SUPPORTING INFORMATION

SI Materials and Methods

Generation of DNA constructs. The promoter sequences of *G8O* (532 bp) and *STR1* (587 bp) have been published (1, 2). A draft of the *C. roseus* genome sequence (3) was used to obtain the promoter sequences (including the 5' untranslated region (UTR)) of *GES* (1633 bp), *8HGO* (627 bp), *IS* (1497 bp), *IO* (1061 bp), *7DLGT* (1931 bp), *7DLH* (1164 bp), and *LAMT (*963 bp). Promoter sequences were isolated by polymerase chain reaction (PCR) from genomic DNA (prepared from *C. roseus* seedlings or suspension cells) with primers that introduce attB sites (Table S4). All PCR products were gel-purified (ThermoScientific) and recombined into pDONR207. The ENTRY clones were sequenced and recombined into the destination vectors pGWL7 (all promoters) and pKGWL7 (p*G8O* and p*STR1*) to create high-copy and binary *firefly luciferase* (*fLUC*) reporter construct plasmids, respectively (4). For the *C. roseus* cell bombardment experiments, p*G8O* was PCR-amplified thereby introducing *Xba*I/*Nco*I sites (Table S4). The PCR product was digested and *Xba*I/*Nco*I ligated into plasmid pGusSH (5). The full-length open reading frame (FL-ORF) sequence of *BIS1* (*Caros001862*) was retrieved from ORCAE/CathaCyc (6) and was available for MYC2 and ORCA3 (7, 8.). The FL-ORFs of *BIS1*, *MYC2,* and *ORCA3* were PCR-amplified from *C. roseus* cDNA with primers that introduce attB sites (Table S4). ENTRY clones were sequenced and recombined into the destination vectors p2GW7 and pK7WG2D to create high-copy and binary overexpression construct plasmids, respectively (4).

For the VIGS silencing construct, we targeted both UTRs of *BIS1*. Primers were used to amplify the 5'-UTR and 3'-UTR by PCR and to introduce partial attB sites and an *Xba*I site (Table S4). Both fragments were ligated with *Xba*I and FL attB sites were introduced by PCR.

The fragment was recombined into pDONR207 (Invitrogen). The creation of the *chelatase subunit H* (*ChlH*) construct has been described previously (9). The respective entry vectors were sequenced and subsequently recombined with pTRV2-GW (ABRC9080; YL279).

All binary plasmids were transferred to *Agrobacterium tumefaciens* GV3101 (pMP90) and *A. rhizogenes* LBA 9402/12 for *N. benthamiana* agroinfiltration and *C. roseus* VIGS, and for transformation of *C. roseus* hairy roots, respectively. High-copy plasmids were directly used for transfection of *Nicotiana tabacum* protoplasts.

Phylogenetic analysis. The amino acid sequences of the bHLH domains of the *Arabidopsis thaliana* bHLH proteins within clade III and IV as defined (10) and those of BIS1 and MYC2 were aligned with ClustalW. A Neighbor-joining tree was constructed in MEGA5 by means of the Jones, Taylor, and Thorton (JTT) amino acid substitution model (11). Gaps that prevailed in the loop region were dealt with by complete deletion of the corresponding sites. A bootstrap analysis was carried out with 10,000 replicates and an unrooted tree was generated.

Transient expression assays. Transient expression assays in protoplast cells prepared from Bright Yellow-2 (BY-2) *N. tabacum* suspension cultured cells were performed as described (12). The reporter plasmid contained a firefly luciferase (*fLUC*) gene under control of the isolated *C. roseus* promoters. *C. roseus* TF genes were expressed under control of the *pCaMV35S* promoter in effector plasmids. Reporter activities in cells transfected with TF effector plasmids were compared with those of cells transfected with a *pCaMV35S:GUS* control effector plasmid, the value of which was set to 1 to obtain fold-induction caused by the *pCaMV35S:TF* constructs. Plasmids with the Renilla luciferase (*rLUC*) expressed under control of the *pCaMV35S* promoter were cotransfected in each sample and served for

normalization of the transfection efficiency. Protoplast cells were transfected with 2 µg of each plasmid by means of the polyethylene glycol (PEG)/Ca^{2+} method and grown overnight in the dark at room temperature with gentle agitation. After lysis of the cells, the luciferase activities were measured with the Dual-Luciferase® Reporter Assay System (Promega). All transient expression assays in *N. tabacum* protoplasts were conducted in eight biological repeats.

N. benthamiana leaf infiltration assays in nonflowering plants (grown with a 16-h/8-h day/night regime at 21°C) were performed as previously described with minor modifications (13). Following infiltration, analyses were done with the Dual-Luciferase® Reporter System (Promega), in which *rLUC* is used to normalize the fLUC enzyme activity. Four leaf discs per infiltration were homogenized and proteins were extracted in 150 µl cell culture lysis reagent (CCLR; Promega) by vortexing, freeze-thawing twice, and centrifugation. Of the supernatant, 70 µl was analyzed with the Dual-Luciferase® Reporter System, as for the protoplast assays. An effector plasmid with a *pCaMV35S:GFP* construct was used as control and this value was set to 1 to obtain fold-induction caused by the *pCaMV35S:TF* constructs. All transient expression assays in *N. benthamiana* leaves were conducted in eight biological repeats and as in the protoplast assays, a *pCaMV35S:rLUC* construct was cotransformed to enable normalization.

Cell line MP183L was plated on paper filter discs placed on Linsmaier and Skoog medium containing 30 g/l sucrose, 2 mg/l 1-naphthaleneacetic acid, and 0.2 mg/l kinetin (LS-13 medium) solidified with 0.7% agar and was transformed by particle bombardment as described (14) by means of a home-made helium gun and 1.8-µm tungsten particles (Pioneer Hi-Bred). Cells were cobombarded in triplicate with 2 µg of a reporter construct and 8 µg of effector plasmids. GUS activities were determined as described (7) and normalized against protein concentrations that were measured with Bradford protein assay reagent (BioRad).

Generation and metabolite profiling of transformed *C. roseus* suspension cells. *C. roseus* cell line MP183L was transformed by cobombardment of a TF plasmid or the empty overexpression plasmid as a control with a plasmid carrying a hygromycin resistance gene as described (14). Individual calli resistant to 50 mg/l hygromycin B were converted to transgenic cell suspensions maintained weekly in LS-13 medium supplemented with hygromycin. Cell lines were screened for high expression levels of the cotransformed TF by means of RNA-blot analysis.

C. roseus cells from three biological repeats of each selected independent transgenic line were harvested 5 days after transfer, frozen in liquid nitrogen, and freeze-dried. Of the dried cell mass, 100 mg was extracted in two steps with 10 ml methanol and centrifuged. The dried supernatant was resuspended in 1 M phosphoric acid and filtered. Extracts were analyzed by high-performance liquid chromatography (Agilent Technologies) For the (seco-)iridoids, tryptophan, and tryptamine, a Zorbax eclipse XDB-C18 (4.6 × 250 mm 5-µm column) was used (Agilent Technologies) with a flow rate of 1.5 ml/minutes and a 50-µl injection volume with solvents: A (5 mM disodiumhydrophosphate) and B (acetonitrile). MIAs were analyzed with a Luna C18(2) 4.6 × 150 mm 5-µm Axia packed column (Phenomenex) with a flow rate of 1.5 ml/minute and a 25 µl injection volume with the solvents A (0.1% trifluoroacetic acid [TFA] in H_2O) and B (acetonitrile with 0.1% TFA). Seco-iridoids and MIAs were identified based on comparison of retention times and UV spectra to those of pure standard compounds. Calibration lines for the detected compounds were made using pure standards, with a correction for strictosidine, which was only 13.76% pure as determined by ^{1}H-NMR in methanol-d_4 (99.80%; Cambridge Isotope Laboratories) containing 0.088 mM tetramethylsilane as an internal quantification reference standard.

Generation and metabolite profiling of transformed *C. roseus* hairy roots. Hairy roots were initiated by infecting axenically grown *C. roseus* (Würzburg) leaves (15) with 2-day-old *A. rhizogenes* carrying the respective constructs. After incubation for 48 h on Murashige and Skoog medium containing 50 µM acetosyringone, the leaves were transferred to solid modified Gamborg B5 medium supplemented with 500 ppm cefotaxime (Duchefa) and 10 ppm meronem (Astra Zeneca) to eliminate *Agrobacterium*. Well-growing root clones were individually subcultured on the same medium without antibiotics. The transgenic nature of the resulting root clones and the absence of *Agrobacterium* were confirmed by PCR. Samples for further analysis were taken 3 weeks after inoculation of fresh plates and immediately shock-frozen in liquid nitrogen.

Pulverized freeze-dried roots (12.2-51.9 mg) were spiked with 20 µl of 2,4 dipyridyl and mixed with 2 ml of MilliQ water in glass tubes. The samples were alkalinized with 75 µl of 10% ammonia and after 1 h incubation, they were vortexed and mixed with 2 ml of dichloromethane (DCM) for 30 min. The samples were centrifuged and extracted again. The separated DCM layers were combined and evaporated to dryness under nitrogen flow and the residue was dissolved into 250 µl DCM.

The roots were analyzed with a 7890A gas chromatograph (Agilent Technologies) combined with a 5975C MSD and a Gerstel MPS autosampler. The runs were done on an Rtx-5ms column (15 m, 0.25 mm, 0.25 µm; Restek). The column oven was programmed from 70°C (1 min) to 270°C (14 min) at 10°C/min. The total run time was 35 min. Helium was used as carrier gas and the split ratio was 1:25. The data were collected within a range of 40 to 800 amu and the EM voltage was 1.306 kV. The injection volume was 2 µl and every second run was a solvent run. The injector temperature was 250°C and the MSD transfer line 240°C.

Identification was based on retention times and mass fragmentation of reference compounds or on comparison to library spectra found in the Nist 08 or Wiley libraries. The

similarity indices varied from 91 to 99 and from 757 to 967 when compared to Wiley and Nist 08 libraries, respectively.

Virus-induced gene silencing (VIGS) in *C. roseus* plants. Virus-induced gene silencing was done essentially as described (16). Plant material was harvested approximately 21 days after pinching, *i.e.* after appearance of leaf bleaching in control lines that had been silenced for *Protoporphyrin IX magnesium chelatase subunit H* (*ChlH*). As a control, plants were infected with an empty vector (EV) construct. Ten plants per construct were used and the harvested material was split in two for subsequent metabolite and transcript profiling. Metabolite profiling was done as described previously (9).

Transcript profiling. *C. roseus* RNA and cDNA were prepared with the RNeasy mini kit (Qiagen) and iScript cDNA synthesis kit (BioRad), respectively, according to the manufacturers' instructions. One µg of DNase-treated total RNA was used for cDNA synthesis. Primers for quantitative PCR (qPCR) were designed by means of the Primer3 software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi) (Table S4). *N2227* and *SAND* were used as reference genes for *C. roseus* (17). qPCR was done with a Lightcycler 480 (Roche) with SYBR Green QPCR master Mix (Stratagene). Reactions were done in triplicate and qBase was used to quantify the relative expression (18).

For the tissue-specific expression analysis, the samples were generated as follows. Whole stem tissue was collected between the mature leaves of greenhouse grown plants. Stem epidermis enriched tissue and peeled stems (pith) were obtained by peeling mature stems with a potato peeler. Central leaf vein tissue was cut out from leaves with a scalpel. To obtain veinless leaf tissue from leaves, the central vein was removed from the leaf, then the tissue between the secondary veins was cut out with a scalpel. For every biological replicate (n=3),

tissues from 5-7 leaves or stems from different plants were pooled together. All stem and leaf tissue samples were snap-frozen in liquid nitrogen and total RNA was extracted as described above.

For RNA-blot analysis, total RNA was extracted from frozen cells by hot phenol/chloroform extraction followed by overnight precipitation with 2 M lithium chloride and two washes with 70% (v/v) ethanol, and resuspended in water. Ten-µg RNA samples were subjected to electrophoresis on 1.5% (w/v) agarose/1% (v/v) formaldehyde gels and blotted onto Genescreen nylon membranes (Perkin-Elmer Life Sciences). Probes were ^{32}P-labeled by random priming. (Pre-)hybridization and subsequent washings of blots were previously described (19) with minor modifications.

RNA (50 bp, single-end) was sequenced with an Illumina HiSeq2500 by GATC Biotech (http://www.gatc-biotech.com/). The raw RNA-Seq reads were quality-trimmed, mapped on the 'artificial *C. roseus* genome' (6) with TopHat (version 2.0.6) (20) and uniquely mapped reads were counted and fragments per kilobase of exon per million fragments mapped (FPKM) values were calculated with Cufflinks (version 2.2.1) (21) with default parameters as described (22). The multiple experiment viewer (MeV) software was used for average linkage hierarchical clustering with Pearson correlation. FPKM values were first normalized against control samples as indicated (see Figure legends) and LOG-transformed.

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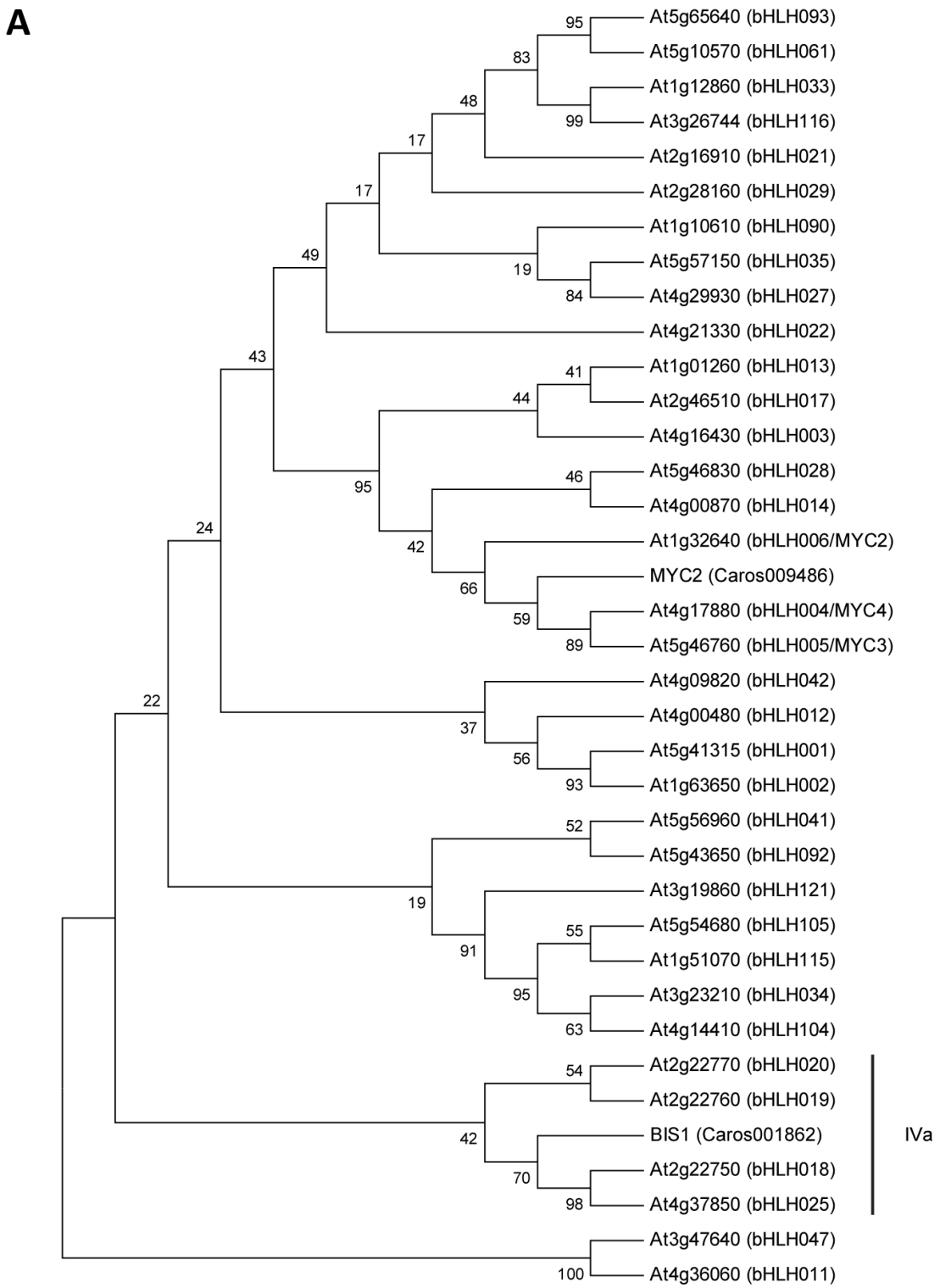
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MYC2 (Caros009486)
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BIS1 (Caros001862)
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B

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BIS1 (Caros001862)         QTYDHIIAERKRREQLSQHFVALSAIVPGLK--KMDKTSVLGDAITYLK-HMQERVKSL
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MYC2 (Caros009486)         EPLNHVEAERQRREKLNQRFYALRAVVPNVS--KMDKASLLGDAISYIN-ELKAKLQTT
At1g32640 (bHLH006/MYC2)   EPLNHVEAERQRREKLNQRFYALRAVVPNVS--KMDKASLLGDAIAYIN-ELKSKVVKT
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At4g16430 (bHLH003)        EALNHVEAERQRREKLNQRFYALRAVVPNIS--KMDKASLLADAITYIT-DMQKKIRVY
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Fig. S1. Phylogenetic analysis of *C. roseus* BIS1 and MYC2. (*A*) A bootstrap consensus tree for *C. roseus* BIS1 and MYC2 and the *Arabidopsis* bHLH proteins of clades III and IV (as defined) (1) was constructed in MEGA5. The Neighbor-joining clustering method with the Jones, Taylor, and Thorton (JTT) amino acid substitution model was used and a bootstrap analysis was carried out with 10,000 replicates as described (*SI Materials and Methods*). Bootstrap confidence values are shown at the nodes. (B) ClustalW multiple alignment of bHLH domains of the bHLH proteins within *Arabidopsis* clades III and IV and *C. roseus* BIS1 and MYC2. The *Arabidopsis* bHLH sequences were obtained from the online TAIR database (http://www.arabidopsis.org).

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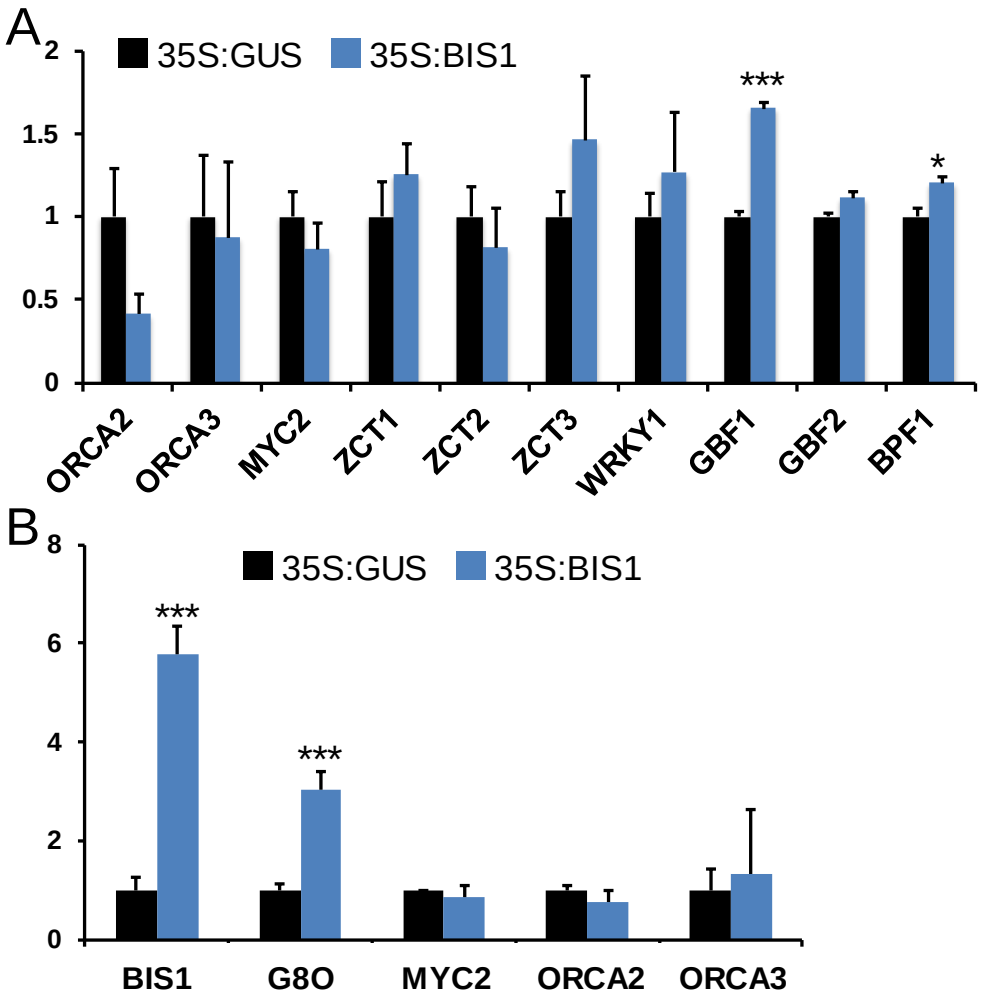

Fig. S2. Expression profiling of TF encoding genes in *BIS1*-overexpressing *C. roseus* hairy roots. (A) Genome-wide expression profiling by RNA-Seq analysis. Effect on expression of selected TF encoding genes indicated as fold induction relative to the control (35S:GUS) lines set at 1. (B) Quantitative PCR (qPCR) analysis showing TF gene expression relative to the control lines set at 1. The error bars designate SE of the mean (n=3). Statistical significance was determined by the Student's *t*-test (*** $P < 0.0005$).

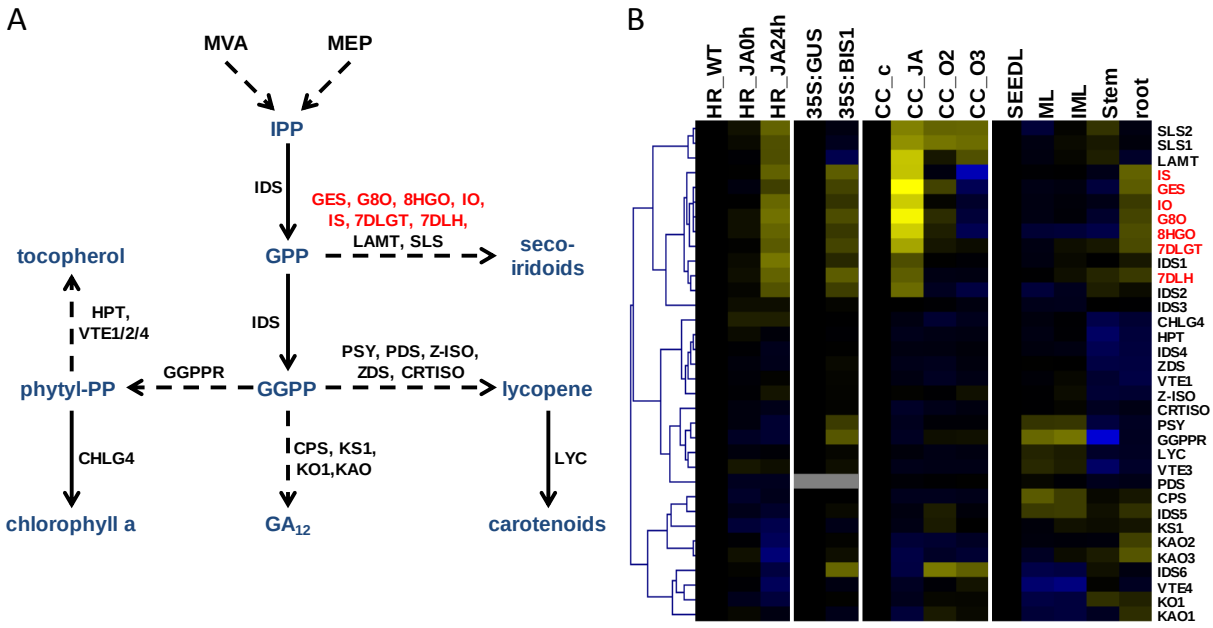

Fig. S3. Regulation of GPP-dependent terpenoid pathways in *C. roseus*. (*A*) Geranyl diphosphate (GPP) is a central compound in the biosynthesis of (seco-)iridoids, carotenoids, gibberellins (GA_{12}), and phytyldiphosphate (phytyl-PP) derived compounds such as chlorophyll and tocopherol. Corresponding *C. roseus* genes of non-curated pathways (1) were automatically retrieved via CathaCyc (http://www.cathacyc.org/) where possible or via BLAST in ORCAE (http://bioinformatics.psb.ugent.be/orcae/overview/SmartCell). Gene abbreviations, gene annotations and corresponding Caros numbers can be found in Table S3. IDS2 corresponds to GGPS-SSU in Fig. 1 and Fig. 4. Solid and dashed arrows represent single and multiple enzymatic steps, respectively. (*B*) Hierarchical clustering of the genes depicted in (*A*) using selected conditions from the Medicinal Plant Genomics Resource (MPGR) database (http://medicinalplantgenomics.msu.edu) and the ORCAE database from the SmartCell consortium (http://bioinformatics.psb.ugent.be/orcae/overview/Catro). FPKM values were normalized and log-transformed prior to hierarchical cluster analysis. HR_WT, 35S:GUS, CC_c and SEEDL samples were used to normalize the hairy root, 35S:BIS1, cell culture and tissue samples, respectively. Blue and yellow denote relative down-regulation and

up-regulation, respectively. FKPM values along with Caros and gene ID can be found in Table S3.

1. Van Moerkercke A, et al. (2013) CathaCyc, a metabolic pathway database built from *Catharanthus roseus* RNA-Seq data. *Plant Cell Physiol* 54(5):673-685.

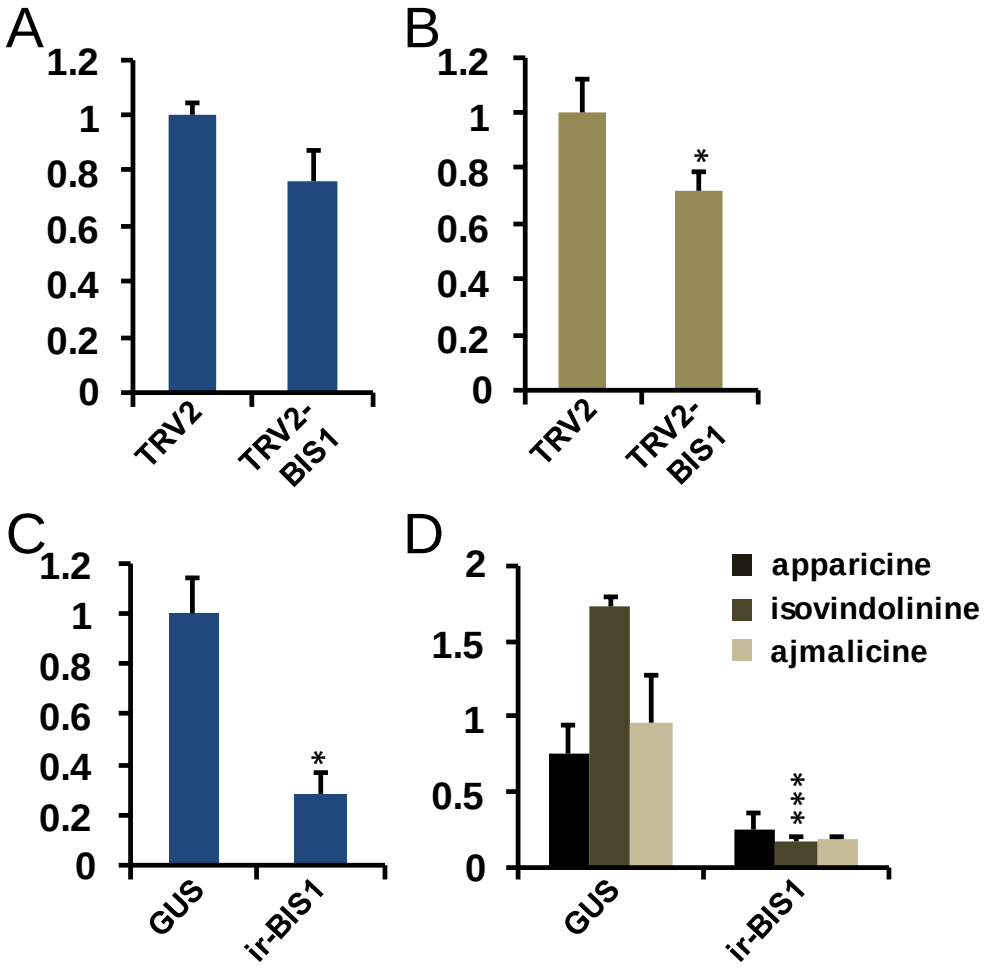

Fig. S4. Silencing of *BIS1* reduces MIA accumulation *in planta*. (*A* and *B*) VIGS silencing of *BIS1* in *C. roseus* seedlings. (*A*) qPCR showing relative *BIS1* mRNA levels in empty vector control lines (TRV2; set at 1) and lines silenced for *BIS1*. The error bars designate SE of the mean (n=10). (*B*) Secologanin levels in control and *BIS1*-silenced lines. Values are shown relative to the control levels. Statistical significance was determined by the Student's *t*-test (* $P < 0.05$). (*C* and *D*) Silencing of *BIS1* in *C. roseus* hairy root lines. (*C*) qPCR analysis showing *BIS1* expression relative to the control (GUS) lines set at 1. The error bars designate SE of the mean (n=3 for GUS and n=2 for BIS1). (*D*) Metabolite profiling on the *BIS1*-silenced and control lines. Alkaloid levels are indicated in mg/g dry weight. The error bars designate SE of the mean (n=3 for GUS and n=2 for BIS1). Statistical significance was determined by the Student's *t*-test (* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$).

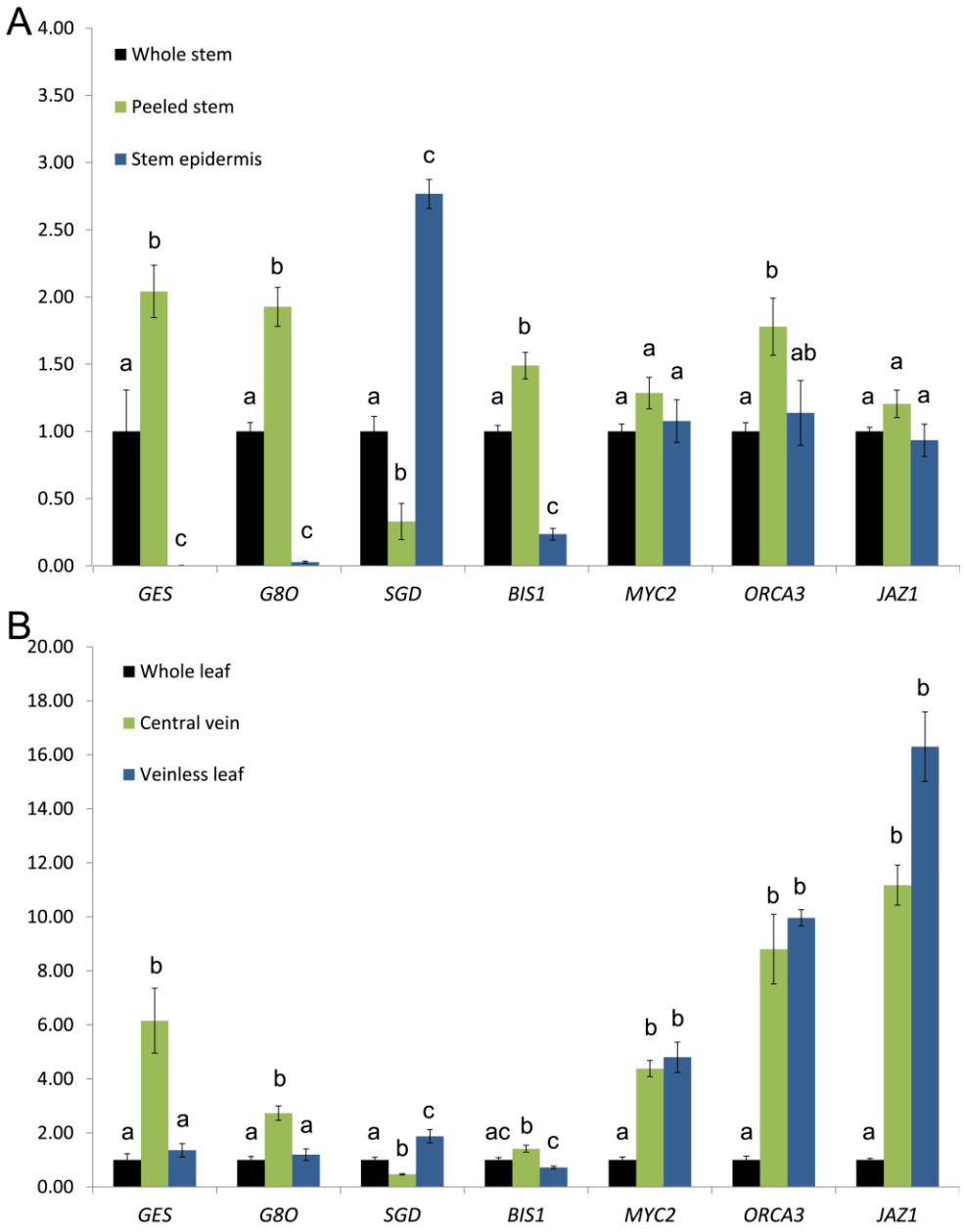

Fig. S5. Expression of *BIS1* and *ORCA3* in *C. roseus* tissues. Quantitative PCR (qPCR) analysis showing gene expression in stem (*A*) and leaf derived tissues (*B*), relative to the control whole stems (*A*) and leaves (*B*) set at 1. Dissection of the leaf material takes several minutes, in contrast to dissection of the stem material that occurs in less than a minute. Therefore, expression of *MYC2* and *JAZ1* was assessed to illustrate that a wounding and endogenous JA response is likely taking place in the leaf but not in the stem samples. The error bars designate SE of the mean (n=3). Bars with different letters differ at $P < 0.05$ (Tukey's HSD test).

Table S1. FPKM (fragments per kilobase of exon per million fragments mapped) values of *C. roseus* MIA and triterpenoid pathway genes in plant organs. FKPM values were extracted from the ORCAE database (http://bioinformatics.psb.ugent.be/orcae/overview/Catro) and were generated through RNA-Seq analysis on different *C. roseus* plant organs (1).

Caros ID	Gene ID	SEEDL	ML	IML	ST	R
Caros009839.1	7DLGT	18.86	13.62	24.96	29.28	73.23
Caros005234.1	7DLH	46.69	44.85	62.75	93.59	134.56
Caros003452.1	8HGO	138.56	53.34	49.52	37.16	569.65
Caros020508.1	AACT1	47.94	14.86	32.24	36.53	44.60
Caros021607.1	AAS	72.79	176.15	200.81	1.81	80.57
Caros022753.1	AO	28.11	179.96	106.91	15.06	61.14
Caros001862.1	BIS1	4.08	1.82	2.68	1.80	13.58
Caros011438.1	CMK	51.81	58.87	48.25	28.05	81.84
Caros009631.1	CMS	1.47	2.19	3.38	1.82	4.70
Caros006133.1	D4H	98.44	349.41	318.89	1.29	37.07
Caros024662.1	DAT	15.46	40.19	58.57	0.06	8.32
Caros006067.1	DXR	80.26	101.35	68.30	56.05	149.30
Caros000586.1	DXS1	82.18	296.85	230.65	18.05	83.34
Caros003623.1	DXS2A	82.60	34.48	46.78	35.33	208.59
Caros016072.1	DXS2B	14.52	7.64	13.47	14.69	40.06
Caros006766.1	G8O	142.78	103.09	123.95	64.34	498.03
Caros003727.1	GES	44.38	30.46	33.50	14.76	241.93
Caros000035.1	GPPS-SSU	45.25	15.71	26.69	78.63	57.15
Caros004608.1	HDR	115.14	193.68	153.96	89.07	265.52
Caros004555.1	HDS	120.41	167.51	147.70	57.77	241.10
Caros022480.1	HMGR1	23.16	13.46	20.40	8.54	40.45
Caros005615.1	HMGR2	66.13	63.51	101.70	160.19	80.90
Caros004529.1	HMGS	78.99	97.86	139.56	69.19	134.75
Caros003676.1	IO	73.87	54.25	61.95	50.49	215.85
Caros002904.1	LAMT	121.73	91.33	139.33	214.13	57.51
Caros016117.1	MAT	5.05	0.01	0.02	62.68	0.10
Caros003433.1	MECS	55.25	86.55	77.27	26.04	88.14
Caros003648.1	MVD	43.67	19.91	26.29	51.47	36.88
Caros004544.1	MVK	22.31	18.89	17.07	36.50	28.18
Caros009486.1	MYC2	21.43	28.92	42.59	230.68	40.58

Caros ID	Gene ID	SEEDL	ML	IML	ST	R
Caros009463.1	NMT	60.63	34.57	31.62	20.41	25.49
Caros004408.1	ORCA3	0.66	2.15	3.70	40.78	1.18
Caros004488.1	PMK	19.37	7.76	8.00	14.17	14.48
Caros008267.1	POR5/IS	48.19	44.99	46.69	31.59	290.91
Caros007312.1	PRX1	152.05	86.45	75.83	13.19	59.63
Caros009426.1	SGD	65.96	15.90	32.77	39.96	23.65
Caros002866.1	SLS1	92.51	67.82	113.56	146.34	74.81
Caros003710.1	SLS2	183.02	66.33	203.73	475.29	134.20
Caros007363.1	SQE1	28.31	55.98	44.86	1.36	37.04
Caros008176.1	SQS	104.94	153.97	127.45	86.50	90.42
Caros011578.1	STR1	107.17	76.97	120.54	190.69	78.28
Caros010005.1	STR2	55.37	26.81	35.52	17.04	30.69
Caros001600.1	T16H1	1.44	0.72	0.92	0.26	0.90
Caros025399.1	T16H2	7.71	42.42	68.61	0.07	13.22
Caros001534.1	T16OMT	9.48	0.44	1.79	22.02	1.75
Caros016367.1	T19H	3.33	0.03	0.00	14.20	0.30
Caros014930.1	TDC	112.14	69.66	195.04	358.59	102.16

SEEDL, seedlings; ML, mature leaf; IM, immature leaf; ST, stem; R, root.

1. Góngora-Castillo E, et al. (2012) Development of transcriptomic resources for interrogating the biosynthesis of monoterpene indole alkaloids in medicinal plant species. *PLoS ONE* 7(12):e52506.

Table S2. FPKM values of *C. roseus* MIA and triterpenoid pathway genes in suspension cells and hairy roots. FKPM values were generated through RNA-Seq analysis on elicited *C. roseus* suspension cell cultures (1, 2), and elicited (3) and transformed (this study) *C. roseus* hairy root cultures and have been extracted or uploaded on the ORCAE database (http://bioinformatics.psb.ugent.be/orcae/overview/Catro).

Caros ID	Gene ID	CC_c	CC_JA	CC_O2	CC_O3	HR_WT	HR_JA0	HR_JA24	HR_GUS	HR_BIS1
Caros009839.1	7DLGT	2.20	44.61	3.33	2.83	104.44	110.43	542.69	5.32	18.66
Caros005234.1	7DLH	25.97	143.05	18.13	18.99	105.71	140.38	654.83	90.58	485.99
Caros003452.1	8HGO	5.31	224.53	9.32	1.23	553.11	742.68	3466.87	557.68	1581.01
Caros020508.1	AACT1	19.17	16.05	22.51	10.92	20.49	30.65	15.37	6.57	4.53
Caros021607.1	AAS	0.33	0.19	0,40	0.36	0.61	1.29	0.70	0.72	0.76
Caros022753.1	AO	0.00	0.00	0.13	0.03	41.44	55.84	4.61	28.54	21.30
Caros001862.1	BIS1	9.31	41.85	4.23	2.28	12.93	16.10	67.35	30.02	163.76
Caros011438.1	CMK	36.01	88.07	36.46	32.36	73.20	73.10	345.51	57.49	157.65
Caros009631.1	CMS	0.76	6.24	0.68	0.73	4.47	3.63	18.23	4.13	12.92
Caros006133.1	D4H	4.86	16.26	1.43	50.58	0.59	0.41	1.38	0.08	0.05
Caros024662.1	DAT	0.10	1.64	0.10	0.30	0.28	0.13	1.45	0.00	0.00
Caros006067.1	DXR	21.10	44.63	43.83	18.79	182.66	262.78	1474.25	165.02	426.35
Caros000586.1	DXS1	21.02	11.43	20.37	15.49	3.91	7.65	2.44	8.80	11.45
Caros003623.1	DXS2A	2.63	125.75	25.12	2.72	101.69	128.30	728.64	130.33	153.14
Caros016072.1	DXS2B	0.15	9.41	2.52	0.47	31.25	39.31	238.10	45.10	98.39
Caros006766.1	G8O	1.68	146.87	3.79	0.61	448.63	604.31	3574.86	461.54	1866.12
Caros003727.1	GES	0.24	51.80	0.79	0.05	198.06	152.34	631.37	133.69	459.93
Caros000035.1	GPPS-SSU	128.36	915.00	71.32	39.18	113.67	126.79	511.91	136.69	430.41

Caros ID	Gene ID	CC_c	CC_JA	CC_O2	CC_O3	HR_WT	HR_JA0	HR_JA24	HR_GUS	HR_BIS1
Caros004608.1	HDR	33.56	77.76	32.80	33.31	238.32	290.35	803.49	304.84	788.62
Caros004555.1	HDS	21.59	151.82	31.38	28.06	231.29	263.15	1235.12	311.72	1061.47
Caros022480.1	HMGR1	15.63	14.78	28.09	23.44	17.96	33.12	14.93	0.38	0.38
Caros005615.1	HMGR2	27.06	42.78	29.75	23.40	90.16	94.49	104.58	70.99	68.64
Caros004529.1	HMGS	72.61	95.30	196.19	118.01	82.94	98.54	45.25	39.71	32.68
Caros003676.1	IO	6.48	265.03	7.33	3.02	246.08	253.71	1635.20	42.40	109.00
Caros002904.1	LAMT	64.49	2319.32	99.24	280.43	393.71	360.60	1650.64	166.80	41.16
Caros016117.1	MAT	0.00	0.00	0.04	0.08	24.60	29.24	364.24	0.94	0.29
Caros003433.1	MECS	59.32	189.96	51.51	55.21	71.60	69.56	386.86	56.50	222.84
Caros003648.1	MVD	45.31	59.06	38.25	40.10	51.65	86.17	60.19	41.71	39.88
Caros004544.1	MVK	27.68	29.57	36.09	21.93	24.25	30.73	33.34	30.05	25.34
Caros009486.1	MYC2	10.43	77.42	10.50	11.50	53.71	193.23	128.42	35.71	28.81
Caros009463.1	NMT	21.77	10.61	16.41	13.44	34.32	39.49	19.55	14.59	13.16
Caros004408.1	ORCA3	16.18	17.27	7.91	680.66	2.03	86.14	29.03	0.29	0.26
Caros004488.1	PMK	14.91	9.46	17.94	13.71	19.72	28.31	26.76	31.48	30.83
Caros008267.1	POR5/IS	2.27	79.44	1.71	0.09	306.87	318.88	1821.86	314.78	1741.14
Caros007312.1	PRX1	0.90	9.01	5.15	4.29	8.58	9.21	24.34	7.77	3.56
Caros009426.1	SGD	163.36	478.93	94.04	152.32	153.15	134.90	423.06	19.37	12.09
Caros002866.1	SLS1	29.02	401.44	244.63	212.40	203.85	248.11	872.36	323.56	190.89
Caros003710.1	SLS2	63.20	670.48	391.68	409.31	518.40	726.19	3173.05	919.06	670.27
Caros007363.1	SQE1	80.47	31.33	40.85	53.74	34.49	39.43	15.03	26.04	25.24
Caros008176.1	SQS	129.21	111.43	116.80	138.45	84.01	120.85	113.50	109.67	97.79

Caros ID	Gene ID	CC_c	CC_JA	CC_O2	CC_O3	HR_WT	HR_JA0	HR_JA24	HR_GUS	HR_BIS1
Caros011578.1	STR1	75.10	300.68	228.80	348.54	215.93	210.59	699.18	140.55	131.32
Caros010005.1	STR2	40.39	36.03	128.71	80.00	36.65	59.06	45.12	26.28	20.08
Caros001600.1	T16H1	73.70	368.88	117.46	26.88	0.00	0.00	0.09	2.45	2.43
Caros025399.1	T16H2	0.15	0.30	0.24	0.02	0.04	0.00	0.00	0.09	0.11
Caros001534.1	T16OMT	0.58	44.70	4.64	0.19	6.31	7.31	31.99	2.42	0.96
Caros016367.1	T19H	6.47	58.72	9.28	142.20	28.10	8.52	33.86	7.42	3.45
Caros014930.1	TDC	493.58	1705.74	1568.42	834.77	1136.53	1160.36	2243.65	1038.66	647.00

CC_c, mock-treated cell culture; CC_JA; JA-treated cell culture; CC_O2 and CC_O3, cell culture overexpressing *ORCA2* and *ORCA3*, respectively; HR_WT, wild-type hairy roots; HR_JA0 and HR_JA24, hairy roots treated with JA for 0 h and 24 h, respectively; HR_GUS and HR_BIS1, hairy roots overexpressing *GUS* and *BIS1*, respectively.

1. Miettinen K, et al. (2014) The seco-iridoid pathway from *Catharanthus roseus*. *Nat Commun* 5:3606 [Erratum *Nat Commun* 5:4175].
2. Van Moerkercke A, et al. (2013) CathaCyc, a metabolic pathway database built from *Catharanthus roseus* RNA-Seq data. *Plant Cell Physiol* 54(5):673-685.
3. Góngora-Castillo E, et al. (2012) Development of transcriptomic resources for interrogating the biosynthesis of monoterpene indole alkaloids in medicinal plant species. *PLoS ONE* 7(12):e52506.

Table S3. FPKM values of *C. roseus* genes involved in MEP-dependent pathways. FKPM values were generated as in Tables S1 and S2.

Gene ID	Name	Caros ID	HR_WT	HR_JA0	HR_JA24	HR_GUS	HR_BIS1	CC_c	CC_JA	CC_O2
CPS	*ent*-copalyl diphosphate synthase	Caros011353.1	4.57	2.17	2.83	11.74	12.11	25.75	15.43	15.25
KS1	*ent*-kaurene synthase	Caros024574.1	2.05	0.75	0.51	0.03	0.02	0.99	0.68	1.64
KO1	*ent*-kaurene oxidase	Caros003905.1	19.15	11.74	6.53	18.78	20.15	51.14	30.88	59.26
KAO1	*ent*-kaurenoic acid oxidase	Caros025043.1	8.21	9.63	5.99	1.85	1.18	1.84	0.66	2.99
KAO2	*ent*-kaurenoic acid oxidase	Caros012022.1	12.76	11.70	2.56	2.21	2.41	10.63	4.00	4.39
KAO3	*ent*-kaurenoic acid oxidase	Caros010842.1	31.17	42.83	3.71	8.59	11.38	20.78	6.07	10.29
PSY	phytoene synthase	Caros009206.1	0.73	0.57	0.30	2.22	6.59	16.94	10.03	15.16
PDS	phytoene desaturase	Caros017455.1	11.76	6.64	6.78	0.00	0.00	6.85	6.11	6.55
Z-ISO	15-cis-zeta-carotene isomerase	Caros009901.1	12.36	11.46	18.39	4.77	5.29	14.58	15.92	13.59
ZDS	zeta-carotene desaturase	Caros007024.1	25.20	20.60	15.94	21.28	25.66	19.36	12.48	11.54
CRTISO	carotenoid isomerase	Caros001945.1	12.99	10.17	8.34	12.04	13.13	6.83	3.36	4.37
GGPPR	geranylgeranyl pyrophosphate reductase	Caros007673.1	3.07	1.60	1.26	7.78	37.48	24.68	13.14	32.66
IDS1	geranyl(geranyl) diphosphate synthase	Caros010143.1	40.21	49.84	347.73	27.83	57.86	8.71	36.95	9.03
IDS2	geranyl(geranyl) diphosphate synthase	Caros000035.1	113.67	126.79	511.91	136.69	430.41	128.36	915.00	71.32
IDS3	geranyl(geranyl) diphosphate synthase	Caros000140.1	29.63	38.58	38.25	21.70	24.20	40.99	41.56	38.02
IDS4	geranyl(geranyl) diphosphate synthase	Caros001657.1	7.07	6.65	4.33	6.10	6.34	18.42	13.79	13.96
IDS5	geranyl(geranyl) diphosphate synthase	Caros015620.1	5.08	3.26	2.29	4.98	5.98	3.51	2.67	6.12
IDS6	geranyl(geranyl) diphosphate synthase	Caros018814.1	7.09	7.81	2.27	0.06	0.36	2.27	0.68	20.63
LYC	lycopene-β-cyclase	Caros009040.1	6.51	5.21	5.76	8.87	10.12	8.98	7.41	6.24
CHLG4	chlorophyll synthase	Caros022566.1	1.32	2.39	2.32	1.14	1.11	6.76	4.90	2.76
HPT	homogentisate phytyltransferase	Caros008321.1	3.97	4.96	3.33	6.61	6.09	16.92	10.51	11.58
VTE3	MPBQ methyltransferase	Caros000123.1	22.52	34.68	29.75	29.75	39.09	39.21	25.29	26.64
VTE1	tocopherol cyclase	Caros000947.1	7.78	6.75	8.40	8.23	9.22	13.81	9.99	7.45
VTE4	tocopherol methyltransferase	Caros004084.1	292.79	271.70	59.48	204.06	148.29	92.04	49.03	81.64

Gene ID	Name	Caros ID	CC_O3	SEEDL	ML	IL	ST	R
CPS	*ent*-copalyl diphosphate synthase	Caros011353.1	16.59	10,53	55,14	32,80	12,53	16,18
KS1	*ent*-kaurene synthase	Caros024574.1	1.00	1,97	1,63	2,73	2,52	2,92
KO1	*ent*-kaurene oxidase	Caros003905.1	58.17	12,99	3,83	4,29	32,01	24,24
KAO1	*ent*-kaurenoic acid oxidase	Caros025043.1	2.22	13,69	5,04	4,53	7,48	31,59
KAO2	*ent*-kaurenoic acid oxidase	Caros012022.1	5.54	13,41	10,66	11,35	10,94	43,97
KAO3	*ent*-kaurenoic acid oxidase	Caros010842.1	8.41	18,85	9,92	24,21	31,45	89,88
PSY	phytoene synthase	Caros009206.1	14.97	54,11	141,25	147,22	17,18	29,03
PDS	phytoene desaturase	Caros017455.1	7.21	20,98	24,41	18,96	12,45	13,92
Z-ISO	15-cis-zeta-carotene isomerase	Caros009901.1	19.82	29,89	28,72	38,06	11,66	12,51
ZDS	zeta-carotene desaturase	Caros007024.1	15.24	68,49	72,13	68,01	20,32	21,25
CRTISO	carotenoid isomerase	Caros001945.1	5.16	14,04	13,54	15,77	7,84	9,64
GGPPR	geranylgeranyl pyrophosphate reductase	Caros007673.1	33.93	82,01	538,78	684,93	1,78	45,45
IDS1	geranyl(geranyl) diphosphate synthase	Caros010143.1	7.83	22,32	16,47	27,10	22,01	35,03
IDS2	geranyl(geranyl) diphosphate synthase	Caros000035.1	39.18	45,25	15,71	26,69	78,63	57,15
IDS3	geranyl(geranyl) diphosphate synthase	Caros000140.1	31.09	32,62	20,71	19,81	32,06	30,94
IDS4	geranyl(geranyl) diphosphate synthase	Caros001657.1	15.38	52,09	40,84	37,75	11,93	17,21
IDS5	geranyl(geranyl) diphosphate synthase	Caros015620.1	2.96	4,16	12,20	12,79	5,72	10,14
IDS6	geranyl(geranyl) diphosphate synthase	Caros018814.1	13.29	4,21	1,13	1,28	5,76	3,14
LYC	lycopene-β-cyclase	Caros009040.1	6.62	27,59	53,24	46,10	12,98	14,80
CHLG4	chlorophyll synthase	Caros022566.1	4.03	18,18	14,21	17,01	4,85	7,35
HPT	homogentisate phytyl transferase	Caros008321.1	12.40	18,41	13,24	16,98	3,12	6,43
VTE3	MPBQ methyltransferase	Caros000123.1	26.75	127,83	269,05	220,58	20,98	60,10
VTE1	tocopherol cyclase	Caros000947.1	9.58	32,02	27,77	37,09	13,67	9,37
VTE4	tocopherol methyltransferase	Caros004084.1	132.48	75,94	10,53	7,75	83,07	41,36

HR_WT, wild-type hairy roots; HR_JA0 and HR_JA24, hairy roots treated with JA for 0 h and 24 h, respectively; HR_GUS and HR_BIS1, hairy roots overexpressing *GUS* and *BIS1*, respectively; CC_c, mock-treated cell culture; CC_JA; JA-treated cell culture; CC_O2 and CC_O3, cell culture overexpressing *ORCA2* and *ORCA3*, respectively; SEEDL, seedlings; ML, mature leaf; IM, immature leaf; ST, stem; R, root.

Table S4. Oligonucleotide primers used for cloning and qPCR.

For Cloning	
Name	**Sequence***
XbaI-BIS1-5'-R	5'-gctctagaTTTTAGATGAGCTCCAACTAAACCTCTC-3'
XbaI-BIS1--3'-F	5'-gctctagaGTGCAGAATTTGAAAGAAAAATAGAC-3'
attB1-BIS1-5'-F	5'-aaaaagcaggctATCCACTTCCCGGAGCTCTTCTTCAG-3'
attB2-BIS1-3'-R	5'-agaaagctgggGGACCTTCTGATGATACTGAAAACTG-3'
attB1-BIS1-F	5'-ggggacaagtttgtacaaaaaagcaggctgtATGACAATGATGATGACGATG-3'
attB2-BIS1-R	5'-ggggaccactttgtacaagaaagctgggtaTCAATCTTCTAAATTTGCACGC-3'
attB_MYC2-F	5'-ggggacaagtttgtacaaaaaagcaggctATGACGGACTATAGGCTACAAC-3'
attB_MYC2-R	5'-ggggaccactttgtacaagaaagctgggTCATACCAAGAGCCTCATCGAG-3'
attB_ORCA3-F	5'-ggggacaagtttgtacaaaaaagcaggctATGTCCGAAGAAATCATTTCC-3'
attB_ORCA3-R	5'-ggggaccactttgtacaagaaagctgggTTAATATCGTCTCTTCTTCCTTC-3'
XbaI-pG80-F	5'-gctctagaTCGTTATAGAATTTCTCGCTTC-3'
NcoI-pG8O-R	5'-cccccatggAATGGAAGTGTACAATTTGTTCTC-3'
attB1-pG8O-F	5'-ggggacaagtttgtacaaaaaagcaggctTCGTTATAGAATTTCTCGCTTC-3'
attB2-pG8O-R	5'-ggggaccactttgtacaagaaagctgggAATGGAAGTGTACAATTTGTTCTC-3'
attB1-p7DLGT-F	5'-ggggaccactttgtacaagaaagctggctTATATGAGATAACTCAAGAATTTCG-3'
attB2-p7DLGT-R	5'-ggggaccactttgtacaagaaagctgggtTGGCCACTTCAGAATCTATGGAG-3'
attB1-pGES-F	5'-ggggacaagtttgtacaaaaaagcaggctAGTGTTAAGGACATCGAATCC-3'
attB2-pGES-R	5'-ggggaccactttgtacaagaaagctgggtGCTATTTTTAATGTTCTAATTTTAAAAG-3'
attB1-p8HGO-F	5'-ggggacaagtttgtacaaaaaagcaggctGAGAAGTAAAAATTGATGACTTTTTC-3'
attB2-p8HGO-R	5'-ggggaccactttgtacaagaaagctgggtCTTGTTGGGGTTGGCAAAATTAG-3'
attB1-pIS-F	5'-ggggacaagtttgtacaaaaaagcaggcTCTTAGATTAATTTATATTTCG-3'
attB2-pIS-R	5'-ggggaccactttgtacaagaaagctgggtaTATGTCTAATCTTTGGTCTAAAG-3'
attB1-pIO-F	5'-ggggacaagtttgtacaaaaaagcaggctGAACTTCACCTCAACTCATCTTC-3'
attB2-pIO-R	5'-ggggaccactttgtacaagaaagctgggtTGTTTTTCTTAAGTGACAGTTC-3'
attB1-p7DLH-F	5'-ggggacaagtttgtacaaaaaagcaggctCAAAGTAACAGAGAAGAACACAC-3'
attB2-p7DLH-R	5'-ggggaccactttgtacaagaaagctgggtaTTCAGTCCAAAGTCAGGCAAGA-3'
attB1-pLAMT-F	5'-ggggacaagtttgtacaaaaaagcaggctgtTACAATGGTCAAATATGAAACC-3'
attB2p-LAMT-R	5'-ggggaccactttgtacaagaaagctgggtATTTTCTTTCTTCTCTGTATGAGTTTTTTG-3'
attB1-pSTR1-F	5'-ggggacaagtttgtacaaaaaagcaggctGATCTTTATTGTTTGTATTTTC-3'
attB2-pSTR1-R	5'-ggggaccactttgtacaagaaagcTGGGTGGTTGACTGTAAATGATGGGAG-3'
attB1 adapter	5'-ggggacaagtttgtacaaaaaagcaggct-3'
attB2 adapter	5'-ggggaccactttgtacaagaaagctgggt-3'
For qPCR	
Name	**Sequence***
7DLGT-F	5'-CCACCACAAGACCTGAAGAAAT-5'
7DLGT-R	5'-CTAGTCTGAGCATGGAGTTCACA-5'

7DLH-F	5'-CCAGGCAAGGATTTCATTATTC-3'
7DLH-R	5'-TTCAGTCCAAAGTCAGGCAAG-3'
8HGO-F	5'-GAGATAGATCATTCATTCAAATG-3'
8HGO-R	5'-CTCATCTCATTTCTCATTTCTC-3'
BIS1-F	5'-ATGGAATCAGTGGTGCTAGTGA-3'
BIS1-R	5'-TTCAATTTCAGGGAGCTGTGAC-3'
DXR-F	5'-CTGCCCTTGTATGACGATGAAC-3'
DXR-R	5'-ACACTTTTTGCATCAACCCATC-3'
G8O-F	5'-GTACTCTCTCCCTCATGGTTGG-3'
G8O-R	5'-CCGTCCATTACTCCCATAAAGA-3'
GES-F	5'-GCTTTGTTTTTCACACCTTGT-3'
GES-R	5'-CTTAGCACATTTTACTCTCTC-3'
HDS-F	5'-GATTTTGGTTATGTTGGTGGTG-3'
HDS-R	5'-CTCCTCCACTGCGTAATGTTTT-3'
IO-F	5'-CCGGTTTTCTTCTCCTCCTTAT-3'
IO-R	5'-CCGTATTTGGACTTGAGCTTGT-3'
IS-F	5'-TCTTGGGTTTTAGGAATTCGATGA-3'
IS-R	5'-AAACCAAACCCAAAGCAGAAAA-3'
JAZ1-F	5'-TTTGGAGATCTCAGCCTTGG-3'
JAZ1-R	5'-TGGCCAGATTTATCCATCGT-3'
MYC2-F	5'-AGTGGTGAAGGAGGCAGAGA-3'
MYC2-R	5'-ATGGCTCTTCCCTTCCATTT-3'
N2227-F	5'-GGTTGCTCTTCATTACGGATTT-3'
N2227-R	5'-TGCAGCATAGTAATGGTTTTGC-3'
ORCA2-F	5'-TCTTCATCGTGTCATCTTCGTC-3'
ORCA2-R	5'-TAATATCACCTTCCCCATGCTC-3'
ORCA3-F	5'-CTGTCAGGAGGATTCTGTTGTG-3'
ORCA3-R	5'-TCGTATGTACCCAACCAAATCC-3'
SAND-F	5'-CAGTTCCACAATGCTTTCTGAC-3'
SAND-R	5'-GGGACTGATCAATCGAAGTAGC-3'
SGD-F	5'-TGGTCATTCTTTGACAACTTCG-3'
SGD-R	5'-TCTCTTTTTAGCCGTGTTCGTA-3'
SLS1-F	5'-TGCTCCTTTTACCATTCTCACA-3'
SLS1-R	5'-GGGCTCCATTTCTCTTTATTTG-3'
STR1-F	5'-TCTCTTCCATAGCTCTGTGGGTA-3'
STR1-R	5'-GCAGCAGACACTCAAAATCTCC-3'

*Gene-specific and adapter sequences are indicated in upper and lower case, respectively.