

*Supplementary Material*

**Effects on plant growth and reproduction of a peach R2R3-MYB transcription factor overexpressed in tobacco**

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## 1 Supplementary Tables

**Supplementary Table 1.** Primers used for gene expression analysis

Gene	GeneBank Accession no.	Forward primers (5'-3')	Reverse primers (5'-3')
<i>MYB10.1</i>	ppa026640m*	CAGGAAGGACAGCGAATGATG	TCGGGGTTGAGGTCTTATTACG
<i>MYB10.2</i>	ppa016711m*	ATGGAGGGTTATGACTTGAGTGT	TTACTTTCTATATTCTTCATTTGAAT
<i>MYB10.3</i>	ppa020385m*	ATGGGGGGAAATAACTTGGATGT	TTATTCTTCTTTTGAATGATTCCA
<i>MYB24</i>	ppa011751m*	CAAGTGGGGAAACAGGTGGTC	CTGCTTGCTTAATGTGCTTTTGAA
<i>PpN1</i>	ppa009483m*	CCAGGAGAATCGGTGAGCAGAAAA	TCGAGGGTGGAGGACTTGAGAATG
<i>NtMYB305</i>	EU111679.1	GGACAAGGATTCAGAAGCACATA	GTTGGACCAGCAGACGCACATA
<i>NtPAL</i>	D17467.1	CCCCCTCGCGGCACCATCAC	TGCTTTAGAATTAGGCCGACCAGT
<i>NtAN2</i>	FJ472647	GAAGAAAGGTGCATGGACTG	TCTGCAGCTCTTTCTGCATC
<i>NtAN1b</i>	HQ589209	CTTGAACACTTCTCAAACCGA	TGCTAGGGCACAATGTGAAG
<i>NtCHS</i>	AF311783.1	CACGGTCATGGCTATCGGAACA	GCTCAACCTTATGCTCGCTATTA
<i>NtCHI</i>	AB213651	GCCGGCGCAGGAAATAGAGGT	TAGCGGCGAGAAAAGGAAGAGCA
<i>NtF3H</i>	AB289450	CTTACCCTTGGACTGAAACGACAC	CAACGGGCTGAACAGTAATCCA
<i>NtDFR</i>	AB289448	CAGAGAAGGCCGCAATGGAAGC	GGTGGGAATGTAGGCGTGAGGAAT
<i>NtFLS</i>	AB289451	GCTGCGAGAAGTTGTGGAGAAAA	CCTTGGGCATGGTGGGTAATAA
<i>NtLAR</i>	AM827419.1	CTTCAAGGTCCTTACGCCATC	GCTGCAGAGAATATCAACCCC
<i>NtANR1</i>	AM791704.1	GCAATCTTTGACAGGGAATGAAT	TGGGCGCGACAAACATCTT
<i>NtANS</i>	AB289447	TTAACTACTACCCCAAATGTCC	TGCCGTTACCCACTGTCCTTC
<i>NtUFGT</i>	FG627024.1	CAATGAGTGCATTGGATGCC	CCAGCTCCATTAGGTCCTTG
<i>NtJAZd</i>	JQ172762.1	TTGCGAGACGAAATTCATTACA	TGCCTTATTTTCCTCATTCTTAGC
<i>NtAOS</i>	AB778304.1	TCGTAGGTGAAGAAGGGGAAAAGT	TCTCGAAACCAGCACCACAAAATC
<i>NtNEC1</i>	AF132671	AAGCCAGGAGCCACAAACAACAAA	TCAATTCGAGCGAGGGGATACACCA
<i>NtACO</i>	X98493	CTCGTTGAGAAAGAGGCAGC	GGATCCATCTTGACATCAGA
<i>NtUBC2</i>	AB026056	TGAGAACAAGCGGAATACAACAG	AACAGATTAAGAGTGCGGGAGATG

\* Phytozome accession numbers ([www.phytozome.net](http://www.phytozome.net))

**Supplementary Table 2.** Comparison of peach MYB10.1 (an R2R3-MYB) TF with anthocyanin promoting *Arabidopsis* and apple R2R3-MYB TFs.

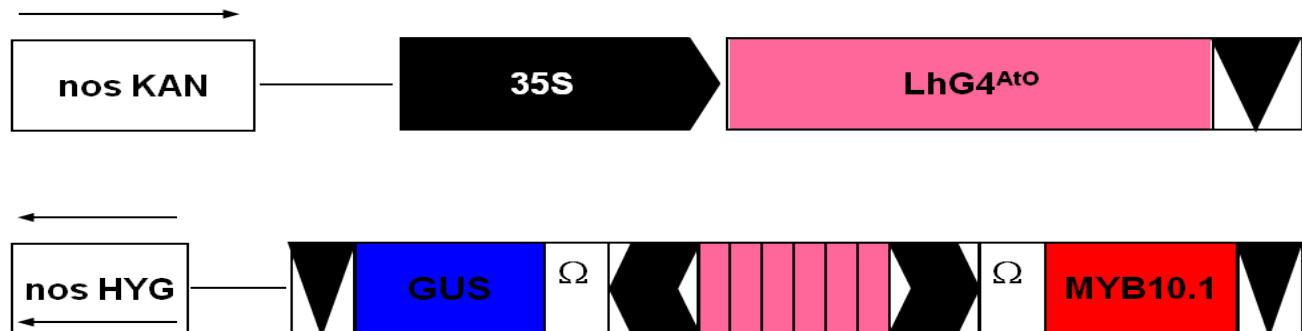
Name of the TFs	GeneBank/TAIR/ Phytozome accessions	gDNA (bp)	CDS (bp)	Protein (aa)	MYB10.1
					Similarity (%)*
<b>MYB10.1</b>	Prupe ppa026640m	1858	720	239	100
<b>AtPAP1/AtMYB75</b>	AT1G56650/AAG42001	1543	747	248	58.6
<b>AtPAP2/AtMYB90</b>	AT1G66390/NP 176813	1585	750	249	63.0
<b>AtPAP3/AtMYB113</b>	AT1G66370/NP 176811	971	741	246	52.6
<b>AtPAP4/AtMYB114</b>	AT1G66380/NP 176812	1078	420	139	57.7
<b>MdMYB10</b>	EU518249.2	4050	732	243	79.2

\* CDS for each TFs were compared with MYB10.1 using ClustalW available within the “Lasergene” software package (DNASTAR).

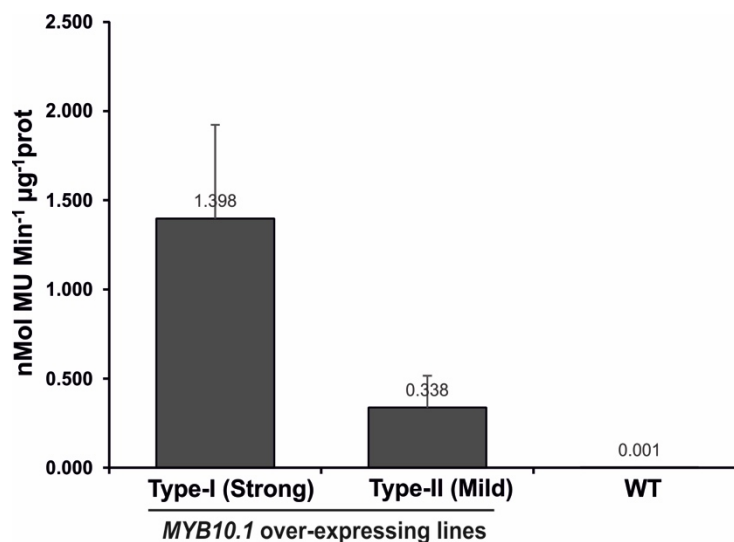
**Supplementary Table 3.** Pistil fertility test by manual pollination. Twenty flowers for each cross were pollinated and their fertility was assessed as ability to set fruits carrying seeds.

	♀ <b>Type-I (Strong)</b> × ♂ <b>WT</b>	♀ <b>WT</b> × ♂ <b>Type-I (Strong)</b>
<b>% fertilized flowers</b>	0	85

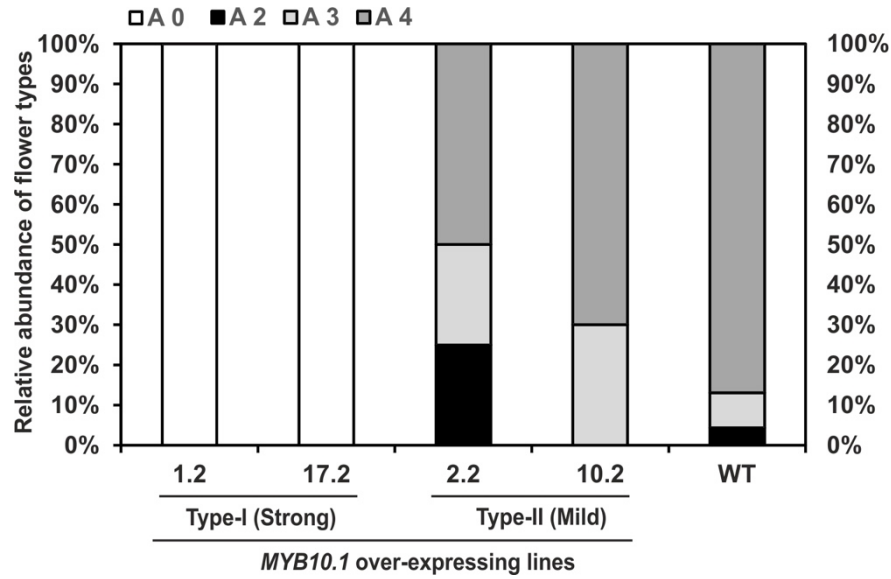
## 2 Supplementary Figures



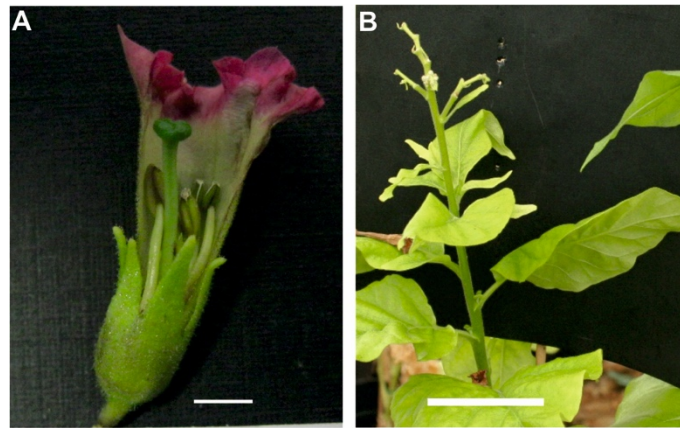
**Supplementary Figure 1.** Schematic maps of the T-DNA regions of the two binary vectors used for the tobacco transformation. The “35S::LhG4” (top) is the LhG4 cassette (activator construct) described in Fig. 1 of (Craft et al., 2005), as it was modified with an improved version of the LhG4 transcriptional activator (i.e. Gal4<sup>AtO</sup>), described by the Moore laboratory in (Rutherford et al., 2005). The “*pOp::MYB10.1*” (bottom) is the pH-TOP-derived cassette (expression construct) described in Fig. 1 of (Craft et al., 2005) in which the peach cDNA encoding the MYB10.1 transcription factor has been cloned, as described previously (Rahim et al., 2014). A different marker gene is present on each cassette to allow the simultaneous selection of both transgenes in co-transformation experiments. The two vectors (Gal4<sup>AtO</sup> and pH-TOP) were a kind gift of prof. Moore.



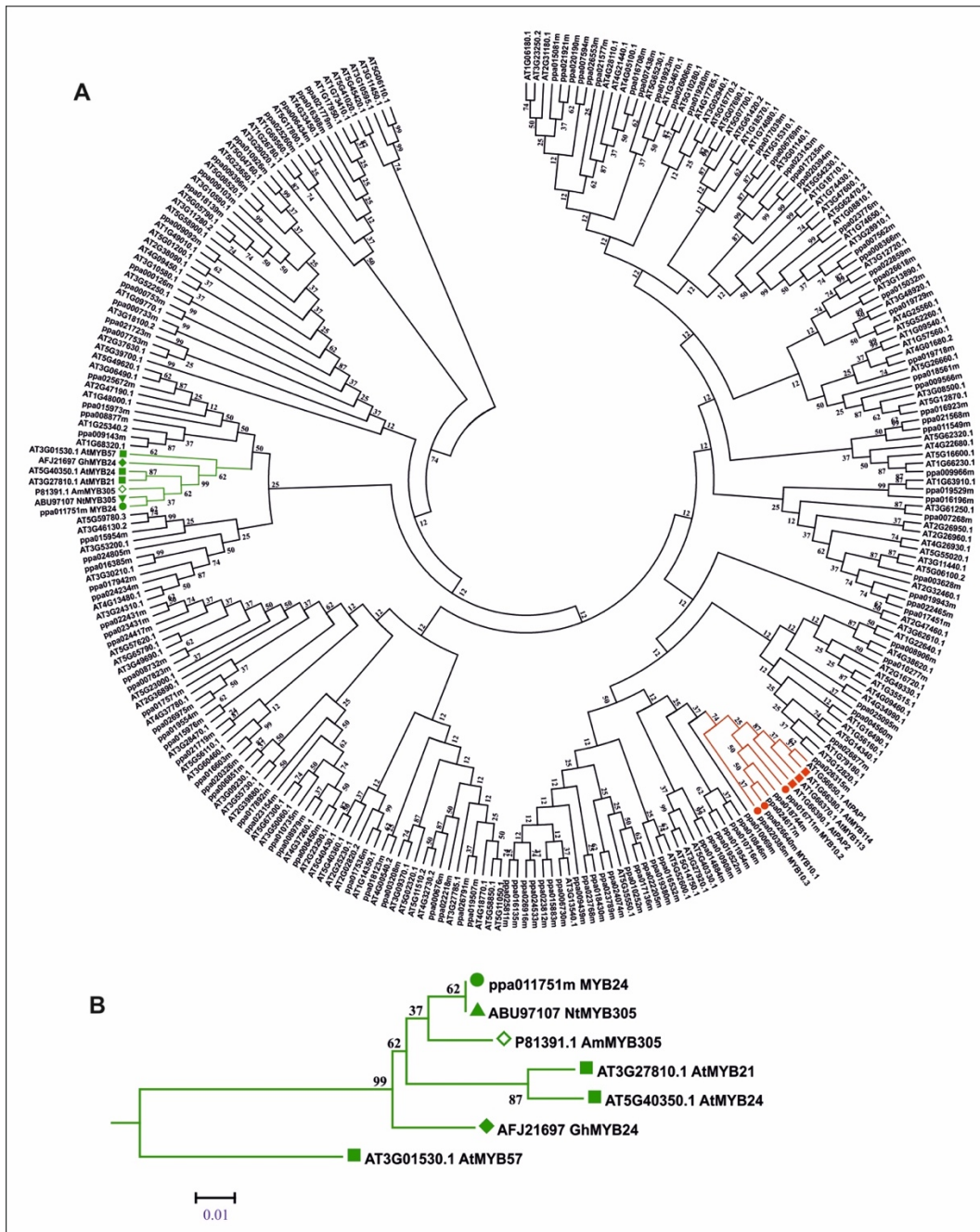
**Supplementary Figure 2.** Enzymatic GUS activity in MYB10.1 over-expressing transgenic tobacco lines and WT. Error bars are  $\pm$  SE of the means of three independent plants.



**Supplementary Figure 3.** The anther filament elongation pattern of *MYB10.1* over-expressing transgenic tobacco flowers. A0, A2, A3 and A4 indicate flowers with 0, 2, 3 and 4 stamens touching the stigmas, respectively.



**Supplementary Figure 4.** Shattering of anthers at extremely late stages of type-I flower development when they are going to abscise (A); due to failure of pollination, fruit setting does not occur and only petioles of the floral inflorescence without capsules remain on type-I transgenic plants (B). Bars are 1 cm (panel A) and 5 cm (panel B).



**Supplementary Figure 5.** Phylogenetic tree showing relationships between *Arabidopsis*, tobacco and peach MYB TFs (A). Magnification of the clade where only MYB TFs related to floral development were clustered (B). Red solid boxes: *Arabidopsis* anthocyanin promoting MYB TFs; green solid boxes: *Arabidopsis* MYB TFs related to flower development (*AtMYB21*, *AtMYB24*, *AtMYB57*); red solid circles: peach MYB10-like TFs; green solid circle: flower specific peach MYB TF, green solid diamond: cotton GhMYB24 (ASJ21697), green unfilled diamond: snapdragon AmMYB305 (P81391) and green solid triangle: tobacco NlxNsMYB305 (ABU97107). The protein sequences of *Arabidopsis* and peach were downloaded from [www.phytozome.net](http://www.phytozome.net). Other protein sequences were retrieved from GeneBank. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5.

Description	Mean normalized expression			
	<i>MYB10.1</i>	<i>M2YB10.2</i>	<i>MYB10.3</i>	<i>MYB24</i>
Complete flower	0.290	0.143	0.038	1.876
Sepal	0.152	0.242	0.028	1.387
Petal	0.842	0.327	0.129	2.516
Androecium	1.354	0.195	0.160	1.783
Gynoecium	0.006	0.022	ND	0.123
Mesocarp around the stone	0.593	ND	0.062	ND

**Supplementary Figure 6.** Expression levels of *MYB10.1*, *MYB10.2*, *MYB10.3* and *MYB24*, determined by qRT-PCR, in peach flower. RNA from flowers at anthesis (Complete flower) and from their parts (sepals, petals, androecium, and gynoecium) was compared with RNA from previous experiments extracted in fruit mesocarp around the stone (see (Rahim et al., 2014)) for the expression levels of the MYB genes belonging to the AtPAP1/MYB10 clade (known to be activators of the anthocyanin pathway) and MYB24, belonging to the AtMYB21/AtMYB24/NtMYB305, known to regulate flower development. The expression values of the target genes were normalized by the expression values of the *PpNI* gene, used as internal standard. To facilitate the reading and comparison of the expression values, the highest one has been arbitrarily set to 100 (blue), and the others accordingly (0 = white).

The expression levels of the peach *MYB24* (likely orthologous of *NtMYB305*, *AmMYB305*, and *AtMYB21*, *AtMYB24* and *AtMYB57*) and of the tree previously identified *MYB10s* (*MYB10.1*, *MYB10.2* and *MYB10.1*) genes (Rahim et al., 2014) were analyzed in different parts of the peach flower by qRT-PCR. The expression profiles revealed that in peach, *MYB24* expression was high in sepal, petal, and androecium but low in gynoecium and was undetected in the fruit mesocarp suggesting that *MYB24* is flower specific. In addition, among the three anthocyanin promoting *MYB10* genes, the expression level of *MYB10.1* was the highest, being higher in the androecium followed by petal whereas almost undetectable in the gynoecium. The high transcript level of *MYB10.1* in androecium is in agreement with the anthocyanin content accumulation in purple stamens, highest among other parts of the peach flower.

## References

- Craft, J., Samalova, M., Baroux, C., Townley, H., Martinez, A., Jepson, I., et al. (2005). New pOp/LhG4 vectors for stringent glucocorticoid-dependent transgene expression in Arabidopsis. *The Plant Journal* 41, 899–918. doi:10.1111/j.1365-313X.2005.02342.x.
- Rahim, Md. A., Busatto, N., and Trainotti, L. (2014). Regulation of anthocyanin biosynthesis in peach fruits. *Planta* 240, 913–929. doi:10.1007/s00425-014-2078-2.
- Rutherford, S., Brandizzi, F., Townley, H., Craft, J., Wang, Y., Jepson, I., et al. (2005). Improved transcriptional activators and their use in mis-expression traps in Arabidopsis. *The Plant Journal* 43, 769–788.