New Phytologist Supporting Information

Article title: A single MYB transcription factor with multiple functions during flower development

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Article acceptance date: 22-May-2023

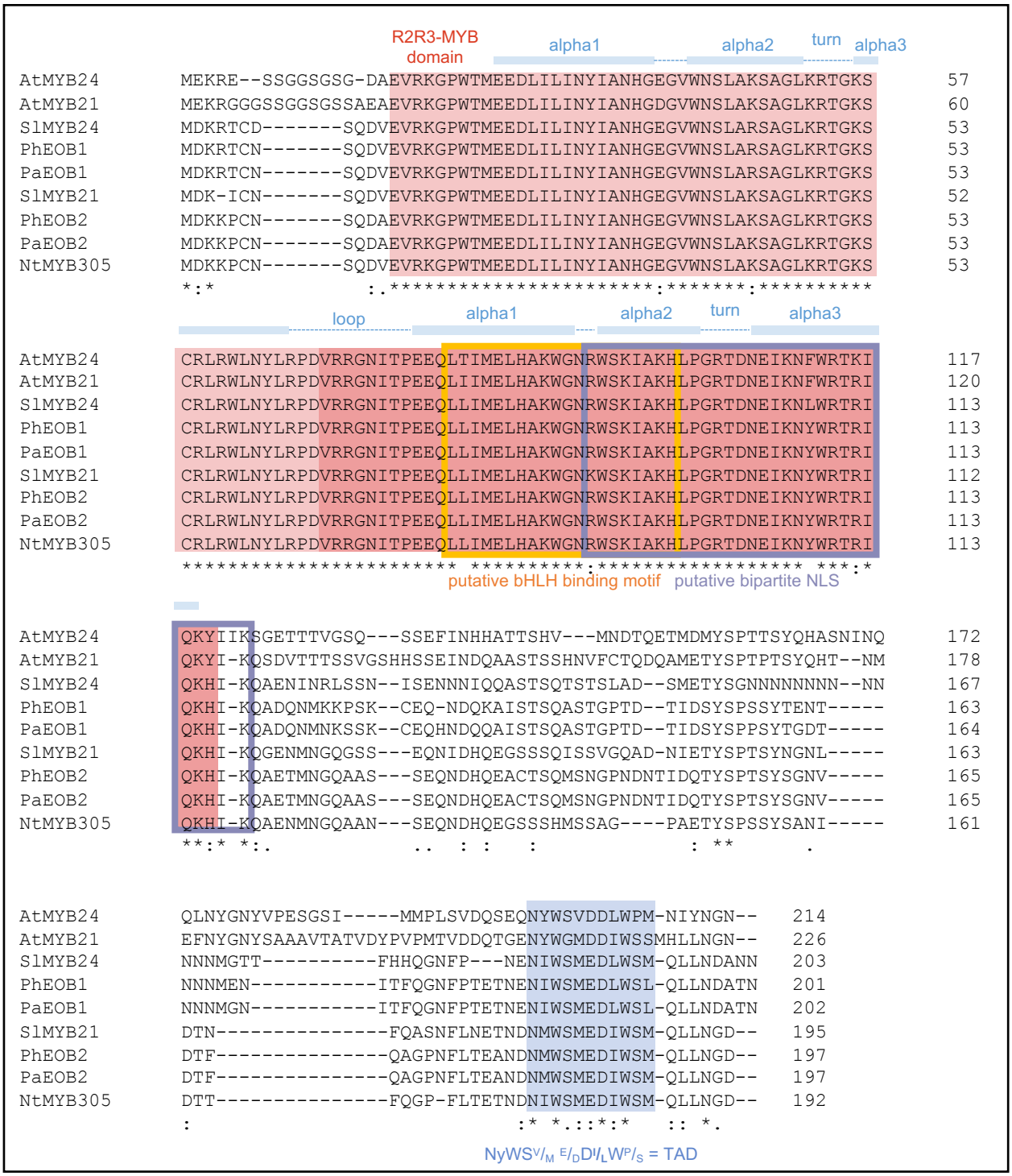
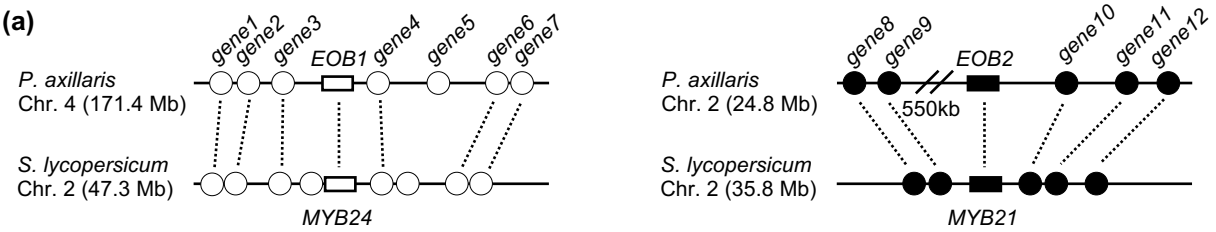


Fig. S1 Multiple sequence alignment of SG19 R2R3-MYB proteins.
Sequence analysis of SG19 R2R3-MYB transcription factors from *Arabidopsis thaliana (At)* and different Solanaceae species: *Petunia axillaris (Pa), Petunia hybrida (Ph), Solanum lycopersicum (Sl)* and *Nicotiana tabacum (Nt)*. The conserved R2R3 MYB-DNA-binding domain is highlighted in red (R2 in light red, R3 in dark red) and the C-terminal motif $NyWS^{V}/_{M}\ ^{E}/_{D}D^{I}/_{L}W^{P}/_{S}$ which is a transcriptional activation domain (TAD) is highlighted in blue. The amino acid (AA) differences observed in the TAD most likely have no impact on the proteins transcriptional activation due to conservative substitutions (M/Y/V/I/L are hydrophobics, E/D are negatively charged) (Figure 5 from Liu *et al*., 2009). The putative bHLH binding motif and bipartite NLS are framed by an orange or purple rectangle respectively.

(a)



(b)

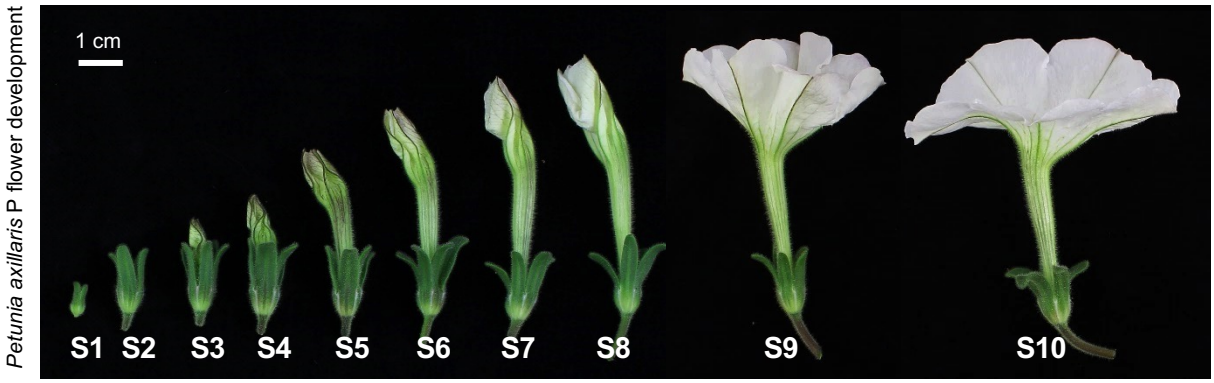
		Petunia axillaris _ **Peax402INV**		*Solanum lycopersicum* _ **ITAG2.4**	
	Functional Description	Gene ID	Genomic Location	Gene ID	Genomic Location
gene1	Unknown protein	Peaxi162Scf00129g00124.1	Chr4:171432749-171433249	Solyc02g086760.1	Chr2:49371895-49372563
gene2	Tudor/PWWP/MBT superfamily protein	Peaxi162Scf00129g01229.1	Chr4:171437850-171441526	Solyc02g086750.1	Chr2:49367024-49369531
gene3	monodehydroascorbate reductase 4	Peaxi162Scf00129g00129.1	Chr4:171444959-171452687	Solyc02g086710.2	Chr2:49352636-49358130
EOB1/MYB24	myb domain protein 21	Peaxi162Scf00129g01231.1	Chr4:171460403-171464096	Solyc02g086690.2	Chr2:49341189-49343806
gene4	peptide deformylase 1B	Peaxi162Scf00129g01227.1	Chr4:171473578-171476063	Solyc02g086680.2	Chr2:49328459-49331889
gene5	Unknown protein	Peaxi162Scf00129g01225.1	Chr4:171480931-171496169		
gene6	DEAD-box ATP-dependent RNA helicase 46	Peaxi162Scf00129g01332.1	Chr4:171502311-171508033	Solyc02g086660.2	Chr2:49307052-49317070
gene7	Phosphoenolpyruvate/phosphate translocator 2, chloroplastic	Peaxi162Scf00129g01336.1	Chr4:171508847-171511745	Solyc02g086650.2	Chr2:49302952-49306789
gene8	Unknown protein	Peaxi162Scf01109g00005.1	Chr2:24282695-24287866	Solyc02g067740.2	Chr2:37856678-37862132
gene9	carbonic anhydrase 1	Peaxi162Scf01109g00003.1	Chr2:24365827-24366905	Solyc02g067750.2	Chr2:37862140-37864509
EOB2/MYB21	myb domain protein 21	Peaxi162Scf00080g00064.1	Chr2:24927180-24931201	Solyc02g067760.2	Chr2:37876383-37879998
gene10	Cysteine-rich receptor-like protein kinase 2	Peaxi162Scf00080g00811.1	Chr2:25134071-25139163	Solyc02g067770.2	Chr2:37893770-37895247
gene11	high affinity nitrate transporter 2.7	Peaxi162Scf00080g00095.1	Chr2:25249937-25251086	Solyc02g067790.2	Chr2:37900235-37903379
gene12	LOB domain-containing protein 25	Peaxi162Scf00080g01010.1	Chr2:25330952-25331925	Solyc02g067800.2	Chr2:37911930-37913905

Fig. S2 Microsynteny analysis comparing genomic regions surrounding *EOB1* or *EOB2* in *P. axillaris* and *S. lycopersicum.* **(a)** The microsynteny analysis was performed using GEvo from CoGe. The SG19 MYB's genomic locations are indicated in the left part. Rectangles indicate SG19 MYB genes, circles indicate the surrounding genes and lines indicate gene-poor regions. Syntenic genes are linked by dashed lines. Left: Genomic region surrounding *PaEOB1* (Peaxi162Scf00129g01231.1) compared with *SlMYB24* (Solyc02g086690.2). Right: Genomic region surrounding *PaEOB2* (Peaxi162Scf00080g00064.1) compared with *SlMYB21* (Solyc02g067760.2). No clear syntheny was observed comparing *P. axillaris* and *A. thaliana***.** Concerning *N. tabacum*, the genome assembly quality did not allow to perform the analysis. **(b)** Gene ID and coordinates of the genes used in the microsynteny analysis.

(a)

Stage	Description	Corresponding day
S1	<6 mm	Day 1
S2	6-15 mm	~ Day 2
S3	15-20 mm	~ Day 3
S4	20-30 mm	~ Day 4
S5	30-40 mm nectary glands and stigmas start their maturation	~ Day 4 - Day 5
S6	40-50 mm	~ Day 5
S7	55 mm	~ Day 6
S8	petals start to unfurl	~ Day 6 - Day 7
S9	petals partially open and anthers start to dehisce	~ Day 7
S10	petals fully open	~ Day 7
Senescence	Petal limb senescence	Around 5 days after flower opening

(b)



(c)

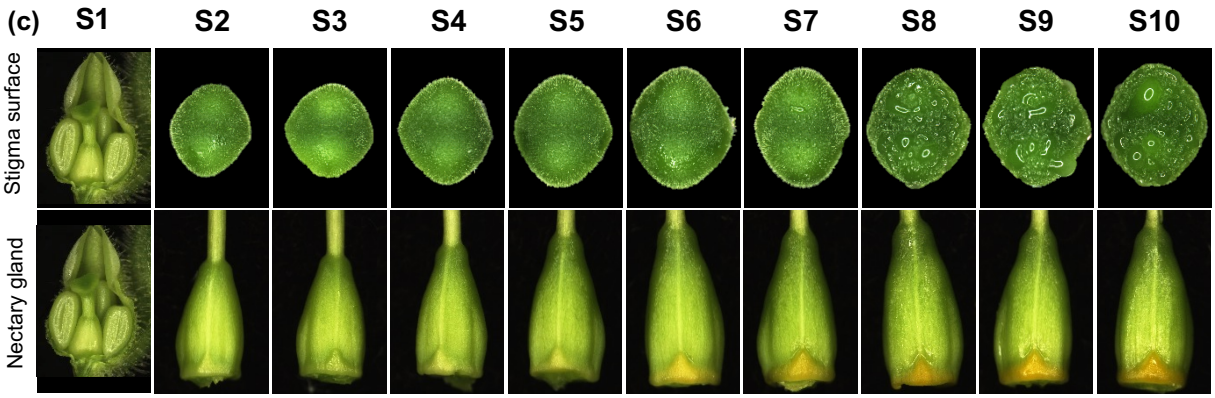


Fig. S3 Description of the different *Petunia axillaris* P floral developmental stages used in this study.
(a) Table describing the different floral developmental stages used in this study over time, S1 to S10 = Stage 1 to Stage 10. **(b)** Pictures of the floral developmental stages of *P. axillaris P*. Scale bar = 1 cm. **(c)** Overview of the stigma surface and the nectary gland over the different developmental stages. The maturation phase of the stigma and nectary gland start at S5. The stigma secretes exudate after S5 and from S8 the stigma surface is covered by exudate droplets resulting in a wet stigma surface. The nectary gland starts to turn orange after S5 due to an accumulation of carotenoids and this process is accompanied by progressive nectar secretion, visible from S6 in the picture series. A metabolic switch may happen in nectary glands and stigma after stage 5.

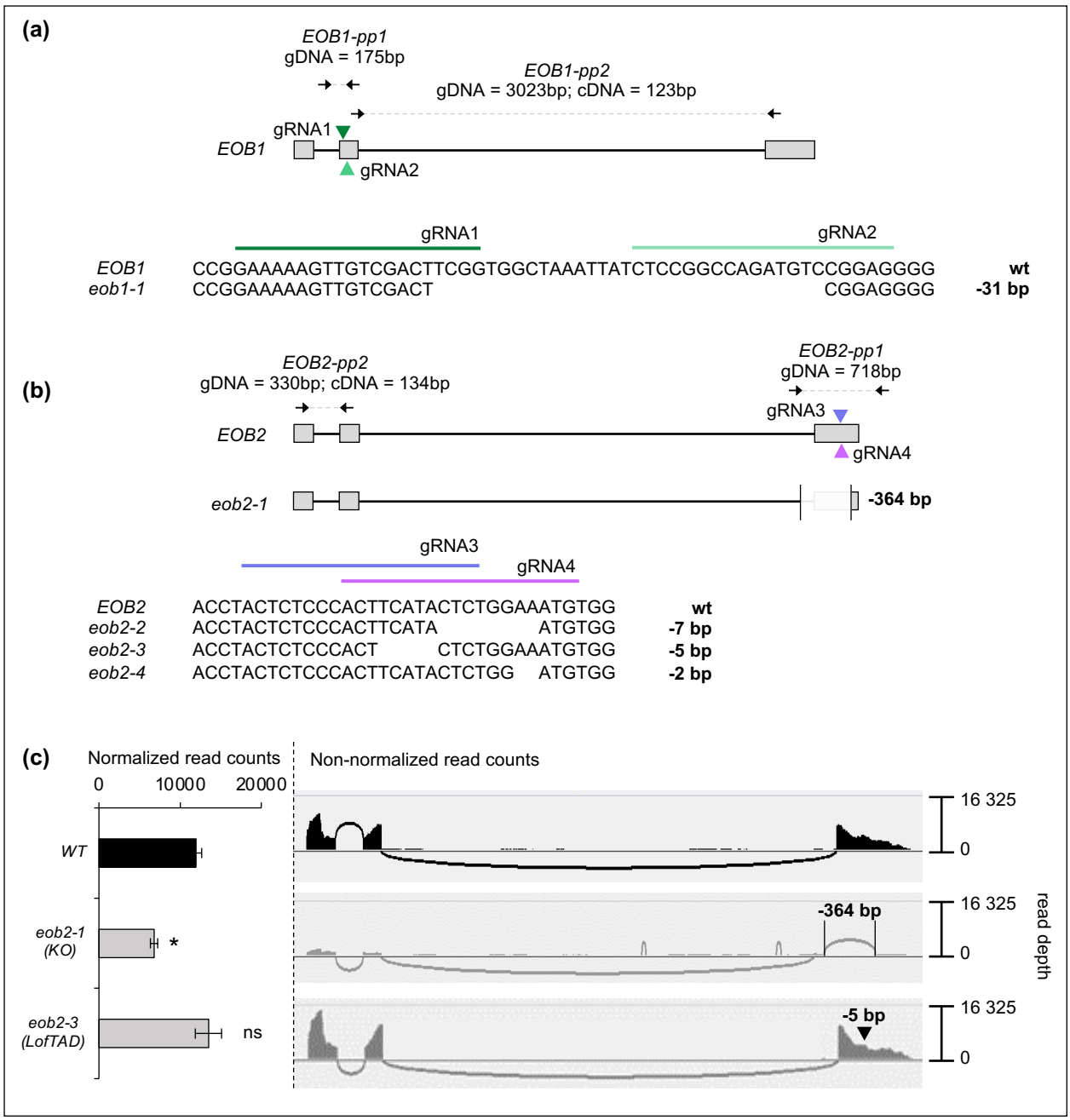


Fig. S4 Construction of *eob1* and *eob2* mutant alleles by CRISPR-Cas9.
(a) *eob1-1* allele generated by CRISR-Cas9. **(b)** *eob2-1* to *4* alleles generated by CRISPR-Cas9. Allele *eob2-1* consists of a 364bp deletion, leading to the removal of the last intron-exon junction affecting the normal splicing process. The primer pairs used for the genotyping (*EOB1-pp1* and *EOB2-pp1*) and RT-qPCR (*EOB1-pp2* and *EOB2-pp2*) are indicated. **(c)** Left: RNA sequencing analysis of *EOB2* expression in WT, *eob2-1* and *eob2-3* S5 petal limb (error bar = SD; significant differences *, $P<0.05$; ns, non-significance; Student's t-test). Right: IGV software was used to construct Sashimi plots depicting splice junctions for the *EOB2* gene, from aligned RNA-seq data derived from S5 limb tissue. The library sizes (13 – 15 M for *eob2-1* and 25 – 60 M for WT) can explain the difference between the number of counts before and after normalization. But overall EOB2 is lower compared to the WT.

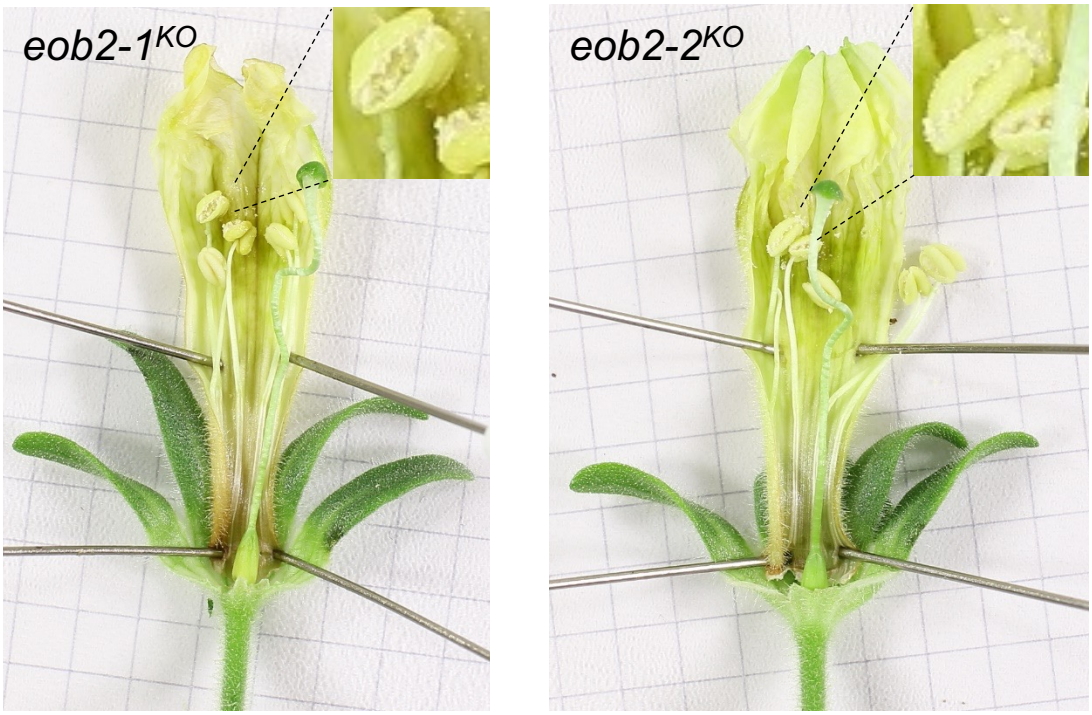


Fig. S5 Observation of the internal floral organs of *eob2-1*^{KO} and *eob2-2*^{KO} flower buds.
The dissected flower buds revealed that styles are twisted, nectary are not mature (yellow) and stamens can released fertile pollen several days after senescence.

Fig. S6 The *eob2-3*^{LofTAD} and eob2-4^{LofTAD} are semi-dominant alleles.
(a) Phenotype of the heterozygous mutant flowers. *eob2-3*^{LofTAD}/+ or *eob2-4*^{LofTAD}/+ displayed an intermediate phenotype between the WT and homozygous mutants. *eob2-1*^{KO}/+ or *eob2-2*^{KO}/+ resembled the WT. **(b)** Nectar measurements (n = 20; error bar = SD). **(c)** Methylbenzoate measurements using a PTR-MS (n = 15 ; error bar = SD).

(a)

>*PaEOB1*_2kb_promoter

ATTAAAAGGAATCTTATTTCTTTTAACGTATATGCACGTCTTGCCAAACTTTTCTTTTCTGAACGCAATATTAATTCTATATCATACATCTCTAATAATGA
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>*PaEOB2*_2kb_promoter

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ACAAAGTACCATATCTTCACTCCCTATCCCATCTCTTTCTCTCTCTCCCTCTCCTTTTTTTTTTCCTTCATCTCTTAAAAAAG

(b)

Percent Identity Matrix - created by Clustal2.1

1: EOB1	EOB1	EOB2
1: EOB1	100.00	49.32
2: EOB2	49.32	100.00

Fig. S7 *EOB1* and *EOB2* promoter sequences are highly divergent.
(a) *Petunia axillaris EOB1* and *EOB2* promoter sequences (2kb). The defined “SG19 MYB-binding site” in the EOB1 promoter is underlined. **(b)** percent identity matrix created by Clustal2.1.

Fig. S8 Additional phenotyping of *eob2-3*^{LofTAD}.
Visible and UV pictures of WT and *eob2-3*^{LofTAD}. All flowers contain UV-absorbing pigments.

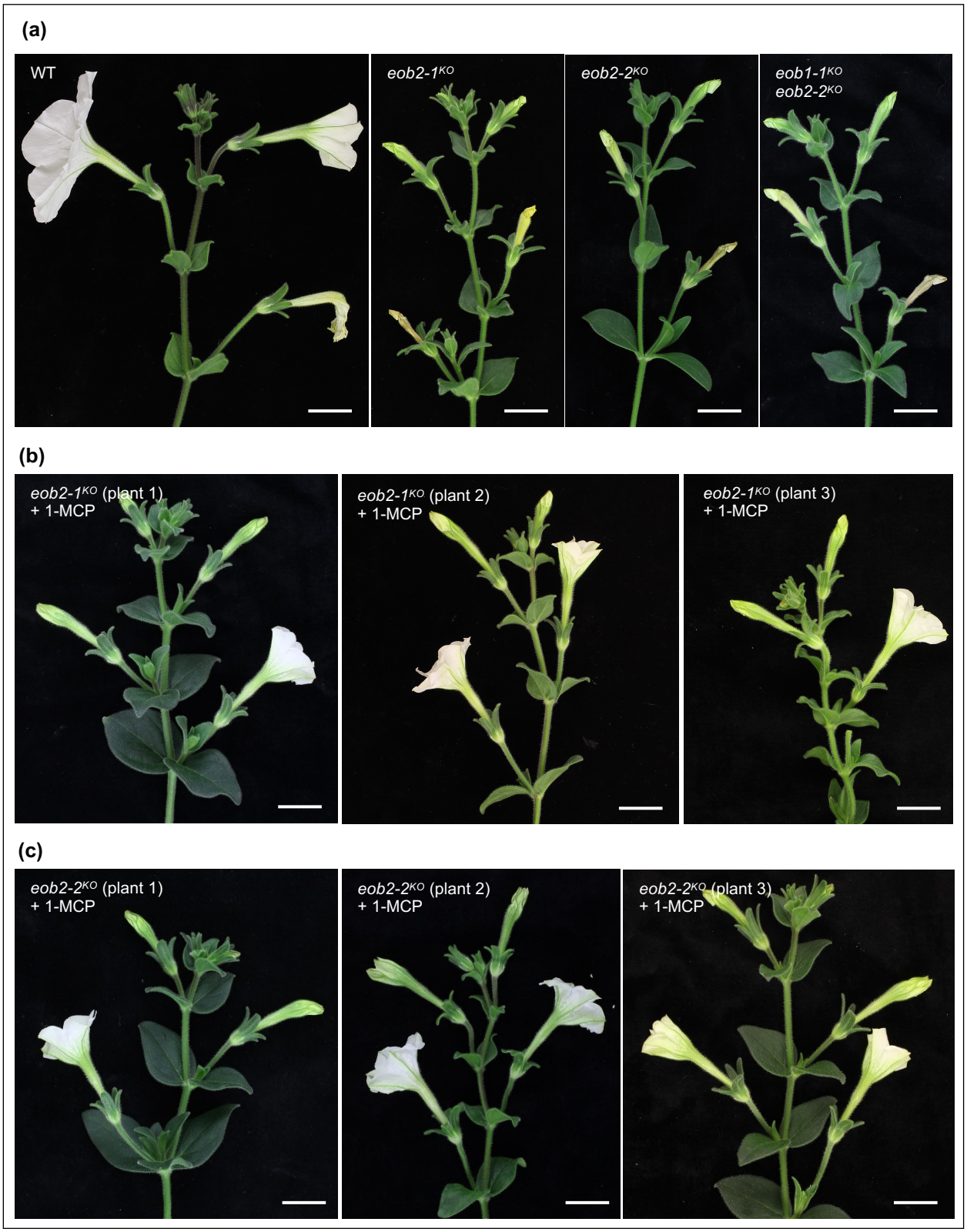


Fig. S9 Branches of WT, *eob2-1*^{KO} and *eob2-2*^{KO} before and after 1-MCP treatment.
(a) Phenotypes of untreated WT, *eob2-1*^{KO}, *eob2-2*^{KO}, and the double mutant *eob1-1*^{KO} *eob2-2*^{KO} branches. **(b)** and **(c)** Partial rescue of the *eob2-1*^{KO} **(b)** and *eob2-2*^{KO} **(c)** phenotypes on 3 independent plant branches after 1-MCP treatment. Scale bars = 2cm.

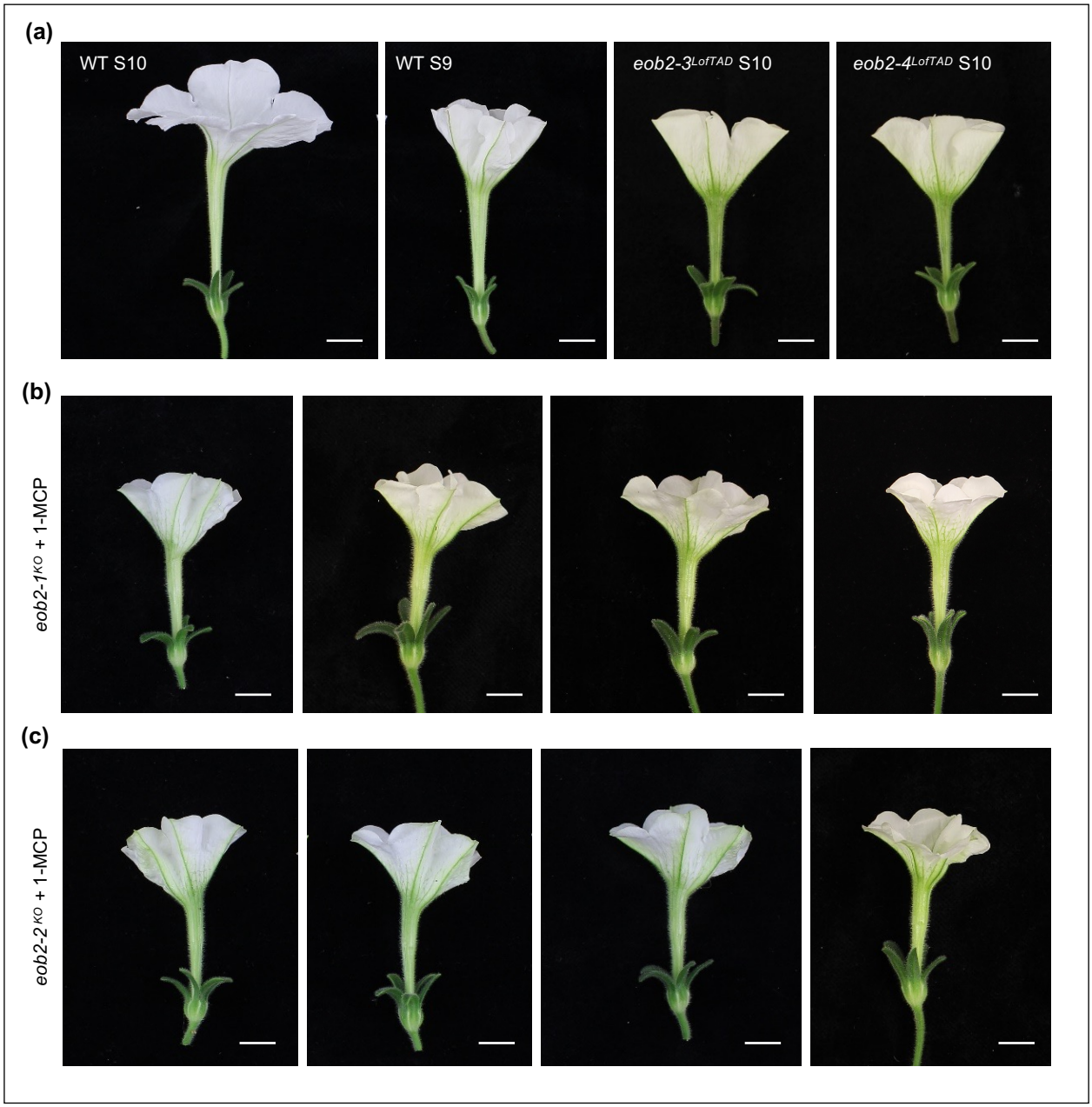


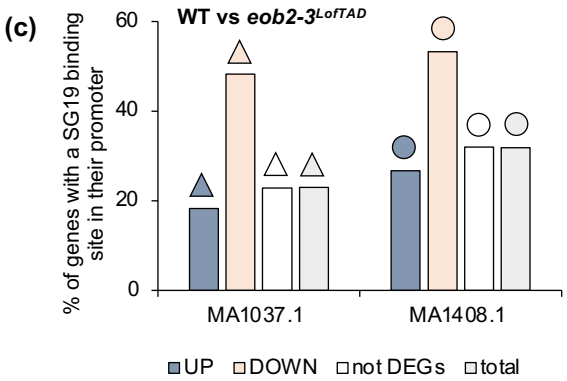
Fig. S10 *eob2-1*^{KO} and *eob2-2*^{KO} flowers after 1-MCP treatment, looked similar to *eob2-3*^{LofTAD} and *eob2-4*^{LofTAD}.
(a) Phenotype of untreated WT, *eob2-3*^{LofTAD} and *eob2-4*^{LofTAD} flowers. **(b)** and **(c)** Partial rescue of the *eob2-1*^{KO} (**b**) and *eob2-2*^{KO} (**c**) phenotypes on 4 independent flowers after 1-MCP treatment. Scale bars = 1cm.

(a) **MA1037.1** (*AtMYB24*)

	**WT vs *eob2-3*** limb S5	**WT vs *eob2-3*** nec S5	**WT vs *eob2-3*** limb S10	**WT vs *eob2-3*** nec S10	**WT vs *eob2-3*** common to the 4 conditions		**WT vs *eob2-1*** limb S5
UP-regulated *eob2-3*^{LofTAD}>WT	**19.6%** 212 over 1084	**18.1%** 179 over 989	**21.1%** 686 over 3255	**19.2%** 485 over 2523	**18.3%** 22 over 120 △	UP-regulated *eob2-1*^{KO}>WT	**21%** 944 over 4485 ✚
DOWN-regulated *eob2-3*^{LofTAD}<WT	**39.3%** 153 over 389	**35%** 292 over 834	**30.5%** 501 over 1645	**28.5%** 529 over 1854	**48.3%** 29 over 60 △	DOWN-regulated *eob2-1*^{KO}<WT	**27.4%** 867 over 3162 ✚
common not DEGs padj>0.01; -1<log2FC<1	**22.9%** 1267 over 5522 △					not DEGs padj>0.01; -1<log2FC<1	**22.9%** 1393 over 6085 ✚
total in the *Pax* genome	**23%** 7517 over 32768 △					total in the *Pax* genome	**23%** 7517 over 32768 ✚

(b) **MA1408.1** (*FaEOBII*)

	**WT vs *eob2-3*** limb S5	**WT vs *eob2-3*** nec S5	**WT vs *eob2-3*** limb S10	**WT vs *eob2-3*** nec S10	**WT vs *eob2-3*** common to the 4 conditions		**WT vs *eob2-1*** limb S5
UP-regulated *eob2-3*^{LofTAD}>WT	**28.5%** 309 over 1084	**26.3%** 260 over 989	**30.9%** 1005 over 3255	**26.8%** 677 over 2523	**26.7%** 32 over 120 ●	UP-regulated *eob2-1*^{KO}>WT	**30.9%** 1387 over 4485 ◆
DOWN-regulated *eob2-3*^{LofTAD}<WT	**40.6%** 158 over 389	**44.6%** 372 over 834	**39.4%** 648 over 1645	**39.6%** 734 over 1854	**53.3%** 32 over 60 ●	DOWN-regulated *eob2-1*^{KO}<WT	**36.7%** 1162 over 3162 ◇
common not DEGs padj>0.01; -1<log2FC<1	**32%** 1767 over 5522 ○					not DEGs padj>0.01; -1<log2FC<1	**32.3%** 1963 over 6085 ◇
total in the *Pax* genome	**31.9%** 10439 over 32768 ○					total in the *Pax* genome	**31.9%** 10439 over 32768 ◇



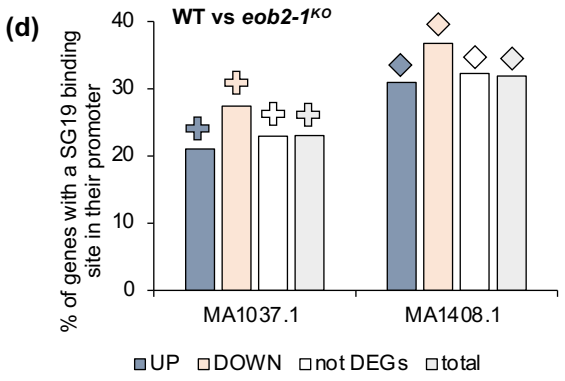


Fig. S11 Percentage of genes with a 2kb promoter containing at least one putative R2R3-MYB SG19 binding site. The presence of a R2R3-MYB SG19 binding site in 2kb promoter was predicted using FIMO and two independent SG19 predicted motifs **(a)** MA1037.1 (*AtMYB24*) and **(b)** MA1408.1 (*FaEOBII*) (P < 1e-4). The symbols indicate the percentage values used in **(c)** WT vs *eob2-3*^{LofTAD} and **(d)** WT vs *eob2-1*^{KO}.

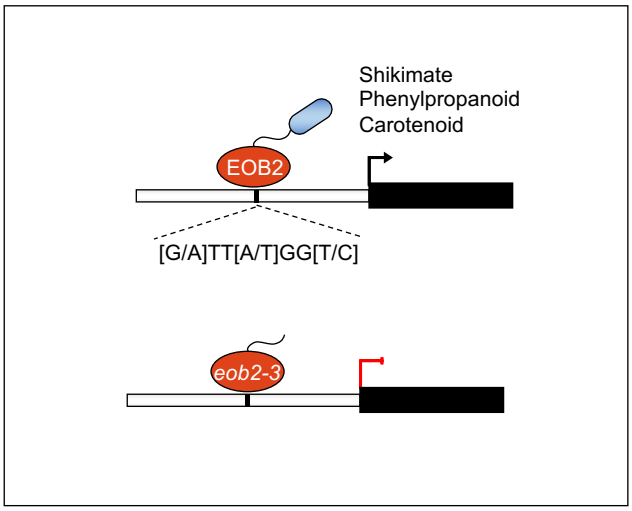


Fig. S12 Model showing the direct activation of secondary metabolite related genes by EOB2.
The promoter of secondary metabolite related genes is represented in white. The reduced expression of these genes in *eob2-3*^{LofTAD} limb and nectary tissues is most likely due to the missing TAD. Both EOB2 and eob2-3^{LofTAD} protein can bind the MYB-DNA-binding site ([G/A]TT[A/T]GG[T/C]) but only EOB2 can activate the transcription.

Organism	Protein-protein interactions	Methods	N- or C-terminus part interaction	Transcriptional function influence	Publication
Arabidopsis thaliana	JAZs* - AtMYB21 JAZs* - AtMYB24 AtMYB21 - AtMYB21 AtMYB24 - AtMYB24 AtMYB21 - AtMYB24	Y2H, LCI, BiFC	N-terminus	inhibition	Song *et al*. (2011)
	AtIIIe bHLH - AtMYB21 AtIIIe bHLH - AtMYB24	Y2H, Co-IP, BiFC	N-terminus	bHLH-MYB transcription complex	Qi *et al.* (2015)
	JAZs** - AtMYB21 JAZs** - AtMYB24 AtMYB24 - AtMYB21 AtMYB24 - AtMYB24	Y2H, LCI	N-terminus N- and C-terminus	inhibition	Huang *et al.* (2017)
	AtMYC2 - AtMYB21	Y2H, BiFC, *in vitro* pull-down	not tested	inhibition	Yang *et al.* (2020)
	DELLAs - AtMYB21 DELLAs - AtMYB24 AtJAZ1 - AtMYB21	Y2H, *in vitro* pull-down	N-terminus	inhibition	Huang *et al.* (2020)
	AtSnRK2.4 - AtMYB21	Y2H, *in vitro* pull-down, BiFC	N- and C-terminus	act together to mediate salt stress responses	Zhang *et al.* (2021)
Petunia hybrida	PhERF6 – EOB1	Y2H, BiFC, Co-IP	N-terminus	inhibition	Liu *et al.* (2017)
Solanum lycopersicum	SlJAZ9 – SlMYB21	Y2H, BiFC, splitTALE	not tested	not tested	Schubert *et al.* (2019)
Freesia hybrida	FhMYC2 - FhMYB21L2	Y2H, BiFC,	not tested	inhibition	Yang *et al.* (2020)
Hedychium coronarium	HcMYB1 - HcIAA4	Y2H, BiFC	not tested	inhibition	Ke *et al.* (2021)

Table S1 Summary of protein-protein interactions involving members of the R2R3-MYB SG19 reported in the literature. List of abbreviations: Bimolecular Fluorescence Complementation (BiFC), Yeast-two-Hybrid (Y2H), Co-Immunoprecipitation (Co-IP), Luciferase Complementation Imaging (LCI), Jasmonate zim-domain (JAZ), Ethylene Response Factor (ERF), repressors of gibberellin (DELLAs), Sucrose non-fermenting-1-related protein kinase 2 (salt tolerance, SnRK2). JAZs* = JAZ1, JAZ8, JAZ11, JAZs** = JAZ1, JAZ2, JAZ3, JAZ4, JAZ5, JAZ6, JAZ8, JAZ10, JAZ11, JAZ12, DELLAs = AtRGA, AtGAI, AtRGL1, AtRGL2, AtRGL3 and IIIe bHLH = bHLH TFs of the IIIe clade such as MYC2, MYC3, MYC4, MYC5.

Organism	Direct target gene	Methods	Publication
Arabidopsis thaliana	AtMYB21 → *PAL1, PAL2* AtMYB21 → *UGT73B2* AtMYB21 → *UGT79B6* AtMYB21 → *AtMYB24* AtMYB24 → *AtMYB21*	DL	Battat *et al.* (2019)
	AtMYB21 → *AtTPS14* AtMYB21 → *AtTPS21*	transient protoplast, EMSA	Yang *et al.* (2020)
	AtMYB21 → *AtFLS1*	Y1H, DL, ChIP, EMSA	Zhang *et al.* (2021)
Nicotiana tabacum *Antirrhinum majus*	MYB305 → *PAL2* MYB305 → *CHS*	Transactivation, EMSA	Sablowski *et al.* (1994) Moyano *et al.* (1996)
Nicotiana tabacum	MYB305 → *NEC1* MYB305 → *NEC5*	EMSA	Liu *et al.* (2009)
	MYB305 → *AGP*	indirect	Liu *et al.* (2012)
	NtMYB305a → *NtPMT1a*	Y1H, ChIP, EMSA	Bian *et al.* (2021)
Petunia hybrida	PhEOB2 → *PhPAL* PhEOB2 → *PhIGS*	Transient protoplast transfection	Spitzer-Rimon *et al.* (2010)
	PhEOB2 → *PhODO1*	Transactivation, EMSA	Moerkercke *et al.* (2011)
	PhEOB2 → *PhEOB1* PhEOB1 → *PhPAL* PhEOB1 → *PhIGS* PhEOB1 → *PhODO1*	Transient protoplast transfection, EMSA, Y1H,	Spitzer-Rimon *et al.* (2012)
Freesia vesca	FaEOB2 → *FvCAD1* FaEOB2 → *PhODO1*	Transactivation	Medina-Puche *et al.* (2015)
Freesia hybrida	FhMYB21L2 → *FhTPS1*	Transient protoplast transfection, ChIP, EMSA	Yang *et al.* (2020)
Malus domestica	MYB305 → *Machi3-1*	Expression correlation analysis	Kurilla *et al.* (2019)
Lilium longiflorum	LlMYB305 → *LlHSC70*	Y1H, DL	Wu *et al.* (2021)
Hedychium coronarium	HcMYB2 → *HcBSMT2* HcMYB1 → *HcBSMT2* HcMYB1 → *HcTPS5*	Y1H, DL	Ke *et al.* (2021)
Vitis vinifera	VvMYB24 → 20 terpene related genes (*VvTPS35)* VvMYB24 → 6 carotenoid related genes (*GGPS1, CRTISO2, LCYE)* VvMYB24 → 30 photosynthesis and light-response genes *VvHYH*	DAP-seq, DL	Zhang *et al.* (2021) (PREPRINT)
Chrysanthemum morifolium	CmMYB21 --| *CmDFR*	Y1H, DL	Wang *et al.* (2022)

Table S2 Summary of the direct target genes of members of the R2R3-MYB SG19 reported in the literature.
R2R3-MYB SG19 are prominently activators, with one exception identified in *Chrysanthenum morifolium*. List of abbreviations: Chromatin ImmunoPrecipitation (ChIP), Electrophoretic Mobility Shift Assay (EMSA), Yeast one-Hybrid (Y1H), Dual-Luciferase (DL), DNA Affinity Purification sequencing (DAP-seq). Shikimate/Phenylpropanoids/Benzaldehyde: Emission Of Benzenoids (EOB), Phenylalanine Ammonia-Lyase (PAL), Chalcone Synthase (CHS), Isoeugenol Synthase (IGS), Cinnamyl Alcohol Dehydrogenase (CAD), Odorant 1 (ODO1). Nectary related: ADP-glucose pyrophosphorylase (small subunit, AGPs), Chitinase (Machi). Terpene related: Terpene synthase (TPS). Flavonoid related: Dihydroflavonol 4-Reductase (DFR), UDP-Glucuronosyltransferase (UGT), Flavonol Synthase (FLS). Carotenoid related: Geranylgeranyl Diphosphate Synthase 1 (GGPS1), Carotenoid Isomerase (CRTISO2), Lycopene Epsilon Cyclase (LCYE). Photosynthesis and light-response genes: HY5 Homolog (HYH). Nicotine related: Putrescine *N*-Methyltransferase (PMT). Others: Heat Shock protein (HSC).

Target gene	gRNA#	gRNA sequence (from 5'-3')
EOB1 _ Peaxi162Scf00129g01231.1	gRNA1	GAAAAAGTTGTCGACTTCGG
EOB1 _ Peaxi162Scf00129g01231.1	gRNA2	CTCCGGCCAGATGTCCGGAG
EOB2 _ Peaxi162Scf00080g00064.1	gRNA3	TACTCTCCCACTTCATACTC
EOB2 _ Peaxi162Scf00080g00064.1	gRNA4	CACTTCATACTCTGGAAATG

Table S3 gRNA sequences used to target *EOB1* and *EOB2*.

Target gene	Application	Primer pair	Forward primer (from 5'-3')	Reverse primer (from 5'-3')
EOB1 _ Peaxi162Scf00129g01231.1	Genotyping	EOB1-pp1	CGACAACTTATTTGAGATTGAGACG	CACTTAGCATGCAGTTCCATAATC
EOB2 _ Peaxi162Scf00080g00064.1	Genotyping	EOB2-pp1	CAAATACATGGTGTACAGGGC	TAACCATAGGCACCTCCATG
EOB1 _ Peaxi162Scf00129g01231.1	RT-qPCR	EOB1-pp2	CAGCTCTTGATTATGGAACTGC	GTGCTTCTGTATCCTAGTCCTC
EOB2 _ Peaxi162Scf00080g00064.1	RT-qPCR	EOB2-pp2	GAGGAAAGGACCTTGGACTATG	ACCGAAGCCGACAACTTT
ODO1 _ Peaxi162Scf00002g00037.1	RT-qPCR	ODO1-pp1	TGCTTCAACCATGTCGAATTG	TCCGTGCCTGTTCTCTACGTT
TPS1 _ Peaxi162Scf00074g00143.1	RT-qPCR	TPS1-pp1	GCAACTGAAGCGCCTATGTT	TGTGTATCCATCCGCCTCTT
RAN1 _ Peaxi162Scf01372g00049.1	RT-qPCR	RAN1-pp1	AAGCTCCCACCTGTCTGGAAA	AACAGATTGCCGGAAGCCA
ACTIN11 _ Peaxi162Scf00258g00618.1	RT-qPCR	ACT11-pp1	TGCACTCCCACATGCTATCCT	TCAGCCGAAGTGGTGAAAGAG
EOB1 _ Peaxi162Scf00129g01231.1	RT-qPCR for transient	EOB1-pp3	TGTGAGCACAATGATCAACAAG	TCCAGTGTATGAAGATGGAGAATAG
EOB2 _ Peaxi162Scf00080g00064.1	RT-qPCR for transient	EOB2-pp3	GCCAAATGTCTAATGGTCCAAAT	TTAGGGCCTGCTTGGAAAG
CAS9 _ pHSE401 vector	Genotyping	CAS9-pp1	CTGCAGAATGAGAAGCTCTAC	GACGATATTCACTTGTGGCATG
EOB1 _ Peaxi162Scf00129g01231.1	Cloning	EOB1-pp4	**GGGGACAAGTTTGTACAAAAAAGCAGGCTT** **C**ATGGATAAAAGAACATGCAATTCTC	**GGGGACCACTTTGTACAAGAAAGCTGGGTC** TTAGTTGGTTGCATCATTAAGC
EOB2 _ Peaxi162Scf00080g00064.1	Cloning	EOB2-pp4	**GGGGACAAGTTTGTACAAAAAAGCAGGCTT** **C**ATGGATAAAAAACCATGCAACTCTC	**GGGGACCACTTTGTACAAGAAAGCTGGGTC** TTAATCACCATTAAGCAATTGCATG
eob2-2 _ Peaxi162Scf00080g00064.1	Cloning	eob2-2-pp1	**GGGGACAAGTTTGTACAAAAAAGCAGGCTT** **C**ATGGATAAAAAACCATGCAACTCTC	**GGGGACCACTTTGTACAAGAAAGCTGGGTC** CTAGCTAACTAGAGGCTTAACTTTTTG
eob2-3 _ Peaxi162Scf00080g00064.1	Cloning	eob2-3-pp1	**GGGGACAAGTTTGTACAAAAAAGCAGGCTT** **C**ATGGATAAAAAACCATGCAACTCTC	**GGGGACCACTTTGTACAAGAAAGCTGGGTC** TTAGGGCCTGCTTGGAAAGTG

Table S4 Sequence description of primers used for this work.
Boldface: recombination site for cloning

Samples	Genotype	Stage	Tissue
Sample 1	*eob2-1*	S5 (flower bud of ~3.5 cm, just before dark)	3 limbs
Sample 2	*eob2-1*	S5 (flower bud of ~3.5 cm, just before dark)	3 limbs
Sample 3	*eob2-1*	S5 (flower bud of ~3.5 cm, just before dark)	3 limbs
Sample 4	*eob2-3*	S5 (flower bud of ~3.5 cm, just before dark)	3 limbs
Sample 5	*eob2-3*	S5 (flower bud of ~3.5 cm, just before dark)	3 limbs
Sample 6	*eob2-3*	S5 (flower bud of ~3.5 cm, just before dark)	3 limbs
Sample 7	PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	3 limbs
Sample 8	PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	3 limbs
Sample 9	PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	3 limbs
Sample 10	*eob2-3*	S7 (flower bud of ~5.5 cm, just before dark)	2 limbs
Sample 11	*eob2-3*	S7 (flower bud of ~5.5 cm, just before dark)	2 limbs
Sample 12	*eob2-3*	S7 (flower bud of ~5.5 cm, just before dark)	2 limbs
Sample 13	PaxP WT	S7 (flower bud of ~5.5 cm, just before dark)	2 limbs
Sample 14	PaxP WT	S7 (flower bud of ~5.5 cm, just before dark)	2 limbs
Sample 15	PaxP WT	S7 (flower bud of ~5.5 cm, just before dark)	2 limbs
Sample 16	*eob2-3*	S10 (open flower; 1 DPA, just before dark)	1 limb
Sample 17	*eob2-3*	S10 (open flower; 1 DPA, just before dark)	1 limb
Sample 18	*eob2-3*	S10 (open flower; 1 DPA, just before dark)	1 limb
Sample 19	PaxP WT	S10 (open flower; 1 DPA, just before dark)	1 limb
Sample 20	PaxP WT	S10 (open flower; 1 DPA, just before dark)	1 limb
Sample 21	PaxP WT	S10 (open flower; 1 DPA, just before dark)	1 limb
Sample 25	*eob2-3*	S5 (flower bud of ~3.5 cm, just before dark)	5 basal ovaries
Sample 26	*eob2-3*	S5 (flower bud of ~3.5 cm, just before dark)	5 basal ovaries
Sample 27	*eob2-3*	S5 (flower bud of ~3.5 cm, just before dark)	5 basal ovaries
Sample 28	PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	5 basal ovaries
Sample 29	PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	5 basal ovaries
Sample 30	PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	5 basal ovaries
Sample 31	*eob2-3*	S10 (open flower; 1 DPA, just before dark)	5 basal ovaries
Sample 32	*eob2-3*	S10 (open flower; 1 DPA, just before dark)	5 basal ovaries
Sample 33	*eob2-3*	S10 (open flower; 1 DPA, just before dark)	5 basal ovaries
Sample 34	PaxP WT	S10 (open flower; 1 DPA, just before dark)	5 basal ovaries
Sample 35	PaxP WT	S10 (open flower; 1 DPA, just before dark)	5 basal ovaries
Sample 36	PaxP WT	S10 (open flower; 1 DPA, just before dark)	5 basal ovaries

Table S5 Description of the samples collected for the RNA sequencing experiment.
Day post anthesis (DPA)

Genotype	Stage	Tissues	Starch	Carotenoids
eob2-3	S5 (flower bud of ~3.5 cm, just before dark)	5 limbs	yes	no
eob2-3	S5 (flower bud of ~3.5 cm, just before dark)	5 limbs	yes	no
eob2-3	S5 (flower bud of ~3.5 cm, just before dark)	5 limbs	yes	no
eob2-3	S10 (open flower; 1 DPA, just before dark)	3 limbs	yes	no
eob2-3	S10 (open flower; 1 DPA, just before dark)	3 limbs	yes	no
eob2-3	S10 (open flower; 1 DPA, just before dark)	3 limbs	yes	no
PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	5 limbs	yes	no
PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	5 limbs	yes	no
PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	5 limbs	yes	no
PaxP WT	S10 (open flower; 1 DPA, just before dark)	3 limbs	yes	no
PaxP WT	S10 (open flower; 1 DPA, just before dark)	3 limbs	yes	no
PaxP WT	S10 (open flower; 1 DPA, just before dark)	3 limbs	yes	no
eob2-3	S5 (flower bud of ~3.5 cm, just before dark)	~67 basal ovaries	yes	no
eob2-3	S5 (flower bud of ~3.5 cm, just before dark)	~67 basal ovaries	yes	no
eob2-3	S5 (flower bud of ~3.5 cm, just before dark)	~67 basal ovaries	yes	no
eob2-3	S10 (open flower; 1 DPA, just before dark)	~55 basal ovaries	yes	yes
eob2-3	S10 (open flower; 1 DPA, just before dark)	~55 basal ovaries	yes	yes
eob2-3	S10 (open flower; 1 DPA, just before dark)	~55 basal ovaries	yes	yes
PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	~40 basal ovaries	yes	no
PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	~40 basal ovaries	yes	no
PaxP WT	S5 (flower bud of ~3.5 cm, just before dark)	~40 basal ovaries	yes	no
PaxP WT	S10 (open flower; 1 DPA, just before dark)	~45 basal ovaries	yes	yes
PaxP WT	S10 (open flower; 1 DPA, just before dark)	~45 basal ovaries	yes	yes
PaxP WT	S10 (open flower; 1 DPA, just before dark)	~45 basal ovaries	yes	yes

Table S6 Description of the samples collected for the starch and carotenoid measurements. Day post anthesis (DPA). yes and no indicate which coumpounds were measured.

Alleles		**T0 plants**	**x**			**T1 plant selected**	**x**			**Final homozygous mutant lines**	
eob1-1 (-31bp)	>	PaxP background *eob1-1*/*EOB1* CAS9 positive	x	PaxP wild-type	>	PaxP background *eob1-1*/*EOB1* CAS9 negative	x	Self	>	PaxP background *eob1-1*/*eob1-1* CAS9 negative	
eob2-1 (-364bp)	>	PaxP background *eob2-1*/*EOB2* CAS9 positive	x	PaxP wild-type	>	PaxP background *eob2-1*/*EOB2* CAS9 negative	x	Self	>	PaxP background *eob2-1*/*eob2-1* CAS9 negative	maintained by cuttings
eob2-2 (-7bp)	>	PaxP background *eob2-2*/*eob2-4* CAS9 positive	x	PaxP wild-type	>	PaxP background *eob2-2*/*EOB2* CAS9 negative	x	Self	>	PaxP background *eob2-2*/*eob2-2* CAS9 negative	maintained by cuttings
eob2-3 (-5bp)	>	PaxP background *eob2-3*/*EOB2* CAS9 positive	x	PaxP wild-type	>	PaxP background *eob2-3*/*EOB2* CAS9 negative	x	Self	>	PaxP background *eob2-3*/*eob2-3* CAS9 negative	
eob2-4 (-2bp)	>	PaxP background *eob2-2*/*eob2-4* CAS9 positive	x	PaxP wild-type	>	PaxP background *eob2-4*/EOB2 CAS9 negative	x	Self	>	PaxP background *eob2-4*/*eob2-4* CAS9 negative	
eob1-1 (-31bp) *eob2-2* (-7bp)	>	PaxP background *eob1-1*/*EOB1* CAS9 positive	x	PaxP background *eob2-2*/*eob2-4* CAS9 positive	>	PaxP background *eob1-1*/*EOB1* *eob2-2*/*EOB2* CAS9 negative	x	Self	>	PaxP background *eob1-1*/*eob1-1* *eob2-2*/*eob2-2* CAS9 negative	maintained by cuttings

Table S7 From the T0 transgenic lines obtained by CRISPR-Cas9 to the final homozygous mutant lines used in this study.

comparison	motif	regulation	with_ regulated	without_ regulated	with	without	fraction	expected_ fraction	p_value	p_adjusted	log10_ padj	fraction_diff
WT vs eob2-3 _ limb S5	MA1408.1	up	309	775	10439	22329	0.285055351	0.318572998	0.016958118	0.020349742	1.69	-0.033517647
WT vs eob2-3 _ nec S5	MA1408.1	up	260	729	10439	22329	0.26289181	0.318572998	0.000117851	0.000217572	3.66	-0.055681188
WT vs eob2-3 _ limb S10	MA1408.1	up	1005	2250	10439	22329	0.30875576	0.318572998	0.211787372	0.23104077	0.64	-0.009817238
WT vs eob2-3 _ nec S10	MA1408.1	up	677	1846	10439	22329	0.268331352	0.318572998	1.20842E-08	2.90021E-08	7.54	-0.050241646
common to the 4 conditions	MA1408.1	up	32	88	10439	22329	0.266666667	0.318572998	0.239775753	0.250200785	0.6	-0.051906331
WT vs eob2-1 _ limb S5	MA1408.1	up	1387	3098	10439	22329	0.309253066	0.318572998	0.15226853	0.174021177	0.76	-0.009319932
WT vs eob2-3 _ limb S5	MA1408.1	down	158	231	10439	22329	0.406169666	0.318572998	0.000290813	0.0004653	3.33	0.087596668
WT vs eob2-3 _ nec S5	MA1408.1	down	372	462	10439	22329	0.446043165	0.318572998	8.07268E-15	9.68722E-14	13.01	0.127470167
WT vs eob2-3 _ limb S10	MA1408.1	down	648	997	10439	22329	0.393920973	0.318572998	3.80814E-11	1.52325E-10	9.82	0.075347975
WT vs eob2-3 _ nec S10	MA1408.1	down	734	1120	10439	22329	0.395900755	0.318572998	4.95218E-13	2.64087E-12	11.58	0.077327757
common to the 4 conditions	MA1408.1	down	32	28	10439	22329	0.533333333	0.318572998	0.000711522	0.001067283	2.97	0.214760335
WT vs eob2-1 _ limb S5	MA1408.1	down	1162	2000	10439	22329	0.367488931	0.318572998	8.82443E-10	2.64733E-09	8.58	0.048915933
comparison	motif	regulation	with_ regulated	without_ regulated	with	without	fraction	expected_ fraction	p_value	p_adjusted	log10_ padj	fraction_diff
WT vs eob2-3 _ limb S5	MA1037.1	up	212	872	7517	25251	0.195571956	0.229400635	0.006554535	0.00873938	2.06	-0.033828679
WT vs eob2-3 _ nec S5	MA1037.1	up	179	810	7517	25251	0.1809909	0.229400635	0.00019159	0.000328441	3.48	-0.048409735
WT vs eob2-3 _ limb S10	MA1037.1	up	686	2569	7517	25251	0.210752688	0.229400635	0.007373347	0.009313701	2.03	-0.018647947
WT vs eob2-3 _ nec S10	MA1037.1	up	485	2038	7517	25251	0.19223147	0.229400635	2.78724E-06	6.08124E-06	5.22	-0.037169164
common to the 4 conditions	MA1037.1	up	22	98	7517	25251	0.183333333	0.229400635	0.276273132	0.276273132	0.56	-0.046067301
WT vs eob2-1 _ limb S5	MA1037.1	up	944	3541	7517	25251	0.210479376	0.229400635	0.001154161	0.001629404	2.79	-0.018921259
WT vs eob2-3 _ limb S5	MA1037.1	down	153	236	7517	25251	0.393316195	0.229400635	3.77189E-13	2.64087E-12	11.58	0.163915561
WT vs eob2-3 _ nec S5	MA1037.1	down	292	542	7517	25251	0.350119904	0.229400635	1.34272E-15	3.22252E-14	13.49	0.120719269
WT vs eob2-3 _ limb S10	MA1037.1	down	501	1144	7517	25251	0.304559271	0.229400635	5.50182E-13	2.64087E-12	11.58	0.075158636
WT vs eob2-3 _ nec S10	MA1037.1	down	529	1325	7517	25251	0.285329018	0.229400635	8.71986E-09	2.32529E-08	7.63	0.055928384
common to the 4 conditions	MA1037.1	down	29	31	7517	25251	0.483333333	0.229400635	1.72634E-05	3.45268E-05	4.46	0.253932699
WT vs eob2-1 _ limb S5	MA1037.1	down	867	2295	7517	25251	0.274193548	0.229400635	5.96085E-10	2.04372E-09	8.69	0.044792914

Table S8 Fraction of genes with a 2kb promoter containing at least one putative R2R3-MYB SG19 binding site.
The presence of a R2R3-MYB SG19 binding site in 2kb promoter was predicted using FIMO and two independent SG19 predicted motifs MA1408.1 (*FaEOBII*) and MA1037.1 (*AtMYB24*) (P < 1e-4). “with-regulated” is for up or down regulated genes with a motif in the promoter and “with” is for the total number of genes identified in Petunia (32,768) with a motif in the promoter.

	Gene ID _ Figure 5C
ACS #1	Peaxi162Scf00074g01725.1
ACS #2	Peaxi162Scf00620g00121.1
ACS #3	Peaxi162Scf00102g01634.1
ACS #4	Peaxi162Scf00381g00219.1
ACS #5	Peaxi162Scf00192g00920.1
ACS #6	Peaxi162Scf00102g01343.1
ACS #7	Peaxi162Scf00096g01846.1
ACS #8	Peaxi162Scf00822g00212.1
	Gene ID _ Figure 5C
ACO #1	Peaxi162Scf00521g00613.1
ACO #2	Peaxi162Scf00294g00812.1
ACO #3	Peaxi162Scf01096g00025.1
ACO #4	Peaxi162Scf00047g01927.1
ACO #5	Peaxi162Scf01333g00015.1
ACO #6	Peaxi162Scf01333g00016.1
	Gene ID _ Figure 6C
EOB1	Peaxi162Scf00129g01231.1
CM1	Peaxi162Scf00166g00931.1
BSMT1	Peaxi162Scf00047g01123.1
BSMT2	Peaxi162Scf00047g00116.1
EGS	Peaxi162Scf00020g01714.1
IGS1	Peaxi162Scf00889g00229.1
IGS3	Peaxi162Scf00185g01622.1
Z-ISO	Peaxi162Scf00378g00631.1
ZDS	Peaxi162Scf00404g00021.1
LCY B	Peaxi162Scf00091g00064.1
CCD	Peaxi162Scf00953g00316.1
	Gene ID _ Figure 7B
BAM	Peaxi162Scf00715g00216.1

Table S9 Gene ID of genes used in this study.
Gene IDs of the eight *1-Aminocyclopropane-1-Carboxylic Acid Synthase* (*ACS*) genes and six *1-Aminocyclopropane-1-Carboxylic Acid Oxidase* (*ACO*) genes used in Figure 5C, eleven genes used in Figure 6C and one *BETA-AMYLASE* (*BAM*) gene used in Figure 7B.

Method S1 Quantification of sesquiterpene accumulation in Petunia pistils and emission from tube by GC-MS.

Samples were analyzed on an Agilent 9000 Intuvo gas chromatograph system (Agilent Technologies), connected to an Agilent 5977B mass detector (Agilent Technologies). 2 µL of samples were injected in a split/splitless injector set at 250°C with a 2-fold split. Temperature of the guard chip was set to 75°C at the beginning of the run before following the temperature of the oven in track oven mode. Bus temperature was set at 280°C. Samples were analyzed on a HP-5MS-UI column (30 m x 0.25 mm x 0.25 µm) (Agilent Technologies) using a program consisting of 1 min at 50°C, followed by 20°C.min-1 to 310°C, then 4 min at 310°C, with helium as carrier gas set at 1.2 mL.min-1. Detector was set at 250 °C, ionization energy was set at 70 eV and analysis was realized in scan mode for analysis of sesquiterpene accumulation in pistils (Mass spectra scanned from 30 to 300 amu) and in single ion monitoring mode for analysis of sesquiterpene emission from tubes using m/z 161 to quantify sesquiterpenes and m/z 95 to quantify IS. Products were identified based on their retention times and electron ionization mass spectra compared to those of authentic standards (Germacrene D) or those present in the NIST2017 and WILEY libraries. Quantification of compounds was performed using the Mass Hunter quantitative software (Agilent Technologies) using response factors of authentic germacrene D relative to the IS and normalized to the weight of tissues.

Method S2 Carotenoid extraction and quantification with an HPLC system.

Tissues were ground using a ball mill (Retch MM400, Retch). Extraction was performed for 15 min in microcentrifuge tubes containing 400 ul 80% acetone in darkness under slight agitation (50 rpm). After this, samples were centrifuged in a microcentrifuge for 7 min at max speed. 100 ul of the supernatant was transferred to an HPLC tube with insert. Pigments were separated with a YMC carotenoid S-5 µm column (250 x 2.1 mm; YMC Europe GMBH). Pigments were eluted using 100% solvent A (Methanol: Methyl tert-butyl ether: H2O, 90:6:4) for the first 12 min followed by an 84 min linear gradient to 100% solvent B (MeOH/MTBE/H20 (25:71:4)). The column temperature was 35 °C, the flow rate 0.2 ml/min. The pigments were detected by their absorbance at 480 nm. Standards were used to identify carotenoids.

Method S3 Starch extraction and quantification with an HPLC system.

After extraction, tubes were centrifuged at 25k x *g* for 5 min. For starch quantitation, the pellet was washed three times with 80% ethanol. The pellet was vacuum dried, and starch was catalyzed to glucose by a 2-step enzymatic reaction. For the first step, the pellet was resuspended in 0.8 mL α-amylase solution (1 mg/ml in water, Rohalase® A3 from *Bacillus subtilis*, 44 U/mg, Serva) and incubated for 30 min at 90 °C while agitating. Subsequently, 0.4 ml amyl-glucosidase solution (0.5 mg/ml in 50 mM citrate buffer, pH 4.6, from *Aspergillus Niger*, 70U/mg, Sigma) was added, shortly mixed by vortex, and incubated for 10 min at 60 °C under agitation. Finally, samples were centrifuged, the supernatant was transferred to a new tube and diluted 20-fold. Glucose levels were analyzed with the HPLC, as described in **Method S2**, this time eluted with 100 mM NaOH + 25 mM sodium acetate instead of only 100 mM NaOH.