

Supplemental data for:

Metabolomics analysis reveals both plant variety and choice of hormone treatment modulate vinca alkaloid production in *Catharanthus roseus*.

Valerie N. Fraser^{†‡}, *Benjamin Philmus*^{§[⊥]*}, *Molly Megraw*^{†[⊥]*}.

[†]Molecular and Cellular Biology Program, Oregon State University, Corvallis, Oregon 97331, United States.

[‡]Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331, United States.

[§]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, Oregon 97331, United States.

[⊥]Center for Genomics and Biocomputing, Oregon State University, Corvallis, Oregon 97331, United States.

Table of Contents

Supplemental Figures

Supplemental Figure S1: Relative intensities of ajmalicine/tetrahydroalstonine.....	3
Supplemental Figure S2: Expression of upstream genes HMGS & DXS2.....	4
Supplemental Figure S3: Expression of more key enzymes in TIA pathway.....	5

Supplemental Tables

Supplemental Table S1: Available 'omics data available for selected varieties.....	6
Supplemental Table S2: ANOVAs for absolute concentration of selected alkaloids.....	7
Supplemental Table S3-1: Welch's t-test pairwise analyses of absolute concentrations between varieties.....	8
Supplemental Table 3-2: Welch's t-test pairwise analyses of treatment effect on absolute concentrations.....	9
Supplementary Table S4-1: Welch's t-test pairwise comparisons for relative intensities between varieties.....	10
Supplemental Table 4-2: Welch's t-test pairwise comparisons of treatment effects on relative intensities.....	11
Supplementary Table S5: ANOVAs for normalized RT-qPCR data.....	12
Supplementary Table S6-1: Welch's t-test pairwise comparisons between varieties for normalized RT-qPCR.....	13
Supplemental Table 6-2: Welch's t-test pairwise comparisons of treatment effects for normalized RT-qPCR.....	14
Supplemental Table S7: qPCR primers information.....	15

Supplemental Figures

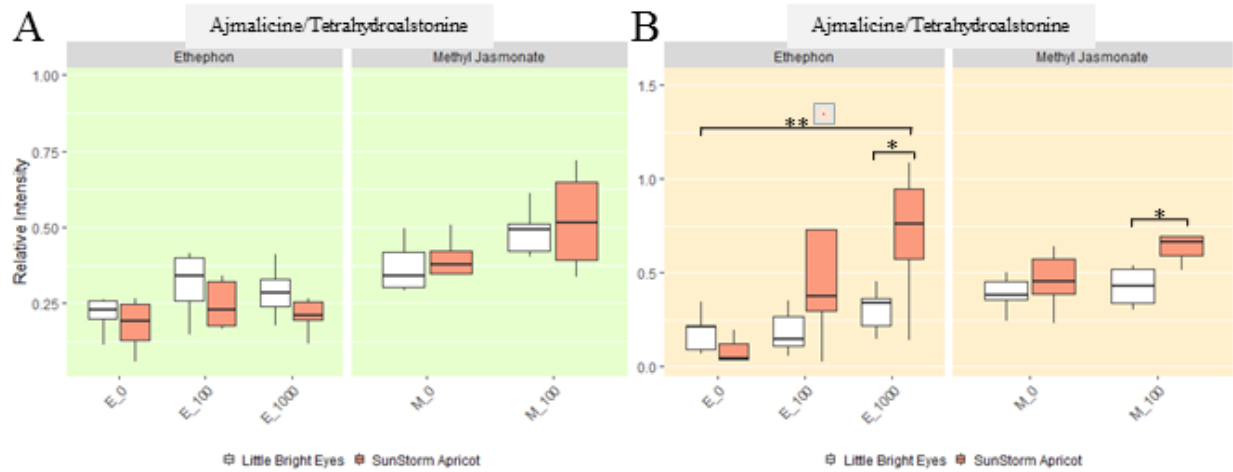


Figure S1 The peak intensity of ajmalicine/tetrahydroalstonine relative to the internal standard (ajmaline) appears to increase upon hormone treatment. * denotes a p-value ≤ 0.05 ; ** denotes a p-value ≤ 0.01 ; all represented statistics are from Welch's t-test post-hoc analyses. Significance markers with a white flower represent treatment differences in LBE, while those with a peach flower represent treatment differences in SSA. (A) Peak intensity relative to internal standard in shoots; (B) peak intensity relative to internal standard in roots.

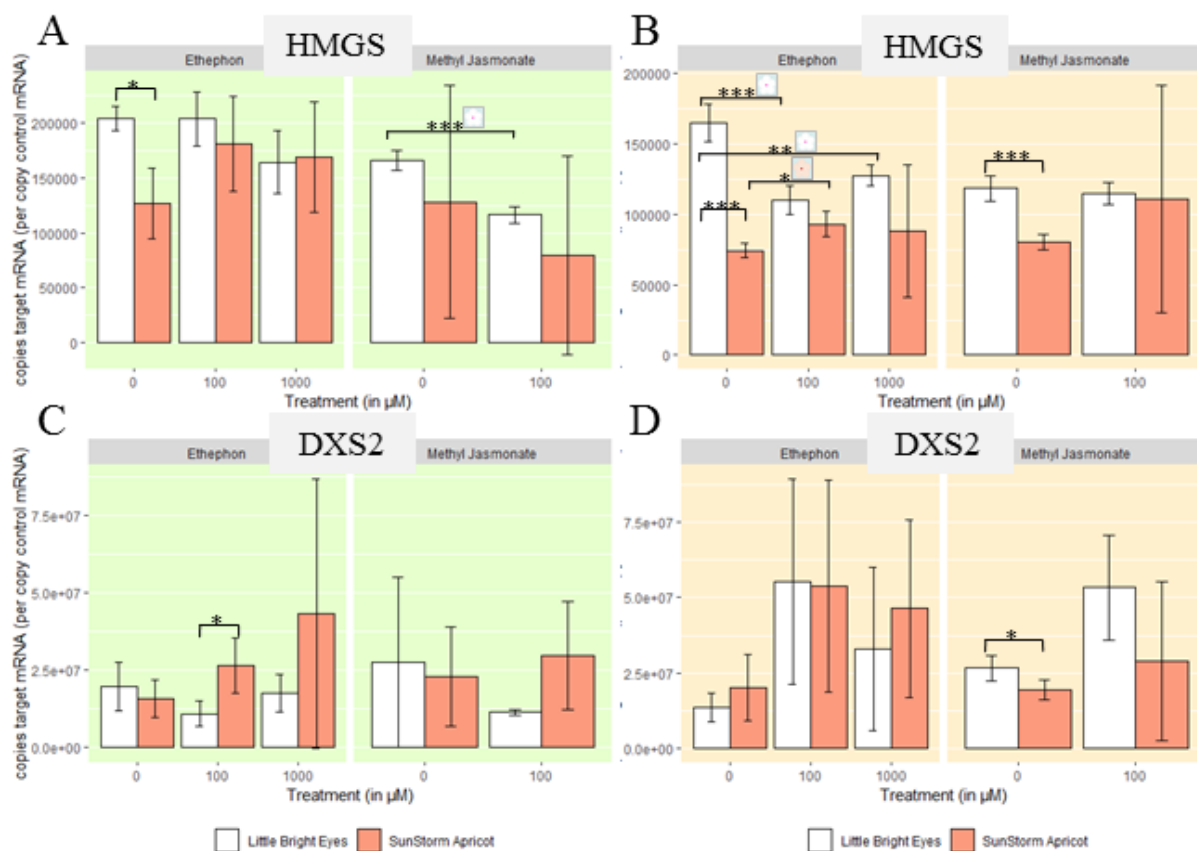


Figure S2 Expression of genes from pathways upstream of the TIA pathway appear to be transcriptionally regulated by hormone treatment. * denotes a p-value ≤ 0.05 ; ** denotes a p-value ≤ 0.01 ; *** denotes a p-value ≤ 0.001 ; all represented statistics are from Welch's t-test post-hoc analyses. Significance markers with a white flower represent treatment differences in LBE, while those with a peach flower represent treatment differences in SSA. (A) HMGS/MVA path shoots (B) HMGS/MVA path roots (C) DXS2/MEP path shoots (D) DXS2/MEP path roots

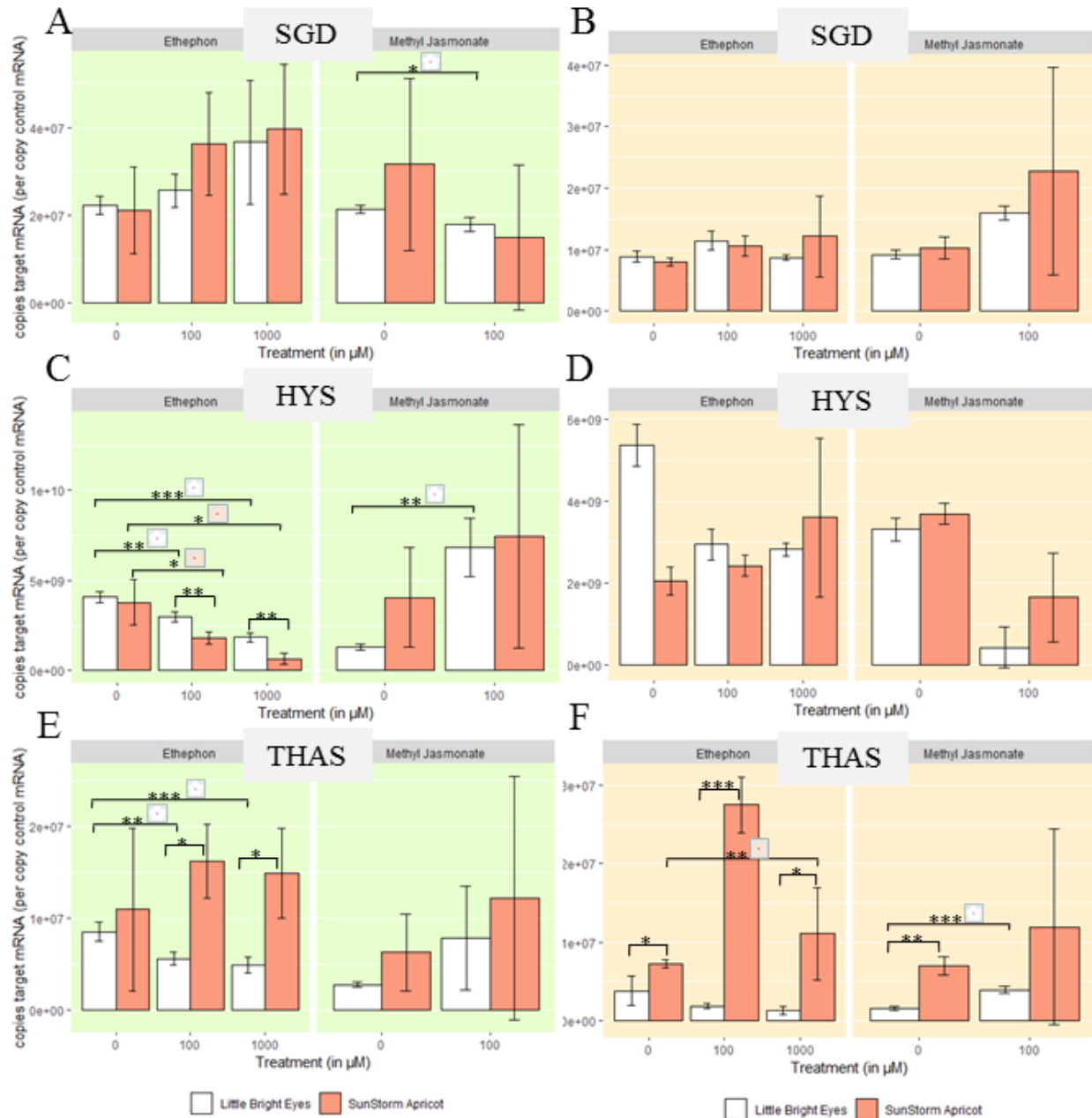


Figure S3 Expression of some key enzymes in the TIA pathway are transcriptionally regulated upon phytohormone treatment. * denotes a p-value ≤ 0.05 ; ** denotes a p-value ≤ 0.01 ; *** denotes a p-value ≤ 0.001 ; all represented statistics are from Welch's t-test post-hoc analyses. Significance markers with a white flower represent treatment differences in LBE, while those with a peach flower represent treatment differences in SSA. (A) SGD expression in shoots (B) SGD expression in roots. (C) HYS expression in shoots (D) HYS expression in roots (E) THAS expression in shoots (F) THAS expression in roots.

Supplemental Tables

Table S1: Available 'omics datasets for selected varieties.

Variety	Genome	Transcriptome	Metabolome	Sources
Sunstorm Apricot	Yes	No	Yes	Kellner 2015; Chung 2011
Little Bright Eye	No	Yes	No	Góngora-Castillo 2012
Prabal	No	Yes	No	Verma 2014
63 other varieties	No	No	Yes	Chung 2011

Table S2: ANOVAs for absolute concentration of selected alkaloids.

Alkaloid	Treatment	Variety	Interaction
Shoot			
Catharanthine	2.67e-05 ***	1.83e-06 ***	0.0128 *
Tabersonine	0.000234 ***	1.31e-09 ***	0.032702 *
Vindoline	0.00026 ***	< 2e-16 ***	0.00029 ***
Root			
Catharanthine	0.203	0.108	0.162
Tabersonine	0.000198 ***	0.048320 *	0.495856

Significance levels: 0 '***' | 0.01 '**' | 0.05 '*' | 0.1 '.'

Table S3-1: Welch’s t-test pairwise analyses of absolute concentrations between varieties.

Significance levels: 0 ‘***’ | 0.01 ‘**’ | 0.05 ‘*’ | 0.1 ‘.’

	Ethephon			Methyl Jasmonate	
	0μM	100μM	1mM	0μM	100μM
Shoot					
Catharanthine	0.6133	0.01656 *	0.001983 **	0.009982 **	0.02625 *
Tabersonine	0.008407 **	0.0449 *	0.001858 **	0.02097 *	0.001604 **
Vindoline	0.1033	0.000813 ***	0.0001867 ***	1.143e-05 ***	0.0006946 ***
Root					
Catharanthine	0.1388	0.6362	0.6143	0.03932 *	0.0954 .
Tabersonine	0.2476	0.4871	0.1831	0.3548	0.08791 .

Table S3-2: Welch’s t-test pairwise analyses of treatment effect on absolute concentrations.

	Shoot			Root	
	Catharanthine	Tabersonine	Vindoline	Catharanthine	Tabersonine
LBE					
Etpn (0:100)	0.002572 **	0.006743 **	0.00582 **	0.8036	0.9903
Etpn (0:1000)	0.002411 **	0.0005549 ***	0.004941 **	0.6636	0.341
MeJA (0:100)	0.2558	0.4912	0.623	0.8765	0.01715 *
SSA					
Etpn (0:100)	0.5329	0.06897 .	0.893	0.2052	0.1716
Etpn (0:1000)	0.9923	0.002391 **	0.3502	0.0239 *	0.009398 **
MeJA (0:100)	0.1951	0.008793 **	0.2516	0.5842	0.06147 .

Significance levels: 0 ‘***’ | 0.01 ‘**’ | 0.05 ‘*’ | 0.1 ‘.’

Table S4-1: Welch’s t-test pairwise comparisons for relative intensities between varieties. * denotes a p-value ≤ 0.05 ; ** denotes a p-value ≤ 0.01 ; *** denotes a p-value ≤ 0.001 ; red boxes are around the uncharacterized m/z = 353 peak.

	Ethephon			Methyl Jasmonate	
	0 μ M	100 μ M	1mM	0 μ M	100 μ M
Shoot					
Ajmalicine/ Tetrahydroalstonine	0.2879	0.8004	0.318	0.8737	0.4245
Catharanthine	0.0491 *	0.0111 *	0.003289 **	0.0105 *	0.0341 *
Tabersonine	0.0252 *	0.03674 *	0.002858 **	0.02719 *	0.001498 **
Vindoline	0.00638 **	4.117e-5 ***	1.997e-6 ***	8.102e-8 ***	2.591e-5 ***
Root					
Ajmalicine/ Tetrahydroalstonine	0.2417	0.1926	0.03341 *	0.4341	0.01938 *
Uncharacterized m/z=353	0.169	0.8337	0.1536	0.6517	0.3356
Catharanthine	0.2881	0.6361	0.616	0.04065 *	0.1079
Tabersonine	0.1592	0.4952	0.1926	0.385	0.07137 .

Significance levels: 0 ‘***’ | 0.01 ‘**’ | 0.05 ‘*’ | 0.1 ‘.’

Table S4-2: Welch's t-test pairwise comparisons of treatment effects on relative intensities. * denotes a p-value ≤ 0.05 ; ** denotes a p-value ≤ 0.01 ; *** denotes a p-value ≤ 0.001 ; red boxes are around the uncharacterized m/z = 353 peak.

	Shoot		Root	
	Ajmalicine/ Tetrahydroalstonine	Uncharacterized m/z =353	Ajmalicine/ Tetrahydroalstonine	Uncharacterized m/z =353
LBE				
Etpn (0:100)	0.2536	N/A	0.9717	0.7975
Etpn (0:1000)	0.4328	N/A	0.1226	0.2184
MeJA (0:100)	0.3265	N/A	0.5281	0.6319
SSA				
Etpn (0:100)	0.2455	N/A	0.1265	0.06465 .
Etpn (0:1000)	0.3365	N/A	0.005827 **	0.001765 **
MeJA (0:100)	0.08547 .	N/A	0.05296 .	0.9346

Significance levels: 0 '***' | 0.01 '**' | 0.05 '*' | 0.1 '.'

Table S5: ANOVAs for normalized RT-qPCR data

	Treatment	Variety	Interaction
Shoot			
SGD	1.09e-06 ***	0.5798	0.0305 *
CS	1.32e-07 ***	3.52e-12 ***	0.00152 **
TS	1.71e-13 ***	0.00928 **	1.73e-06 ***
HYS	5.18e-09 ***	0.0017 **	3.84e-08 ***
THAS	0.028701 *	0.000266 ***	0.006317 **
DAT	0.00329 **	0.18643	0.01432 *
ORCA2	2.73e-08 ***	0.0763 .	0.7814
ORCA3	0.0130 *	4.44e-11 ***	0.0378 *
PRX1	0.000301 ***	0.465309	0.014337 *
DXS2	0.122	0.623	0.518
HGMS	5.26e-05 ***	2.80e-06 ***	0.232
Root			
SGD	2.57e-06 ***	0.00299 **	0.00122 **
CS	5.49e-08 ***	1.05e-12 ***	0.00225 **
TS	1.98e-10 ***	3.02e-07 ***	1.21e-05 ***
HYS	< 2e-16 ***	0.0662 .	4.62e-16 ***
THAS	4.93e-10 ***	3.64e-15 ***	3.39e-10 ***
ORCA2	7.42e-12 ***	1.30e-05 ***	9.96e-13 ***
ORCA3	5.08e-08 ***	0.005728 **	0.000266 ***
PRX1	1.54e-11 ***	2.86e-08 ***	0.00062 ***
DXS2	0.00164 **	0.88019	0.26284
HGMS	1.52e-09 ***	3.79e-10 ***	0.000998 ***

Significance levels: 0 '****' | 0.01 '***' | 0.05 '**' | 0.1 '.'

Table S6-1: Welch’s t-test pairwise comparisons between varieties for normalized RT-qPCR

	Etpn 0μM (control)	Etpn 100μM	Etpn 1mM	MeJA 0μM (control)	MeJA 100μM
Shoots					
SGD	0.8267	0.1690	0.7763	0.3730	0.7357
CS	0.0772 .	0.0282 *	0.0093 **	0.0454 *	0.3712
TS	0.5309	0.0917 .	0.4365	0.4203	0.9399
HYS	0.6547	0.0017 **	0.0013 **	0.1385	0.8606
THAS	0.6229	0.0120 *	0.0246 *	0.1875	0.5828
DAT	0.4679	0.0252 *	0.4589	0.2783	0.4920
ORCA2	0.7190	0.0045 **	0.3258	0.7555	0.7308
ORCA3	0.0102 *	0.00018 ***	0.00784 **	0.0975 .	0.1610
PRX1	0.0493 *	0.149645	0.0727 .	0.9298	0.9335
DXS2	0.4954	0.0309 *	0.3236	0.7836	0.1249
HMGS	0.01204 *	0.4017	0.8788	0.5297	0.4735
Roots					
SGD	0.1741	0.4535	0.3710	0.3053	0.4782
CS	0.000278 ***	0.03403 *	0.0037 **	0.00052 ***	0.0195 *
TS	0.5280	0.7979	0.3571	0.0014 **	0.2743
HYS	0.00011 ***	0.0698 .	0.4782	0.0835 .	0.1028
THAS	0.02918 *	0.00067 ***	0.0447 *	0.00156 **	0.2910
ORCA2	0.00079 ***	0.0166 *	0.3216	0.0013 **	0.8774
ORCA3	0.00016 ***	0.00073 ***	0.1297	0.4201	0.6366
PRX1	0.00033 ***	0.00460 **	0.2854	0.0012 **	0.3041
DXS2	0.334845	0.95096	0.5236	0.0344 *	0.1805
HMGS	0.000305 ***	0.0507 .	0.19485	0.000798 ***	0.9302

Significance levels: 0 ‘***’ | 0.01 ‘**’ | 0.05 ‘*’ | 0.1 ‘.’

Table S6-2: Welch's t-test pairwise comparisons of treatment effects for normalized RT-qPCR.

	LBE			SSA		
	E0 vs E1	E0 vs E4	M0 vs M1	E0 vs E1	E0 vs E4	M0 vs M1
Shoot						
SGD	0.1828	0.1355	0.0166 *	0.0976 .	0.0885 .	0.2403
CS	0.0312 *	0.0076 **	7.517e-05 ***	0.1282	0.1895	0.4377
TS	0.6502	0.1804	0.0260 *	0.2501	0.1257	0.3751
HYS	0.00217 **	3.777e-05 ***	0.00601 **	0.0473 *	0.0125 *	0.3716
THAS	0.0041 **	0.0016 **	0.1682	0.336	0.4746	0.4516
DAT	0.2345	0.0221 *	0.0863 .	0.8151	0.0433 *	0.9716
ORCA2	0.596	0.0017 **	0.1268	0.0484 *	0.0330 *	0.433
ORCA3	7.656e-05 ***	2.460e-05 ***	0.0059 **	0.9285	0.9417	0.4768
PRX1	0.2316	0.7947	0.0116 *	0.4976	0.0125 *	0.745
DXS2	0.1039	0.656	0.3203	0.1209	0.3004	0.5855
HMGS	0.9682	0.0625 .	1.380e-04 ***	0.0944 .	0.2115	0.5112
Root						
SGD	0.0352 *	0.7443	0.00014 ***	0.0482 *	0.2969	0.2366
CS	0.1404	1.0464e-04 ***	0.00255 **	0.0026 **	0.2932	0.8262
TS	0.0102 *	0.1158	0.03599 *	0.0908 .	0.1919	0.4141
THAS	0.1318	0.0736 .	0.00013 ***	0.0012 **	0.2899	0.489
HYS	0.00044 ***	0.00128 **	0.00025 ***	0.1311	0.207	0.0294 *
ORCA2	0.0361 *	0.0022 **	8.820e-05 ***	0.00051 ***	0.0706 .	0.1769
ORCA3	0.00021 ***	0.00016 ***	0.00012 ***	0.00062 ***	0.9302	0.1621
PRX1	0.1349	0.0248 *	0.0016 **	0.00219 **	0.1439	0.8028
DXS2	0.0893 .	0.2474	0.0509 .	0.1488	0.1717	0.5235
HMGS	0.00078 ***	0.00528 **	0.562	0.0180 *	0.5944	0.5005

Significance levels: 0 '***' | 0.01 '**' | 0.05 '*' | 0.1 '.'

Table S7: qPCR primers information. qPCR primers; CS and TS primer sequences were obtained from Caputi et al, 2018. DSX2 and HMGS primer sequences were obtained from Zhang et al, 2012.

Gene	Forward primer	Reverse primer	Genbank Accession	Product length
strictosidine β-D-glucosidase (SGD)	GGAGGATCTGCTTATCAGTGTG	TGGCTGGATATCGGTTTGT	AF112888.1	91nt
catharanthine synthase (CS)	CTCCTGGCGGGATGAATAAC	GGAAACCAGGGTAACCAACA	MF770512	139nt
tabersonine synthase (TS)	AGATGCTCCTGGTGGAAATG	CAACCATGGAAATCAGCAACC	MF770513	104nt
heteroyohimbine synthase (HYS)	AGCAATCAGATTTGCCAAGG	GGGTTACTGTTGAGCAAGAAAG	KU865325.1	120nt
class III peroxidase 1 (PRX1)	TTCCATTGGGAAGAAGAGATGG	TTAGGAGGGCACTTGTGTG	AM236087.1	96nt
tetrahydroalstonine synthase (THAS)	TTTAGGTGCACCAGAAATGC	TTCCTTCATACTCCCAGCAG	KM524258.1	97nt
6-17-O-acetylvindoline O-acetyltransferase (DAT)	AGAGACCTAGTCCTTCCCAAAC	AAAGCAACCGCCAAACCT	AF053307.1	101nt
ORCA2	CGTTTCAACTCCGTGGTTCTA	ATCGGCGTCTAGGACTTACT	AJ238740.1	92nt
ORCA3	CCAGCTCGGAATTGACTTCTAC	GGCTACCGGGTTTCTGTATTT	EU072424.1	96nt
1-deoxy-D-xylulose-5-phosphate synthase (DSX2)	CGAATGGGGTTTTAATGAGG	GAGTGGAGAAATGGGAGGAA	DQ848672.1	61nt
hydroxymethylglutaryl-CoA synthase (HMGS)	CTCAATGAGTATGACGGCAGTT	AGACGACCAATTTGCTTTGG	JF739871.1	72nt
RPS9 (reference gene)	TCAGTTTCTACCGGAACATATGG	GCTTCAACTCTGCATCCAATC	AJ749993	84nt

References

- (1) Caputi, L.; Franke, J.; Farrow, S. C.; Chung, K.; Payne, R. M. E.; Nguyen, T.-D.; Dang, T.-T. T.; Carqueijeiro, I. S. T.; Koudounas, K.; Bernonville, T. D. d.; Ameyaw, B.; Jones, D. M.; Vieira, I. J. C.; Courdavault, V.; O'Connor, S. E., Missing enzymes in the biosynthesis of the anticancer drug vinblastine in Madagascar periwinkle. *Science* **2018**, *360*, (6394), 1235-1239.
- (2) Chung, I. M.; Hong, S. B.; Peebles, C. A.; Kim, J. A.; San, K. Y., Effect of the engineered indole pathway on accumulation of phenolic compounds in *Catharanthus roseus* hairy roots. *Biotechnol. Prog.* **2007**, *23*, (2), 327-32.
- (3) Góngora-Castillo, E.; Childs, K. L.; Fedewa, G.; Hamilton, J. P.; Liscombe, D. K.; Magallanes-Lundback, M.; Mandadi, K. K.; Nims, E.; Runguphan, W.; Vaillancourt, B.; Varbanova-Herde, M.; DellaPenna, D.; McKnight, T. D.; O'Connor, S.; Buell, C. R., Development of transcriptomic resources for interrogating the biosynthesis of monoterpene indole alkaloids in medicinal plant species. *PLOS ONE* **2012**, *7*, (12), e52506.
- (4) Kellner, F.; Kim, J.; Clavijo, B. J.; Hamilton, J. P.; Childs, K. L.; Vaillancourt, B.; Cepela, J.; Habermann, M.; Steuernagel, B.; Clissold, L.; McLay, K.; Buell, C. R.; O'Connor, S. E., Genome-guided investigation of plant natural product biosynthesis. *Plant J.* **2015**, *82*, (4), 680-92.
- (5) Verma, M.; Ghangal, R.; Sharma, R.; Sinha, A. K.; Jain, M., Transcriptome analysis of *Catharanthus roseus* for gene discovery and expression profiling. *PLOS ONE* **2014**, *9*, (7).
- (6) Zhang, X.-N.; Liu, J.; Liu, Y.; Wang, Y.; Abozeid, A.; Yu, Z.-G.; Tang, Z.-H., Metabolomics analysis reveals that ethylene and methyl jasmonate regulate different branch pathways to promote the accumulation of terpenoid indole alkaloids in *Catharanthus roseus*. *J. Nat. Prod.* **2018**, *81*, (2), 335-342.