynthetic transcripts measured in the same samples. It is
12 tempting to speculate that *MYB92* regulates these different gene subcircuits by
13 means of distinct regulatory mechanisms. Secondary cell wall synthesis (Zhong *et*
14 *al.*, 2010) and triacylglycerol production in maturing seeds (Baud and Lepiniec, 2009)
15 were also shown to require the concerted activation of different gene subcircuits
16 through different molecular mechanisms. Some of these mechanisms involve indirect
17 regulations by means of secondary TFs participating in downstream cascades of
18 transcriptional activation. Regarding the regulation of suberin biosynthesis, the
19 existence of such cascades cannot be completely ruled out. However, the present
20 study provides evidence that MYB TFs comprising subgroups S10 and S24 directly
21 activate the FA biosynthesis gene *BCCP2*, while Gou *et al.* (2017) reported a direct
22 transcriptional activation of suberin biosynthesis genes by *MYB107*. Together, these
23 results invite us to consider other types of mechanisms regulating the concerted
24 activation of these pathways. For instance, differential protein-protein interactions
25 could specify the binding of the TFs to their target promoters or modulate their
26 transcriptional activity. Importantly, the regulatory cascades hypothesis and the
27 occurrence of differential protein-protein interactions are not necessarily mutually
28 exclusive, considering that some TFs can directly control the expression of genes
29 coding for their own protein partners. This can be exemplified by the regulation of the
30 synergid gene regulatory network by *MYB98* (Punwani *et al.*, 2008). Identification of
31 interacting partners of the MYB TFs considered in this study combined with an in-
32 depth characterization of the promoter sequences of the suberin biosynthesis genes
33 will be essential to further elucidate the complex regulatory network governing the
34 synthesis of polyesters.
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58 Suberin is an acylglycerol polymer deposited on the inner surface of the cell wall of
59 specific cell types forming a secondary wall structure, in certain endodermal and
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3 peridermal tissues (Kosma *et al.*, 2014; Jenkin and Molina, 2015). Suberized tissues
4 include seed coat layers, tree bark, periderms of tubers and roots, root endodermis,
5 and abscission scars (Franke and Schreiber, 2007; Franke *et al.*, 2012). This
6 polyester is also deposited in response to wounding (Boher *et al.*, 2013). Ectopic
7 expression of *MYB92* in leaves of *N. benthamiana* is sufficient to trigger the massive
8 production and deposition of suberin-type monomers such as 18:1 and very long-
9 chain saturated ω -hydroxy and dicarboxylic FAs. Further studies are now required for
10 a comprehensive understanding of the physiological functions and regulations of this
11 TF in Arabidopsis. Expression of *MYB92* in the endodermis of the root of Arabidopsis
12 (Iyer-Pascuzzi *et al.*, 2011) suggests a role in the regulation of the biosynthesis of
13 Casparian strips. Although seed expression of *MYB92* (Figure S4a) may coincide
14 with suberin deposition, a thorough characterization of *myb92* mutant seeds did not
15 allow detecting any defect in polyester accumulation in this organ (Figure S4c). It is
16 likely that MYB92 and closely related MYB transcription factors have partially
17 redundant functions with respect to polyester synthesis, and that functionally
18 redundant and co-expressed MYBs hamper approaches of reverse genetics.

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20 In agreement with this hypothesis, MYB9 and MYB107 were previously shown to
21 regulate suberin deposition in the integument of Arabidopsis seeds. Likewise, MYB
22 TFs homologous to Arabidopsis MYB factors from subgroups S10 and S24 were also
23 linked to suberin biosynthesis across multiple plant species (Legay *et al.*, 2016;
24 Lashbrooke *et al.*, 2016; Gou *et al.*, 2017). But transcriptional regulation of suberin
25 deposition by MYB TFs seems to involve an even wider array of MYB factors since
26 MYB41, a member of adjacent subgroup S11, can activate suberin synthesis under
27 conditions of abiotic stress (Kosma *et al.*, 2014). The reason why several related TFs
28 participate in the regulation of the same pathway in a given plant species could be
29 due to the necessity to integrate a variety of developmental and environmental
30 signals, hence the partially different expression patterns of the genes encoding these
31 factors (Winter *et al.*, 2007; Gibbs *et al.*, 2014; Kosma *et al.*, 2014; Gou *et al.*, 2017).

32
33 It is then tempting to speculate that these factors do not activate the same array of
34 targets in exactly the same manner, allowing a modulation of the polyester
35 composition thanks to subtle variations in the activation of enzymes participating in
36 the biosynthesis pathway. In agreement with this, analysis of the suberin monomers
37 in different organs of Arabidopsis (e.g. seed integuments and roots) reveals strikingly
38 different compositions (Li-Beisson *et al.*, 2010). This hypothesis is also supported by
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3 the occurrence of cutin regulators (MYB16 and MYB106) in the adjacent subgroup 9
4 of MYB TFs that induce the production of a glycerolipid polyester related to suberin
5 despite characteristic differences in chemical composition (Oshima *et al.*, 2013;
6 Jenkin and Molina, 2015).
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10 The discovery that *MYB93* expression in endodermal cells also participates in the
11 regulation of lateral root development through interaction with ARABIDILLO proteins
12 (Gibbs *et al.*, 2014) suggests another reason for the multiplication of the MYB factors
13 regulating polyester biosynthesis. It could well be that some of these MYBs act as
14 master regulators governing the metabolic and developmental processes required for
15 the specialization of different cell types producing suberin. Different MYB factors
16 interacting with distinct protein partners may lead to the specification of different cell
17 fates.
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25 In conclusion, we have demonstrated that MYB92 is capable of activating FA
26 biosynthetic genes. Whereas expression of *WRI1* in leaves of *N. benthamiana*
27 stimulates FA synthesis and promotes TAG accumulation, expression of *MYB92* in
28 the same system yields a remarkable accumulation of suberin (Figure 10). This
29 efficient channeling of FAs in the polyester biosynthetic pathway lies in the strong
30 transcriptional activation of the actors of the pathway also regulated by MYB92. The
31 demonstration that MYB92, like MYB41 (Kosma *et al.*, 2014), can stimulate the
32 ectopic deposition of suberin-type material provides an original tool for the
33 biotechnological design of crops with improved agronomical properties linked to
34 altered barrier properties. This could also serve as a lever for the production of
35 renewable chemical feedstock, thus contributing to replace fossil oil for some
36 material and chemicals for the industry (Gandini, 2008). Both basic and applied
37 research objectives now require an exhaustive characterization of these closely
38 related MYB TFs to elucidate their specificities and regulations.
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53 **EXPERIMENTAL PROCEDURES**

56 **Constructs**

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58 The sequences of primers used for DNA amplification are indicated in Table S1.
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3 For construction of the *Pro35Sdual:MYB* transgenes, *MYB92* and *MYB107* cDNAs
4 were amplified with the proofreading Phusion High-Fidelity DNA polymerase (Thermo
5 Scientific) from a mixture of seed cDNAs (Col-0 accession). The PCR products were
6 introduced by BP recombination into the pDONR207 entry vector (Invitrogen).
7 Kelemen *et al.* (2015) previously reported the cloning of *MYB9*, *MYB39*, *MYB53*, and
8 *MYB93* cDNAs into the pDONR207. The cDNAs were then transferred into the
9 destination vector pMDC32 (Curtis and Grossniklaus, 2003) by LR recombination.

10
11 Construction of the *Pro35Sdual:WRI1* transgene was previously described by
12 Baud *et al.* (2007a) and that of the *Pro35Sdual:MYB118* transgene by Barthole *et al.*
13 (2014).

14
15 For construction of the *ProACP1:uidA* and *ProLPD1:uidA* transgenes, regions -600
16 to -1 bp and -515 to -1 bp relative to the translational start codon of the genes
17 considered were amplified with the proofreading Phusion High-Fidelity DNA
18 polymerase (Thermo Scientific) from Col-0 genomic DNA. The PCR products were
19 introduced by BP recombination into the pDONR207 entry vector (Invitrogen) and
20 transferred into the destination vector pBI101-R1R2-GUS (Baud *et al.*, 2007a) by LR
21 recombination.

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23 Construction of the *ProBCCP2:uidA* transgene was previously described by Baud
24 *et al.* (2009b) and that of the *ProODD:uidA* transgene by Barthole *et al.* (2014).

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26 For construction of the *Pro35Sdual:MYB92:mGFP6* transgene, *MYB92* cDNA
27 without STOP codon was amplified with the proofreading Phusion High-Fidelity DNA
28 polymerase (Thermo Scientific) from a mixture of seed cDNAs (Col-0 accession).
29 The PCR product was introduced by BP recombination into the pDONR207 entry
30 vector and transferred into the destination vector pMDC83 (Curtis and Grossniklaus,
31 2003) by LR recombination.

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33 For construction of the *ProADH1:GAL4-DBD:MYB* transgenes, cDNAs previously
34 cloned into the pDONR207 entry vector were transferred into the pDEST32 vector for
35 GAL4-DBD fusion (Invitrogen).

52 RNA analyses

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54 Material used for RNA extraction was frozen in liquid nitrogen immediately after
55 harvest, then stored at -80°C. RNA extraction and reverse transcription (RT) were
56 carried out as previously described (Baud *et al.*, 2004).
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3 RT-PCR analyses were performed as described in Baud *et al.* (2004). Primers
4 used for RT-PCR (Figure S2) were as follows: 5'-ATGGGAAGATCTCCTATCTC-3'
5 (*MYB92_atg*) and 5'-CTAAGGAATGTCTGGAAAATA-3' (*MYB92_stop*), 5'-
6 ATGCCCCAGGACATCGTGATTTTCAT-3' (*EF_up*) and 5'-
7 TTGGCGGCACCCTTAGCTGGATCA-3' (*EF_low*).
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11 Real-time RT quantitative PCR reactions were performed in a CFX Connect Real-
12 Time PCR Detection System (Biorad), using the SsoAdvanced PreAmp Supermix
13 Biorad kit according to the manufacturer's protocol. Reactions used 5 µl of 1:50
14 diluted cDNAs in a total volume of 15 µl. The reactions were incubated as follows: a
15 first step at 95°C for 8 min to activate the hot start recombinant Taq DNA
16 polymerase, followed by 40 cycles of 95°C for 10 s and 60°C for 10 s. The specificity
17 of the PCR amplification was checked with a heat dissociation protocol (from 60 °C to
18 95 °C with a temperature transition rate of 0.1°C.s⁻¹) following the final cycle of the
19 PCR. The efficiency of the primer sets was calculated by performing real time PCR
20 on several dilutions of first strands. Efficiencies of the different primer sets used were
21 checked to be almost similar. The results obtained for the different *N. benthamiana*
22 samples analyzed were standardized to the constitutive *NbL23* gene (coding for a
23 60S ribosomal protein) expression level (Liu *et al.*, 2012). Those obtained for the *A.*
24 *thaliana* samples analyzed were standardized to the constitutive *EF1αA4* gene
25 (coding for a translation elongation factor) expression level (Nesi *et al.*, 2000). The
26 sequences of primers used for real-time RT-qPCR are indicated in Table S2.
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41 Yeast one-hybrid experiments

42 Construction of the pHISi reporter plasmid containing a 180-bp fragment of the
43 *BCCP2* promoter was previously described by Baud *et al.* (2009b). Integration of the
44 plasmid into the yeast strain EGY48 and screening of the REGIA cDNA expression
45 library were previously described by To *et al.* (2012).
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49 To validate the candidate genes isolated, *MYB* cDNAs were cloned into the
50 pDEST22 vector (Invitrogen) to be expressed in yeast as GAL4 activating domain
51 (AD) fusions. cDNAs previously cloned into the pDONR207 were transferred into the
52 pDEST22 expression vector by LR recombination. YM4271 yeast cells presenting the
53 *HIS3* reporter gene under the control of a functional *BCCP2* promoter (To *et al.*,
54 2012) were transformed with pDEST22 according to the AcLi/SSDNA/PEG method
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3 (Gietz and Woods, 2002). Transformants were selected on appropriate media.
4 Construction of *ProBAN:HIS* was described previously (Baudry *et al.*, 2004).

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6 The reporter plasmid used for β -galactosidase assays was constructed by
7 insertion of a 180-bp fragment of the *BCCP2* promoter into the pLacZi vector (To *et*
8 *al.*, 2012). After digestion with *Apa*I, this plasmid was integrated into the yeast strain
9 YM4271 at the *URA3* locus. The resulting yeast strains were selected on a medium
10 lacking uracil and then co-transformed with the pDEST22 vector allowing expression
11 of the MYBs using the AcLi/SSDNA/PEG method (Gietz and Woods, 2002).
12 Transformants were selected on appropriate media and β -galactosidase activity was
13 assayed on liquid cultures using o-nitrophenyl- β -D-galactopyranoside (ONPG) as a
14 substrate, as recommended by the constructor (Yeast protocol handbook; Clontech).
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24 Electrophoretic mobility-shift assays

25 The expression plasmid was constructed by transferring *MYB92* cDNA from the
26 pDONR207 to the expression vector pETG20A (<http://www.embl-hamburg.de/>). The
27 resulting vector was electroporated into *Escherichia coli* RosettaBlue(DE3)pLysS
28 strain (Novagen) for expression. After induction by 0.5 mM IPTG in Luria-Bertani
29 buffer, cells were grown overnight at 17°C. Cell lysis and protein purification were
30 adapted from Baud *et al.* (2009b). Briefly, cell pellet was sonicated in lysis buffer A
31 (20 mM NaCl, 20 mM Tris-HCl, pH 8.0, 5 mM imidazole, 5% glycerol) and centrifuged
32 for 40 min at 21 000 *g*. The clear supernatant was incubated for 4 h at 4°C with Ni-
33 NTA resin (Qiagen). The resin was transferred into a column, washed with 5 ml of
34 buffer A plus 20 mM imidazole and eluted with 5 ml of buffer A plus 300 mM
35 imidazole. Recombinant WRI1 and LEC2 proteins were obtained as previously
36 described in Baud *et al.* (2009b) and in Baud *et al.* (2009a), respectively.
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46 To prepare DNA probes, complementary biotin-labeled (at the 5' end)
47 oligonucleotides (Eurofins MWG Operon) were annealed. The DNA binding assays
48 were performed as previously described in Troncoso-Ponce *et al.* (2016).
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53 Lipid analyses

54 To analyze polar lipids and triacylglycerols, 500 mg of leaves were harvested and
55 stored at -80°C prior to lipid extraction. Leaf samples were ground in 7.2 ml of
56 precooled chloroform/methanol/formic acid (10:10:0.5, v/v/v) and incubated at -20°C
57 overnight. The mixture was centrifuged at 7,500 *g* for 10 min at 4°C to pellet the cell
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3 debris. Lipids of the pellet were reextracted in 2.64 ml of precooled
4 chloroform/methanol/water (10:10:1, v/v/v). Then, 3.6 ml of cooled Hajra solution
5 (KCl 2 M and H₃PO₄ 0.2 M) were added to the pooled supernatants. After shaking
6 and centrifugation (7,500 g for 10 min at 4°C), the lower phase containing lipids was
7 collected and evaporated with a stream of N₂. Lipids were finally resuspended in
8 chloroform/methanol (2:1, v/v) and separated on thin-layer chromatography plates
9 developed with hexane/diethylether/acetic acid (35:15:0.01, v/v/v). Polar lipids and
10 triacylglycerols were visualized under UV by staining with sprayed primuline. Lipid
11 spots were ultimately collected and analyzed by gas chromatography as previously
12 described (Baud *et al.*, 2002).
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20 To analyze suberin polyester composition in leaves, fresh material was collected
21 and immersed in hot isopropanol for 10 min at 80°C. For seed coat analyses, 50 mg
22 of dry seeds were ground in isopropanol, and then heated for 3 hours at 85°C. After
23 cooling, samples were extensively delipidated by extracting the soluble lipids, then
24 dried and depolymerized as previously described (Domergue *et al.*, 2010).
25 Extraction, derivatization, and analysis by GC-MS were performed as previously
26 described (Domergue *et al.*, 2010).
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32 For determination of seed oil content, total fatty acid analyses were performed on
33 pools of 20 dry seeds as previously described (Li *et al.*, 2006).
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37 Microscopy

38 Histochemical detection of GUS activity and bright-field microscopy observations of
39 leaf discs were performed as described by Baud *et al.* (2007b). MYB92-GFP fusion
40 proteins were imaged in leaves of *N. benthamiana* with a Zeiss LSM710 confocal
41 microscope as described by Miart *et al.* (2014).
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48 Molecular characterization of T-DNA mutants

49 Plant genomic DNA flanking the left T-DNA borders of the T-DNA mutant lines
50 ordered from the Salk Institute were amplified by PCR and sequenced to confirm the
51 FSTs identified. Molecular characterization of the *wri1-4* allele was previously
52 described (Baud *et al.*, 2007a). In the *myb92-1* line (N116918), the T-DNA insertion
53 was located in the 3rd exon of the *MYB92* gene. To amplify the T-DNA left border,
54 pAt5g10280F (5'-GCAAGCAACCAAATCTCAGAG-3') and JicSMLB2 (5'-
55 CGAATAAGAGCGTCCATTTTAGAG-3') were used. To amplify the left T-DNA
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border in *myb92-2* (N128394; insertion located in the 2nd exon of the *MYB92* gene), pAt5g10280F and JicSMLB2 were used. In the *myb107* line (N107792), the T-DNA insertion was located in the 2nd exon of the *MYB107* gene. To amplify the T-DNA left border, At3g02940R (5'-ATGGCTGGTGATCTTGTGTA-3') and JicSMLB2 were used.

Seed coat permeability test

Tetrazolium red assays used for seed coat permeability tests were performed as previously described (Vishwanath *et al.*, 2013). Dry seeds were incubated in the dark in a solution of 1% (w/v) tetrazolium red (2,3,5-triphenyltetrazolium chloride) at 30°C for 48 h. The seeds were observed for change in color using a binocular magnifier.

Phylogenetic analysis

To perform distance analysis among the MYB TFs, programs (with default values) proposed at <https://mafft.cbrc.jp/alignment/server/> were used: MAFFT version 7 for multiple alignments and NJ (Neighbor Joining, with 1,000 bootstraps) for tree building (Kuraku *et al.*, 2013). Alignments are provided as Figure S5 online.

Materials described in this manuscript will be available on request.

ACCESSION NUMBERS

Arabidopsis sequence data from this article can be found in the EMBL/GenBank data libraries under accession numbers: *AtACP1*, At3g05020; *AtASFT*, At5g41040; *AtBAN*, At1g61720; *AtBCCP2*, At5g15530; *AtCYP86B1*, At5g23190; *AtEF1 α A4*, At5g60390; *AtLPD1*, At3g16950; *AtMYB9*, At5g16770; *AtMYB39*, At4g17785; *AtMYB53*, At5g65230; *AtMYB92*, At5g10280; *AtMYB93*, At1g34670; *AtMYB107*, At3g02940; *AtMYB118*, At3g27785; *AtODD*, At1g04380; *AtWRI1*, At3g54320; *AtWRI3*, At1g16060. *N. benthamiana* sequence data can be found in the Sol Genomics Network data libraries under accession numbers provided in Table S2.

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5 Observatory technological platforms. Suberin analyses were carried out at
6 Metabolome facility of Bordeaux (<https://cgfb.u-bordeaux.fr/>).
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10 11 12 **AUTHOR CONTRIBUTION**

13 Conceived and designed the experiments: AT, JJ, LL and SB. Performed the
14 experiments: AT, JJ, JT and SK. Analyzed the data: AT, JJ, LL and SB. Conceived
15 and wrote the paper: SB.
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20 21 22 **CONFLICT OF INTEREST**

23 The authors declare no competing interests.
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26 27 28 **SUPPORTING INFORMATION**

29 Additional Supporting materials for this article may be found in the online version of
30 this article.

31 **Figure S1.** Schematic representation of the promoter fragments of *BCCP2*, *ACP1*,
32 and *LPD1* used to prepare constructs consisting of the *uidA* reporter gene driven by
33 these different promoters.
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35 **Figure S3.** Fatty acid composition of leaves of *Nicotiana benthamiana* expressing
36 *MYB92*, *WRI1*, or *MYB118*.
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39 **Figure S3.** Complementary information for the characterization of polyester
40 monomer concentrations in leaves of *Arabidopsis thaliana* stably overexpressing
41 *MYB92*.
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44 **Figure S4.** Characterization of *myb92* mutant alleles.
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46 **Figure S5.** Alignment of amino acid sequences corresponding to the MYB
47 transcription factors of subgroups S10 and S24.
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49 **Table S1.** Primers used for construct preparation.
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51 **Table S2.** Primers used for quantitative RT-PCR.
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37 38 39 40 41 42 43 **FIGURE LEGENDS**

44 **Figure 1.** Interaction of MYB92 with the promoter of the *BCCP2* gene.

45 (a) Analysis of MYB92 binding to the promoter of *BCCP2* in yeast one-hybrid
46 experiments. Yeast strains containing the *HIS3* reporter gene under the control of
47 either the *BANYULS* (*BAN*, negative control) or *BCCP2* promoter were transformed
48 with either the empty pDEST22 expression vector or with a version of this vector
49 allowing the expression of MYB92 or WRI1 before being plated on appropriate media
50 to maintain the expression of the vectors (SD-UW) and to test the activation of the
51 *HIS3* reporter gene (SD-UWH). Data presented are representative from the results
52 obtained for five independent colonies. SD, synthetic drop-out medium.

(b) Quantification of MYB92 binding to the promoter of *BCCP2* in yeast one-hybrid experiments. Yeast strains containing the *LacZ* reporter gene under the control of the *BCCP2* promoter were transformed with either the empty pDEST22 expression vector or with a version of this vector allowing the expression of MYB92 or WRI1 before being plated on appropriate media to maintain the expression of the vectors. β -galactosidase activity was then measured to test the activation of the *LacZ* reporter gene. Values are the means and SE of 15 measurements carried out on five independent colonies. Asterisks indicate significant difference from the control according to *t*-test at ****P* < 0.001.

(c) Binding of MYB92 to the proximal upstream region of *BCCP2*. Electrophoretic mobility shift assay (EMSA) of a probe covering a region from -188 to -110 bp upstream from the ATG codon of *BCCP2* with increasing amounts of MYB92 ('+' = 0.4 μ g, '++' = 0.8 μ g). LEC2 was used as a negative control. Competition of MYB92 binding was carried out in the presence of 10-, 20-, and 100-fold amounts of the unlabeled *ProBCCP2* fragment. Position of free probe (open arrowhead) and the shifted bands (closed arrowheads) are indicated.

(d) Concomitant binding of MYB92 and WRI1 to the proximal upstream region of *BCCP2*. EMSA was carried in the same conditions as set out above. s, supershifted band.

Figure 2. Interaction of MYB92 with two adjacent MYB *cis*-regulatory elements in the promoter of *BCCP2*.

(a) Representation of the wild-type DNA probe covering a 79-bp region of the promoter of *BCCP2* (from -188 to -110 bp upstream from the ATG codon) and of mutagenized versions of this DNA probe used for electrophoretic mobility shift assay (EMSA). Positions of the AW (green box) and MYB *cis*-regulatory elements identified (blue boxes) are presented. Mutations are indicated in red.

(b) Identification of the binding sites of MYB92 in the proximal upstream region of *BCCP2*. The EMSA of truncated and partially overlapping wild-type sequences (WT; lanes 1-5) and of mutagenized versions of the full-length probe (lanes 7-10) is presented. Position of free probe (open arrowhead) and the shifted bands (closed arrowheads) are indicated.

(c) Binding site sequence specificity of MYB92. A competition of MYB92 binding to *ProBCCP2* (-188 to -110 bp) probe was carried out in the presence of unlabeled

ProBCCP2 fragments containing single mutations near the MYB elements 1 and 2. Mutations that affected MYB92 binding are indicated in red, while mutations that did not affect MYB92 binding are indicated in blue. Position of free probe (open arrowhead) and the shifted bands (closed arrowheads) are indicated.

WT, wild type.

Figure 3. Transcriptional activation of the *BCCP2* promoter by MYB92.

(a) Confocal micrographs showing localization of MYB92:GFP fusion proteins in *Nicotiana benthamiana* plants stably transformed with a construct coding for RFP:H2B fusion proteins (Martin *et al.*, 2009). Plants were co-infiltrated with the *Pro35Sdual:MYB92:GFP* construct and a vector encoding the p19 protein of tomato bushy stunt virus (TBSV) that prevents the onset of post-transcriptional gene silencing (Shamloul *et al.*, 2014). GFP was observed 4 days after infiltration. Bar = 50 μ m.

(b) Transcriptional activity of MYB92. *MYB92* coding sequence was cloned in frame with the GAL4 DNA-binding domain (DBD). The fusion construct was introduced into reporter yeast containing the *HIS3* and *ADE2* reporter genes, before being plated on appropriate media to maintain the expression of the vectors (SD-L) and to test the activation of the *HIS3* (SD-LH) or *HIS3* and *ADE2* reporter genes (SD-LHA). Data presented are representative from the results obtained for six independent colonies. SD, synthetic drop-out medium.

(c) Transactivation assay in leaves of *Nicotiana benthamiana*. *Pro:uidA* reporter constructs alone or in combination with a vector allowing the expression of *MYB92*, *WRI1*, or *MYB118* (negative control) were co-infiltrated in young leaves of *N. benthamiana* with a vector coding for the p19 protein. Leaf discs were assayed for GUS activity four days after infiltration. Tissues were incubated 4.5 h in a buffer containing 2 mM each of potassium ferrocyanide and potassium ferricyanide. Representative discs (diameter = 0.8 cm) are presented.

(d) Transactivation assay in leaves of *Nicotiana benthamiana* with reporter constructs comprising mutagenized versions of the *BCCP2* promoter. Schematic representations of the reporter constructs used are presented. Blue squares and green circles denote wild-type *cis*-regulatory elements, and asterisks indicate that the MYB-core element was modified as follows: GTTTGGT \rightarrow GCCTAGC (MYB-box 1), GTTAGTT \rightarrow GCCATTC (MYB-box 2), and GTTGGGT \rightarrow GCCATTC (Myb-box 3).

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3 Leaf discs harvested for GUS activity four days after infiltration were incubated 2.5 h
4 in a buffer containing 2 mM each of potassium ferrocyanide and potassium
5 ferricyanide. Representative discs (diameter = 0.8 cm) are presented.
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10 **Figure 4.** Activation of the *BCCP2* promoter by the MYB transcription factors
11 comprising subgroups S10 and S24.

12 (a) Phylogram, with branch lengths in arbitrary units, using the alignment generated
13 by the MAFFT program. Full-length sequences of the MYB transcription factors (with
14 gaps) were used for the distance analyses.
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18 (b) Analysis of MYB binding to the promoter of *BCCP2* in yeast one-hybrid
19 experiments. Yeast strains containing the *HIS3* reporter gene under the control of
20 either the *BANYULS* (*BAN*, negative control) or *BCCP2* promoter were transformed
21 with either the empty pDEST22 expression vector or with a version of this vector
22 allowing the expression of the MYBs before being plated on appropriate media to
23 maintain the expression of the vectors (SD-UW) and to test the activation of the *HIS3*
24 reporter gene (SD-UWH). Data presented are representative from the results
25 obtained for five independent colonies. SD, synthetic drop-out medium.
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29 (c) Quantification of MYB binding to the promoter of *BCCP2* in yeast one-hybrid
30 experiments. Yeast strains containing the *LacZ* reporter gene under the control of the
31 *BCCP2* promoter were transformed with either the empty pDEST22 expression
32 vector or with a version of this vector allowing the expression of the MYBs before
33 being plated on appropriate media to maintain the expression of the vectors. β -
34 galactosidase activity was then measured to test the activation of the *LacZ* reporter
35 gene. Values are the means and SE of 18 measurements carried out on six
36 independent colonies. Asterisks indicate significant difference from the control
37 according to *t*-test at ****P* < 0.001.
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41 (d) Transcriptional activity of the MYB transcription factors. *MYB* coding sequences
42 were cloned in frame with the GAL4 DNA-binding domain (DBD). The fusion
43 constructs were introduced into reporter yeast containing the *HIS3* and *ADE2*
44 reporter genes, before being plated on appropriate media to maintain the expression
45 of the vectors (SD-L) and to test the activation of the *HIS3* (SD-LH) or *HIS3* and
46 *ADE2* reporter genes (SD-LHA). Data presented are representative from the results
47 obtained for six independent colonies.
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3 (e) Transactivation assay in leaves of *Nicotiana benthamiana*. *Pro:uidA* reporter
4 constructs alone or in combination with a vector allowing the expression of the *MYBs*
5 were co-infiltrated in young leaves of *N. benthamiana* with a vector coding for the p19
6 protein. Leaf discs were assayed for GUS activity four days after infiltration. Tissues
7 were incubated 3 h in a buffer containing 2 mM each of potassium ferrocyanide and
8 potassium ferricyanide. Representative discs (diameter = 0.8 cm) are presented.
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15 **Figure 5.** Activation of the promoters of fatty acid biosynthetic genes by MYB92.

16 (a) Genes coexpressed with *MYB92* in Arabidopsis. Output of a query on Expression
17 Angler 2016 (developmental map; with default values) displaying an eFP image
18 depicting the expression data and correlation coefficients for the two genes exhibiting
19 the most similar expression patterns with *MYB92*.
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22

23 (b) Transactivation assay in leaves of *Nicotiana benthamiana*. *Pro:uidA* reporter
24 constructs alone or in combination with a vector allowing the expression of *MYB92*,
25 *WRI1*, or *MYB118* (negative control) were co-infiltrated in young leaves of *N.*
26 *benthamiana* with a vector coding for the p19 protein. Leaf discs were assayed for
27 GUS activity four days after infiltration. Tissues were incubated 2 h in a buffer
28 containing 2 mM each of potassium ferrocyanide and potassium ferricyanide.
29 Representative discs (diameter = 0.8 cm) are presented.
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38 **Figure 6.** Transcriptional activation of lipogenic genes by MYB92 in leaves of
39 *Nicotiana benthamiana*.

40 A vector allowing the expression of *MYB92*, *WRI1*, or *MYB118* was co-infiltrated in
41 young leaves of *N. benthamiana* with a vector coding for the p19 protein. In control
42 leaves, the vector coding for the p19 protein was infiltrated alone. Leaf discs were
43 harvested four days after infiltration and cDNAs obtained from six independent
44 mRNA extractions for each vector studied were used for RT-qPCR experiments.
45 Analyses of transcript abundance are presented for Arabidopsis transgenes
46 (*AtMYB92*, *AtWRI1*, and *AtMYB118*) and for *N. benthamiana* genes. Values are the
47 means and SE of six or twelve replicates. Statistical analyses of the data were
48 performed using a one-way ANOVA, followed by Tukey HSD and Holm-Bonferroni
49 multiple comparison tests ($P < 0.01$).
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3 **Figure 7.** Lipid composition of leaves of *Nicotiana benthamiana* expressing *MYB92*,
4 *WRI1*, or *MYB118*.

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6 A vector allowing the expression of transcription factors was co-infiltrated in young
7 leaves of *N. benthamiana* with a vector coding for the p19 protein. In control leaves,
8 the vector coding for the p19 protein was infiltrated alone. Leaf discs harvested four
9 days after infiltration were used for lipid extractions. (a) Concentrations of polar lipids
10 (PL) and triacylglycerols (TAG) separated by thin-layer chromatography and
11 expressed on a fresh weight (FW) basis.

12
13 (b) Concentrations of suberin monomers expressed on a dry weight (DW) basis of
14 delipidated material. The detailed composition of leaves expressing *MYB92* is
15 presented: each constituent is designated by carbon chain length and labeled by
16 chemical class along the x axis.

17
18 Values are the means and SE of four (a) or five (b) biological replicates. Statistical
19 analyses of the data (PL, TAG, and total polyesters) were performed using a one-
20 way ANOVA, followed by Tukey HSD and Holm-Bonferroni multiple comparison tests
21 ($P < 0.01$). In the box presenting concentrations of suberin monomers, asterisks
22 indicate significant difference from the control according to *t*-test at *** $P < 0.001$ and
23 ** $P < 0.01$, respectively.

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35 **Figure 8.** Transcriptional activation of genes involved in suberin biosynthesis by
36 *MYB92* in leaves of *Nicotiana benthamiana*.

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38 A vector allowing the expression of *MYB92*, *WRI1*, or *MYB118* was co-infiltrated in
39 young leaves of *N. benthamiana* with a vector coding for the p19 protein. In control
40 leaves, the vector coding for the p19 protein was infiltrated alone. Leaf discs were
41 harvested four days after infiltration and cDNAs obtained from six independent
42 mRNA extractions for each vector studied were used for RT-qPCR experiments.
43 Values are the means and SE of six replicates. Statistical analyses of the data were
44 performed using a one-way ANOVA, followed by Tukey HSD and Holm-Bonferroni
45 multiple comparison tests ($P < 0.01$).

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55 **Figure 9.** Characterization of leaves of *Arabidopsis thaliana* stably overexpressing
56 *MYB92*.

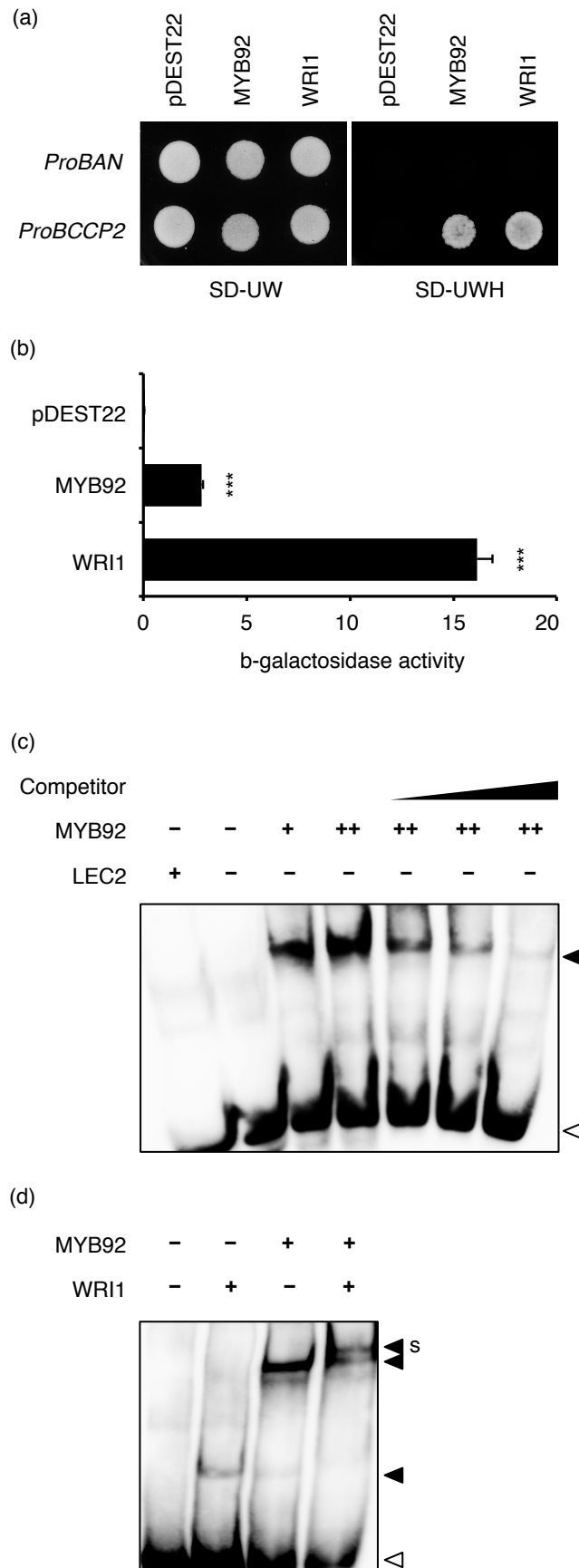
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3 (a) RT-qPCR analysis of transcript abundance in cDNAs prepared from rosette
4 leaves. Values are the means and SE of three to six replicates performed on cDNA
5 dilutions obtained from three independent RNA extractions.
6

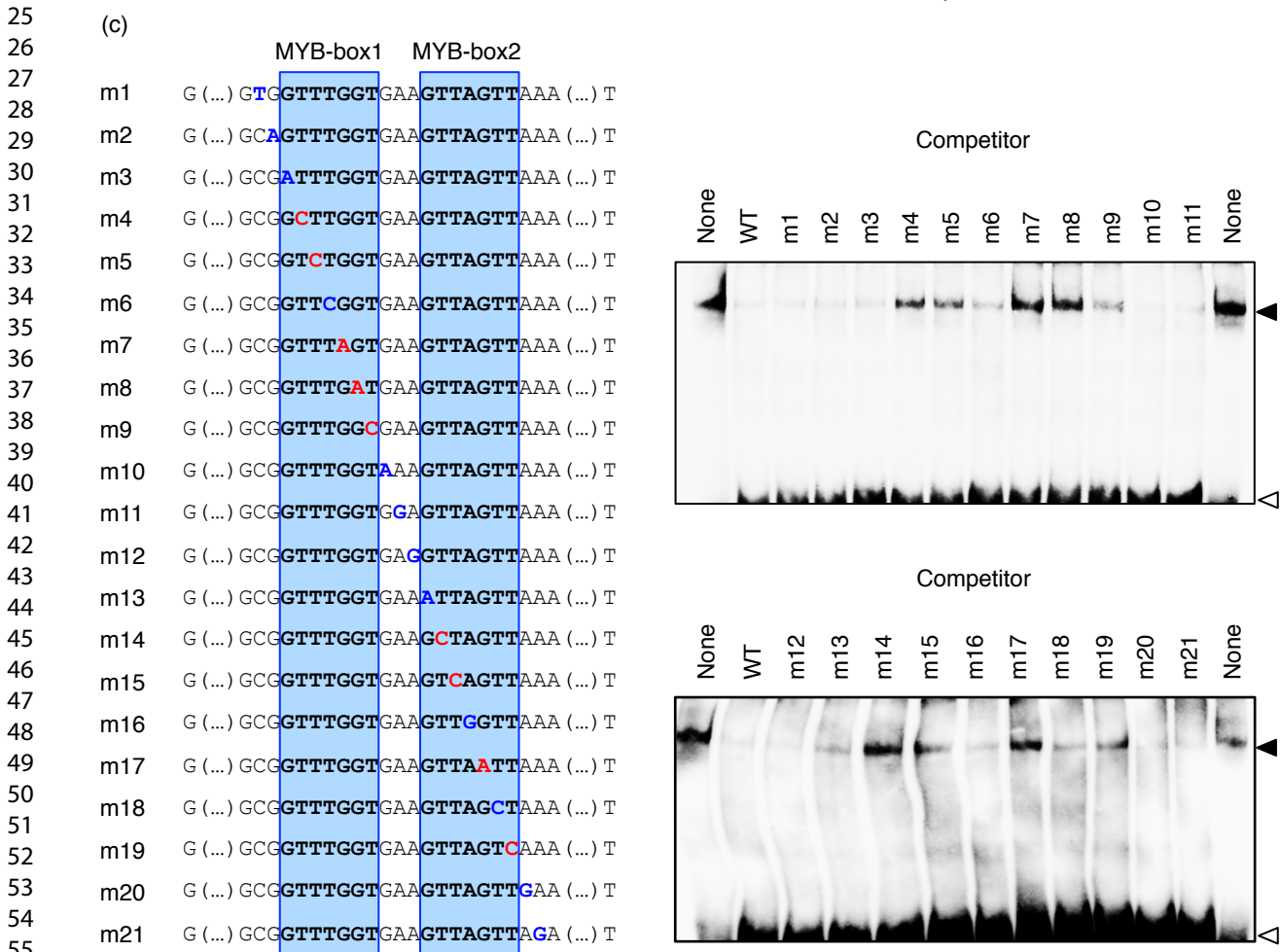
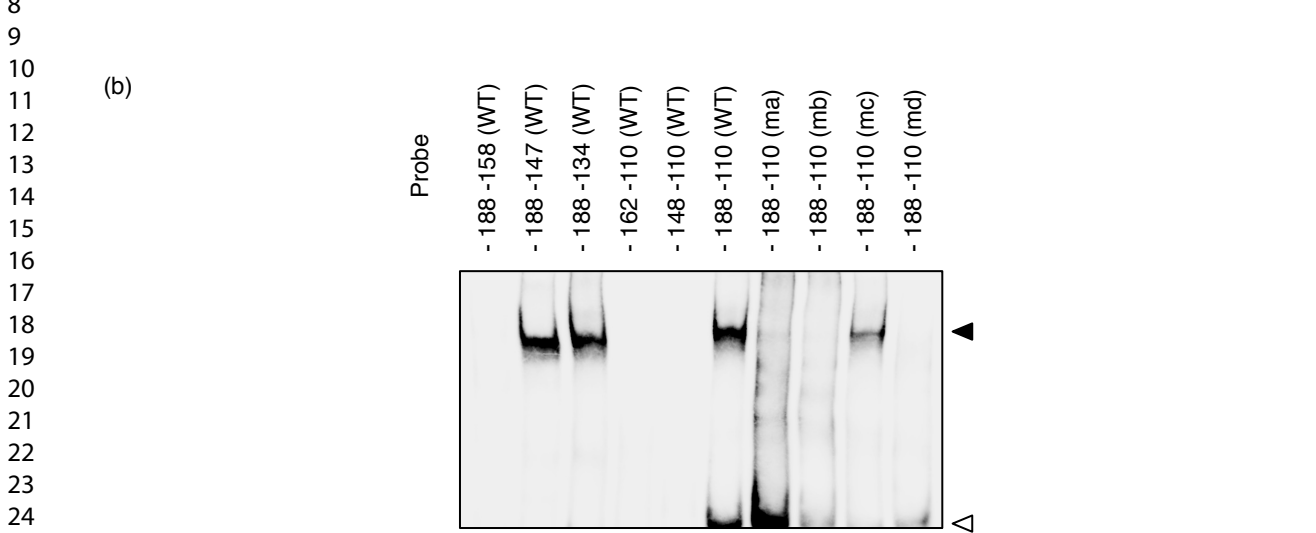
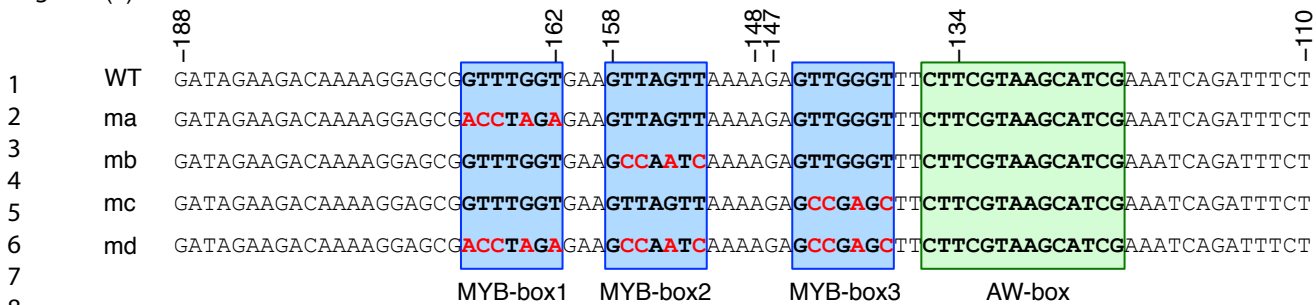
7
8 (b) Content of dicarboxylic fatty acid (DCA) monomers in leaf polyesters as
9 determined by gas chromatography-mass spectrometry and expressed on a dry
10 weight of delipidated material. Values are the means and SE of three to five
11 replicates.
12

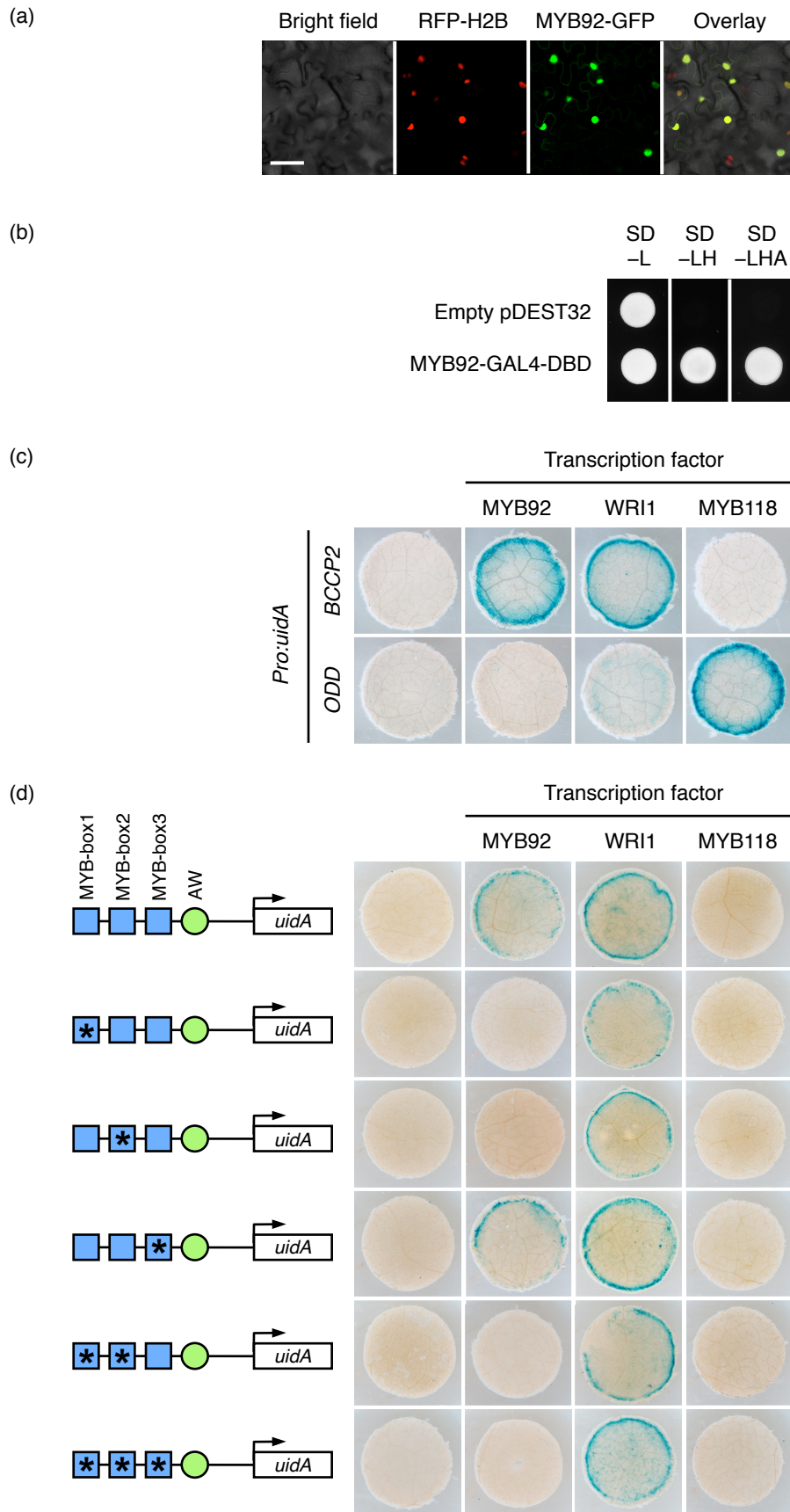
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15 Asterisks indicate significant difference from the wild-type (WT) control according to *t*-
16 test at ****P* < 0.001, ***P* < 0.01, and **P* < 0.05, respectively.
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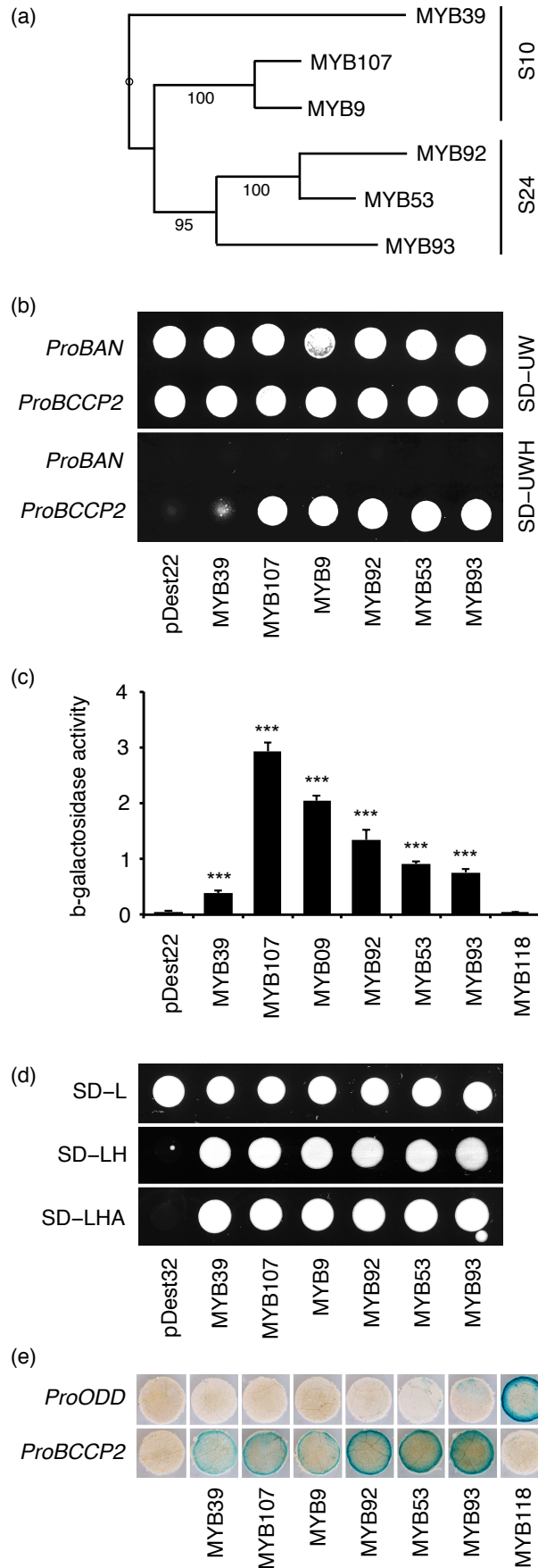
20
21 **Figure 10.** Model for the transcriptional regulation of lipid homeostasis in leaves of
22 *Nicotiana benthamiana*.

23
24 In wild-type leaves, fatty acids produced in the plastids are mainly used for
25 membrane lipid synthesis, and to a lesser extent for the production of surface and
26 storage lipids. Upon *WRI1* expression, the fatty acid biosynthetic pathway is up-
27 regulated. *WRI1* does not dictate the use made of these excess fatty acids though. It
28 seems that triacylglycerol biosynthetic enzymes coded by genes already expressed
29 in untransformed leaves efficiently channel excess fatty acids toward triacylglycerol
30 production, leading to a limited, though significant, over accumulation of storage
31 lipids. On the contrary, *MYB92* not only activates glycolytic and fatty acid biosynthetic
32 genes but also targets genes coding for suberin biosynthetic enzymes. As a
33 consequence, a remarkable increase in suberin monomers is measured in leaves
34 expressing *MYB92*.
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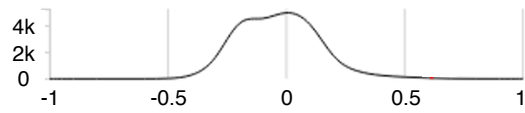






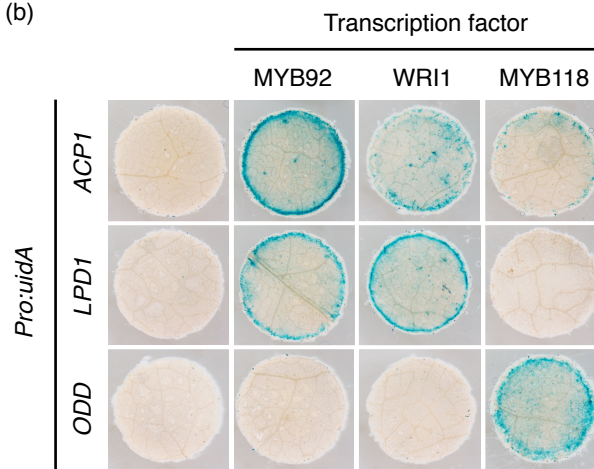


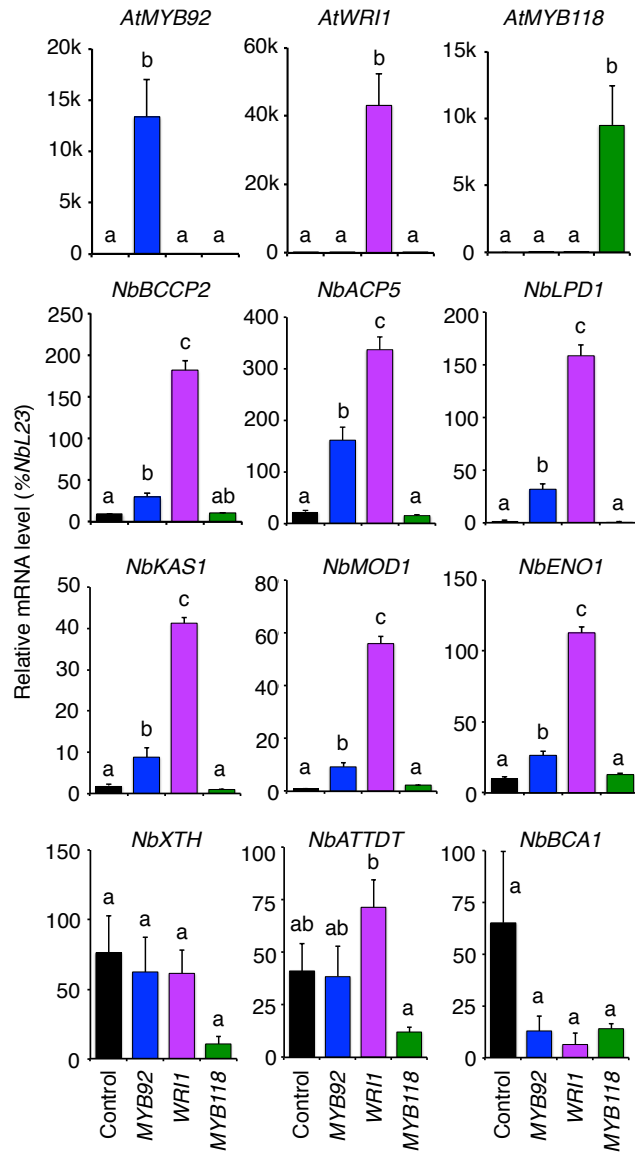
(a) Distribution of r-values for the queried expression pattern:

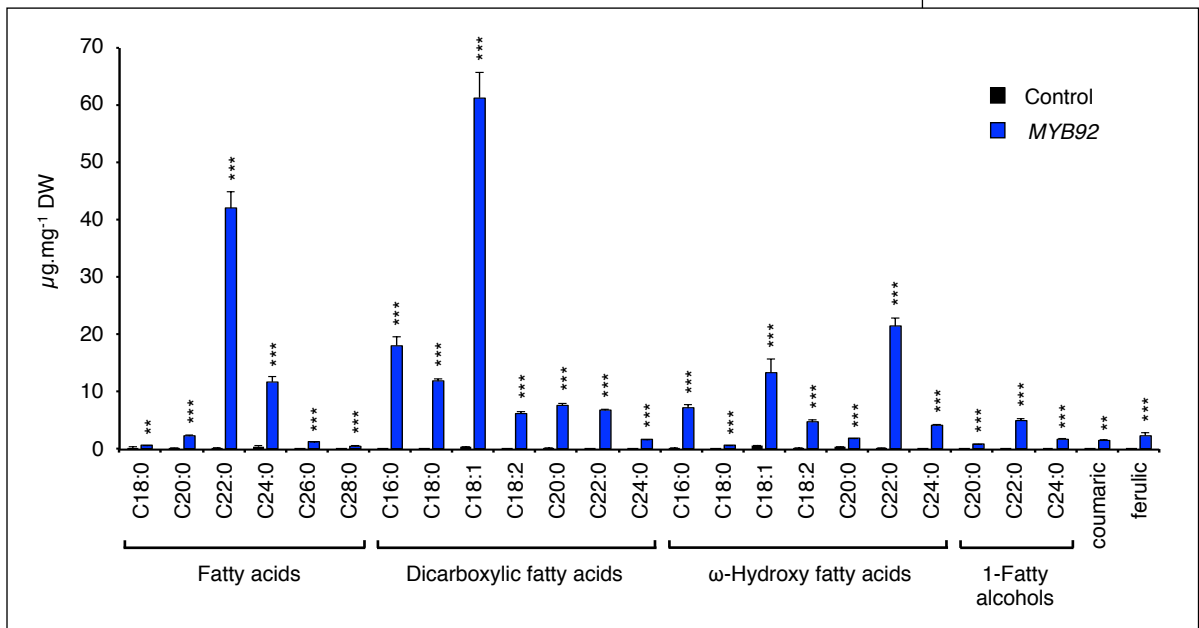
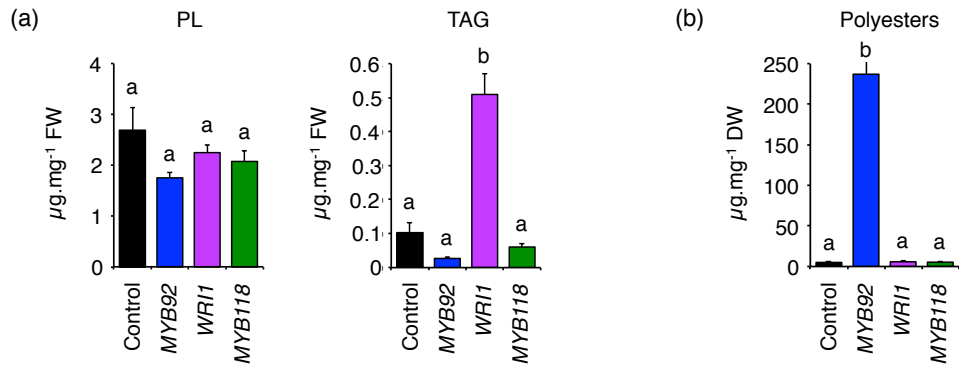


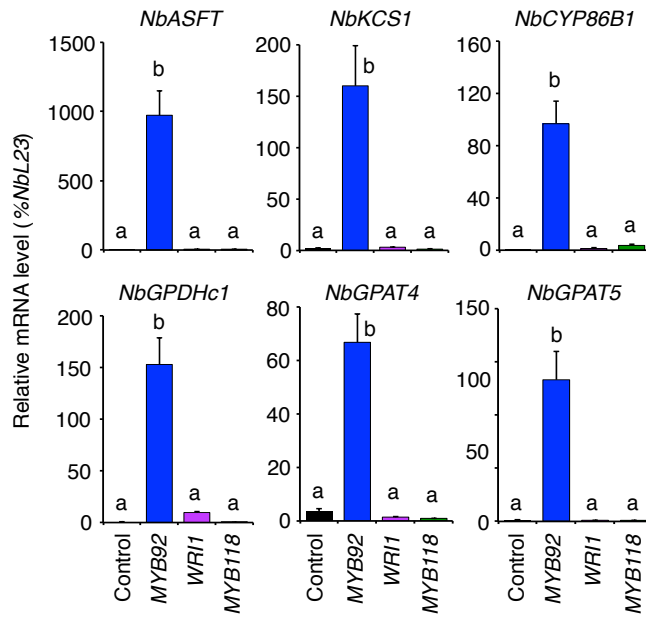
| Gene name | r-value | Heatmap of all samples |
|--------------------------|---------|------------------------|
| AT5G15530 / <i>BCCP2</i> | 1.000 | |
| AT3G05020 / <i>ACP1</i> | 0.881 | |
| AT3G16950 / <i>LPD1</i> | 0.804 | |

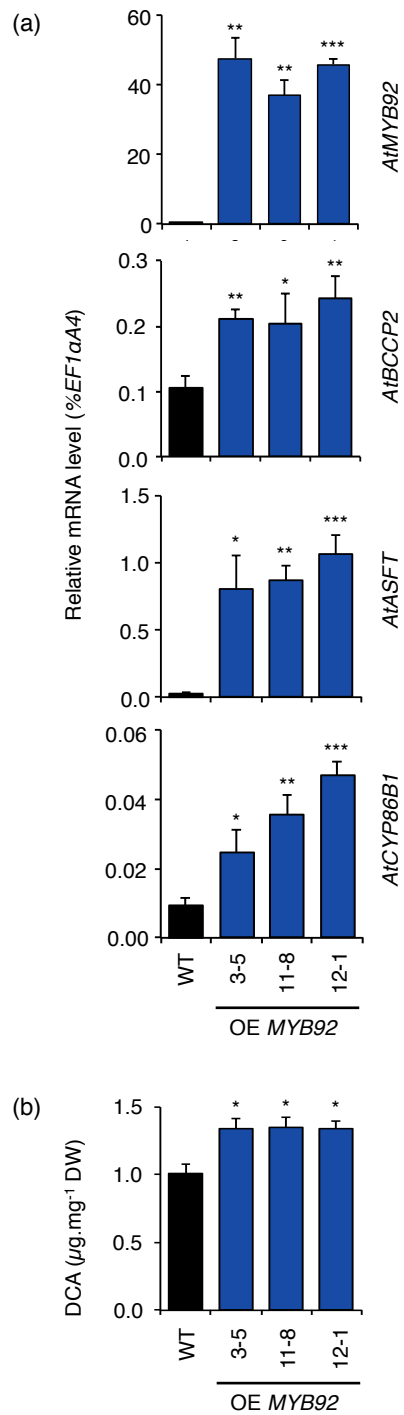
(b)

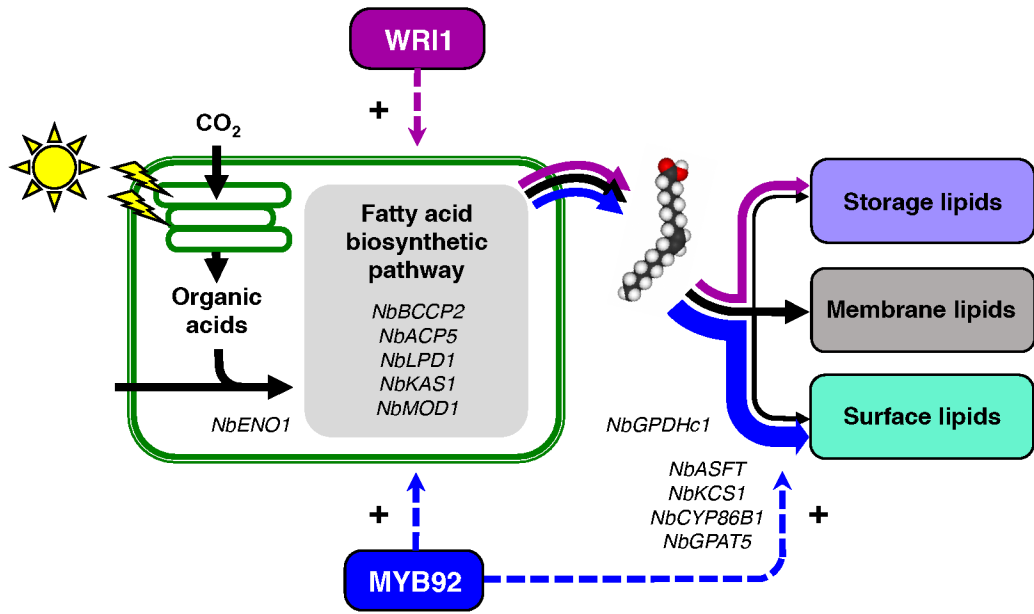


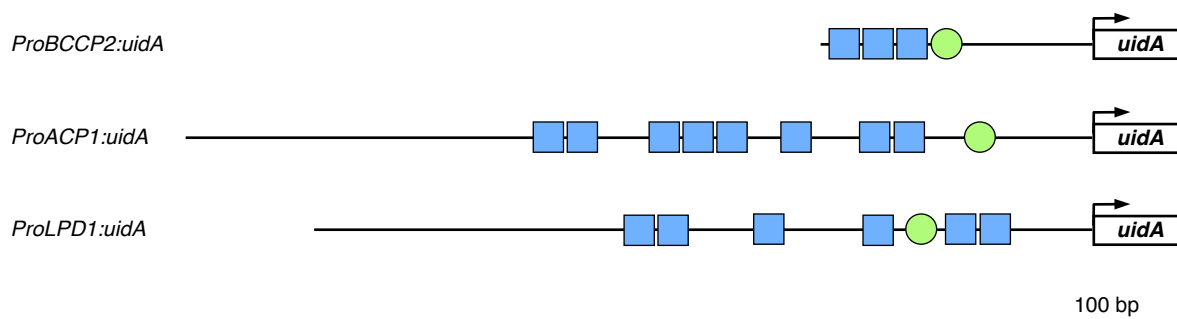


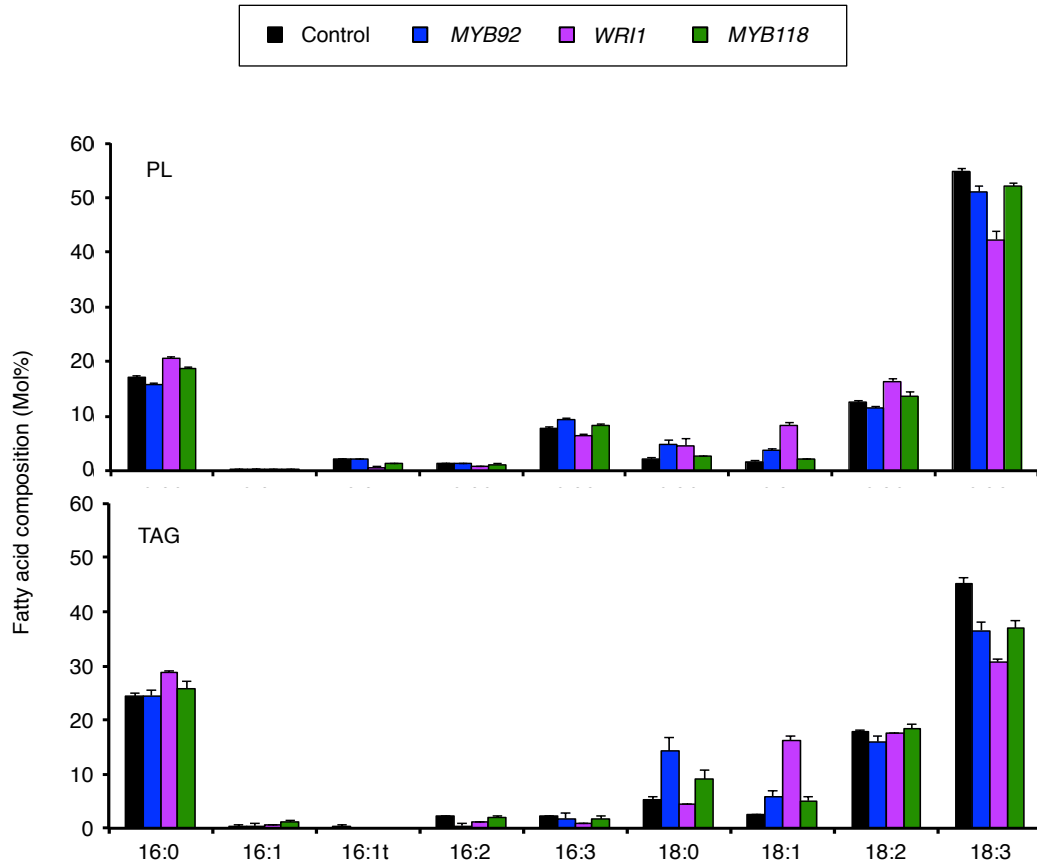


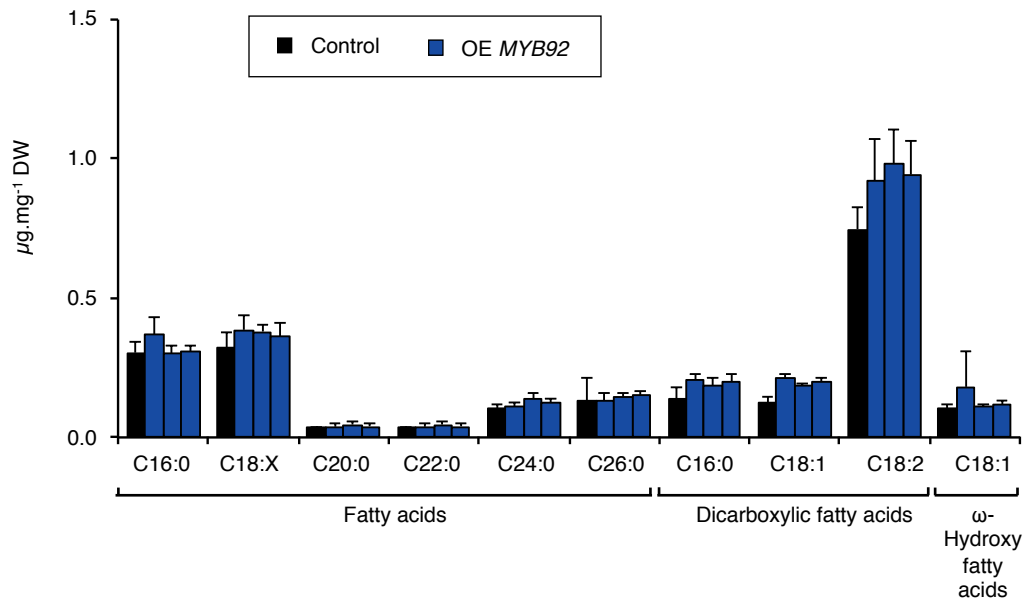












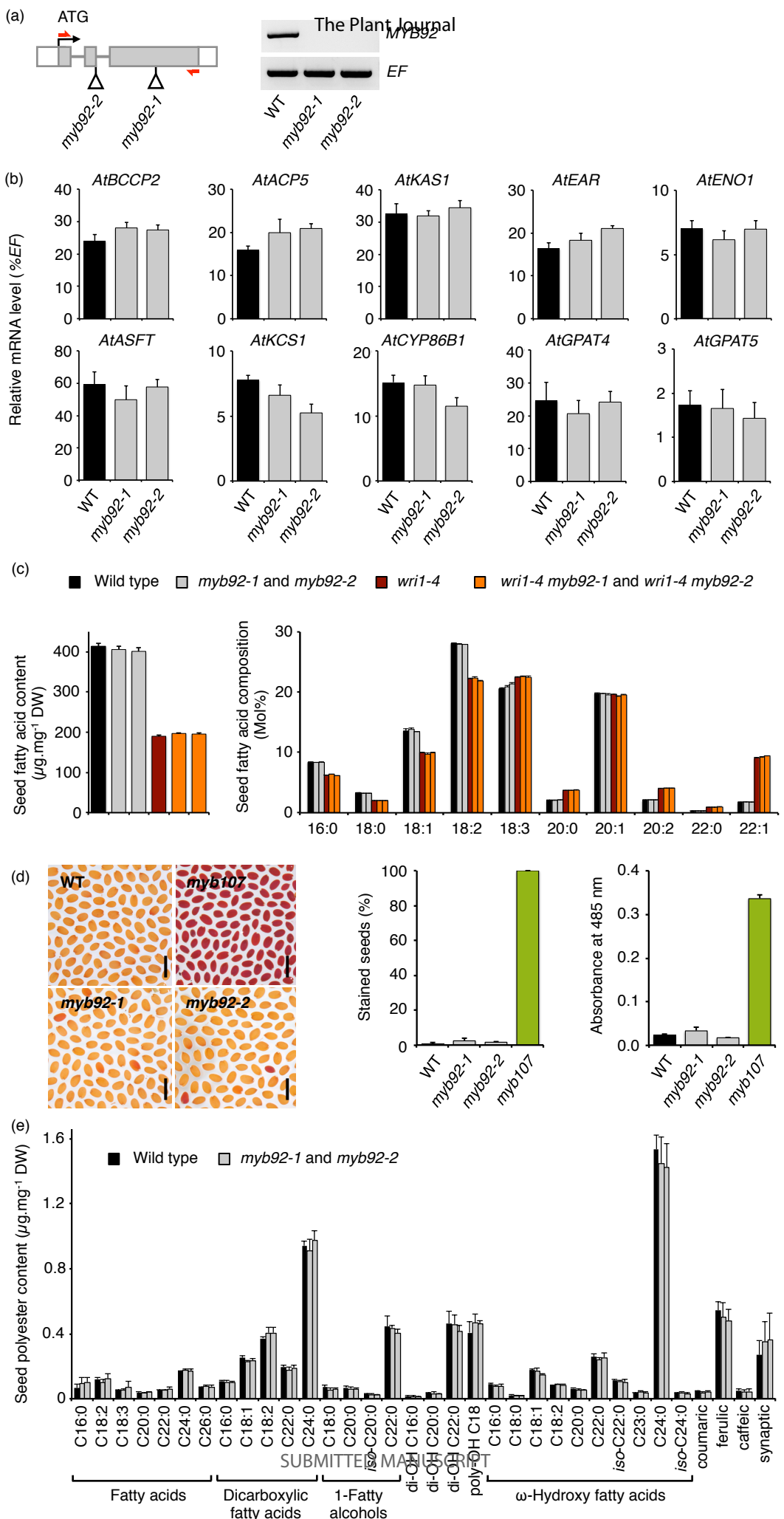


Figure S4

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5 MYB9 MGRSPCCDE-NGLKKGPTQEEEDDKLIDHIQKHGHSWRALPKQAGLNRCGKSCRLRWTN
6 MYB107 MGRSPCCDE-SGLKKGPTPEEDQKLIHIRKHGHSWRALPKQAGLNRCGKSCRLRWTN
7 MYB53 MGRSPSSDE-TGLKKGPTPEEDDKLINYIHKHGSWSALPKLAGLNRCGKSCRLRWTN
8 MYB92 MGRSPISDD-SGLKKGPTPEDEDEKLVNYVQKHGHSWRALPKLAGLNRCGKSCRLRWTN
9 MYB93 MGRSPCCDE-NGLKKGPTPEEDQKLIHYIHKHGSWRALPKLADLNRCGKSCRLRWTN
10 MYB39 MGRSPCCDQDKGVKKGPTPEEDDKLTAYINENGYGNWRS LPKLAGLNRCGKSCRLRWMN
11 -----
12 DNA-binding domain 1
13 MYB9 YLRPDIKRGNFTEEEEQTIINLHSLGKWSIAGNLPGRTDNEIKNYWNTHLRKKLLQM
14 MYB107 YLRPDIKRGNFTAEEEQTIINLHSLGKWSIAGHLPGRTDNEIKNYWNTHIRKKLIQM
15 MYB53 YLRPDIKRGKFSAAAAETILNLHAVLGNKWSMIASHLPGRTDNEIKNFWNTHLKKKLIQM
16 MYB92 YLRPDIKRGKFSPEEEQTIINLHSLVGNKWSITIANQLPGRTDNEIKNFWNTHLKKKLIQM
17 MYB93 YLRPDIKRGKFSAAAAETILHLSILGNKWSAIATHLQGRTDNEIKNFWNTHLKKKLIQM
18 MYB39 YLRPDIRGKFS DGEESTIVRLHALLGNKWSKIAGHLPGRTDNEIKNYWNTHMRKKLLQM
19 -----
20 DNA-binding domain 2
21 MYB9 GIDPVTHRPRTDHLNVLAAALPQLIAA-ANFNSLLNLNQNVLQD-----ATTAK
22 MYB107 GIDPVTHRPRTDHLNVLAAALPQLLAA-ANFNLLNLNQNQLD-----ATSVAK
23 MYB53 GFDPMTHTPRTD--DIFSSLQMLSL-SNLRGLVDLQQQF PME-DQALLNL---QTEMAK
24 MYB92 GFDPMTHTPRTD---IFSSLQMLSLSSNLRGFVDLQQQFPIDQEHTILKL---QTEMAK
25 MYB93 GIDPVTHPRTD---LFASLPQLIAL-ANLKDIEQTSQFS-----SMQGEAAQLAN
26 MYB39 GIDPVTHEPRTN-----DLSPILDV-SQMLAAAINNGQFG---NNLLNNNTALEDILK
27 MYB9 AQLLHTMIQVLSTN---NNTNPSFSSSTM-----QNSNTNLFQASYLEN---QNLFG
28 MYB107 AQLLHSMIQVLSNN---NTS-----SSFDI-----HHTTNNLFQSSFLENLPNIENPYD
29 MYB53 LQLFQYLLQPSAPMSINNINPNILNLLIK-----ENSVTS-----
30 MYB92 LQLFQYLLQPSMS---NNVNPNDFDLTLSL-----LNSIASFKETSN-----
31 MYB93 LQYLQRMFNSSASL---TNNNGNMFSPSSILDIDQHAMNLLNSMVSWNKQDNPAPDPLV
32 MYB39 LQLIHKMLQIITPK---AIPNISSFKT-NLLNPKPEPVVNSFNT-----NSVNPKPDP--
33 MYB9 QSQNFSHILEDENLMVKTQIIDN-----PLDSFSSPIQ-PGFQDDHNSLPLLV
34 MYB107 QTQGLSH-----IDDQ-----PLDSFSSPIRVVAYQHDQNFIPPLI
35 MYB53 -----NIDL---GFLSSH-----LQDFNN---NLPPLK
36 MYB92 --NTTSN---NLDL---GFLGSY-----LQDF---HSLPLK
37 MYB93 ELEANDQ---NQDLFPLGFIIDQ-----P---TQPLQKQYHL---NNSPSEL
38 MYB39 -----PAGLFINQSGITPEAASDFIP---SYENVWDGFED---NQLPGLV
39 MYB9 PASPE-----ESKETQRMINKNDIVDYHHHD
40 MYB107 STSPD-----ESKETQMMVKNKEIMKYNDH-
41 MYB53 T-----LDDNHFSQNT-----SP-----
42 MYB92 TLNSN---MEPSSVFPQNLDDNHFKFST-----QRENLPVSP-----
43 MYB93 PSQGDPLLDHVPFSLQTPLNSEDHFIDN-----LVKHP---TDHEHEH
44 MYB39 TVSQESLNTAKP-GTSTTTKVNHDHIRTGMMPCYGQDQLLETPTSTGVSVSPEPSTSLNHPS
45 MYB9 ASNP-----SSNSTFTQDHHHPWCDTI-----DDGASDSFWKEIEIETC
46 MYB107 TSNP-----SST-STFTQD-HQPWCDII-----DDEASDSYWEKIEIETC
47 MYB53 -----IWLHEPPSLNQTMLPT-HDPCAQSVDGFG---SNQASSSHDQE-VAVTD
48 MYB92 -----IWLSDPSSTT---PA-HVNDLIFNQYGIEDVNSNITSSSGQE-SGASA
49 MYB93 DDNPSSWWLPSLIDNPKTVTSSLPH-NNPA-----DASSSSYG---GCEAA
50 MYB39 TAQ-----HSSGSDFLED---WEKFL-----DDETSDESCWKSFLDLTS
51 MYB9 SEPWP-----FPE--
52 MYB107 SEPWP-----FRE--
53 MYB53 SVDWPDHHLFDSSMFPDISYQS
54 MYB92 SAAWPD-HLLDDSI FSDIP---
55 MYB93 SFYWPD-ICFDESLMNVIS---
56 MYB39 PTSSP-----VPW--

Figure S5

Table S1. Primers used for construct preparation

| Sequence cloned | AGI | Primer | Primer sequence (5'→3') |
|--------------------------|------------------|---------|---|
| ORF | <i>At5g10280</i> | Forward | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGGAAGATCTCCTATCTC |
| | | Reverse | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAAGGAATGTCGGAAAATA |
| ORF minus stop | <i>At5g10280</i> | Forward | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGGAAGATCTCCTATCTC |
| | | Reverse | GGGGACCACTTTGTACAAGAAAGCTGGGTCAGGAATGTCGGAAAATA |
| ORF | <i>At3g02940</i> | Forward | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGGAGATCACCGTGT |
| | | Reverse | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATTACGAAATGGCCA |
| 600-bp promoter sequence | <i>At3g05020</i> | Forward | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAGCCATGATCTTAGTAAACC |
| | | Reverse | GGGGACCACTTTGTACAAGAAAGCTGGGTCGTTTCGTGAAACACGAG |
| 515-bp promoter sequence | <i>At3g16950</i> | Forward | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCTTCATCGAAATCAACATCT |
| | | Reverse | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAAGAGACACAGACAGAGA |

Sequences in blue denote DNA recombination sequences (att sites).

Table S2. Primers used for quantitative RT-PCR

| Gene | AGI | Forward Primer (5'→3') | Reverse Primer (5'→3') |
|-------------------|--|---------------------------------|-------------------------|
| <i>EF1alphaA4</i> | At5g60390 | CTGGAGGTTTTGAGGCTGGTAT | CCAAGGGTGAAAGCAAGAAGA |
| <i>AtMYB92</i> | At5g10280 | Quantitec Primer Assay (QIAGEN) | |
| <i>AtWRI1</i> | At3g54320 | Quantitec Primer Assay (QIAGEN) | |
| <i>AtMYB118</i> | At3g27785 | Quantitec Primer Assay (QIAGEN) | |
| <i>AtBCCP2</i> | At5g15530 | GACCCGGTGAACCCCT | GTCAACGCTGACTGGTTTTCCAT |
| <i>AtASFT</i> | At5g41040 | TCAAAGGAACCAGCTTTGG | TTCCCTCTCTCCTCGGATTT |
| <i>AtCYP86B1</i> | At5g23190 | AGTGACCCTCTGGTTTCACG | CTTCTGACGCAAGGCATGTA |
| Gene | Gene transcript <i>N. benthamiana</i> | Forward Primer (5'→3') | Reverse Primer (5'→3') |
| <i>NbACP5</i> | Niben101Scf04146g02002.1 | TTGCTCCGCTAGCTGTTCA | AATGCACCTGATTCTGCTTG |
| <i>NbATTD1</i> | Niben101Scf02002g01002.1 | CACATTCTTCGTCAGCATGTGG | TGCTGGCTCCGGTTAACC |
| <i>NbBCA1</i> | Niben101Scf06349g00008.1 | GCAAACCATTCGACCCTGTT | TCAGAGCATGCGAAGACCAT |
| <i>NbBCCP2</i> | Niben101Scf12165g00003.1 | AACCACAGTTGCCTCCTGC | CCCTTTCTGGACCTTGTCTCC |
| <i>NbENO1</i> | Niben101Scf11341g00010.1 | GCAGCACTAGACTCCAAAGC | GTGCTCCCGTCTTGATCTGT |
| <i>NbKAS1</i> | Niben101Scf03455g05017.1 | ACCACATCGTTCACATCGAA | GAGTGGAGATGGACGGAAG |
| <i>NbL23</i> | Niben101Scf01444g02009.1 | TGAGGACAACAATACCCTTG | GTCCCATCAGGCCTAATCAA |
| <i>NbLPD1</i> | Niben101Scf00466g04035.1 | TGCTGCTCTAATTGCGACTG | ACCAACTCTCCATTGGCATC |
| <i>NbMOD1</i> | Niben101Scf06661g04001.1 | GCCAAATGGTTCGTTGATG | TCCTGCATAGCGTTTGTGTTG |
| <i>NbXTH</i> | Niben101Scf02990g05004.1 | TCAGGATGTGGGTTTGCTTCC | ACATTCGTCTGGACAGTGACG |
| <i>NbASFT</i> | Niben101Scf14958g00043.1 | GGGACCTGTGGCTTTGC | ATAGCAGAAGCTGGCAATCC |
| <i>NbKCS1</i> | Niben101Scf09024g00004.1 | CGAACGCGCTTGTGTTAGG | CCAATGCACCGAACATAACC |
| <i>NbCYP86B1</i> | Niben101Scf07191g02026.1 | TCGATAACGTGTGCATGATTGC | TCGGTTGCTGACTCGAAAGC |
| <i>NbGPDHc1</i> | Niben101Scf01634g06003.1 | GGCGATCGGACACTATATGC | TTTCAGTCGAGGGCAATCC |
| <i>NbGPAT4</i> | Niben101Scf02264g05017.1 | GCTGCCATTCGGGTTTGC | GCTTGATCCCAACCAATGC |
| <i>NbGPAT5</i> | Niben101Scf08973g01006.1 | TGGATCCGGTGGTCCTATCC | TCAATCGGACGGTTGGTATCG |