



Article

PgMYB2, a MeJA-Responsive Transcription Factor, Positively Regulates the Dammarenediol Synthase Gene Expression in *Panax ginseng*

Tuo Liu ^{1,†}, Tiao Luo ^{1,2,†}, Xiangqian Guo ¹, Xian Zou ¹, Donghua Zhou ², Sadia Afrin ¹, Gui Li ¹, Yue Zhang ¹, Ru Zhang ³, and Zhiyong Luo ^{1,*}

¹ Department of Biochemistry and Molecular Biology, School of Life Sciences, Central South University, Changsha 410008, China; lt1994@csu.edu.cn (Tu.L.); tiaoul96@163.com (Ti.L.); gxq199x@163.com (X.G.); zx13618463547@163.com (X.Z.); nilabotdu@yahoo.com (S.A.); ligui20061029@126.com (G.L.); zhang1045242781@126.com (Y.Z.)

² School of Stomatology of Changsha Medical University, Changsha 410006, China; csyxyzdh@163.com

³ College of Chemistry and Chemical Engineering, Hunan Institute of Engineering, Xiangtan 411104, China; zhangru2002@126.com

* Correspondence: luozhiyong@csu.edu.cn; Tel.: +86-731-8480-5025

† These authors contributed equally to this work.

Supplementary Materials:

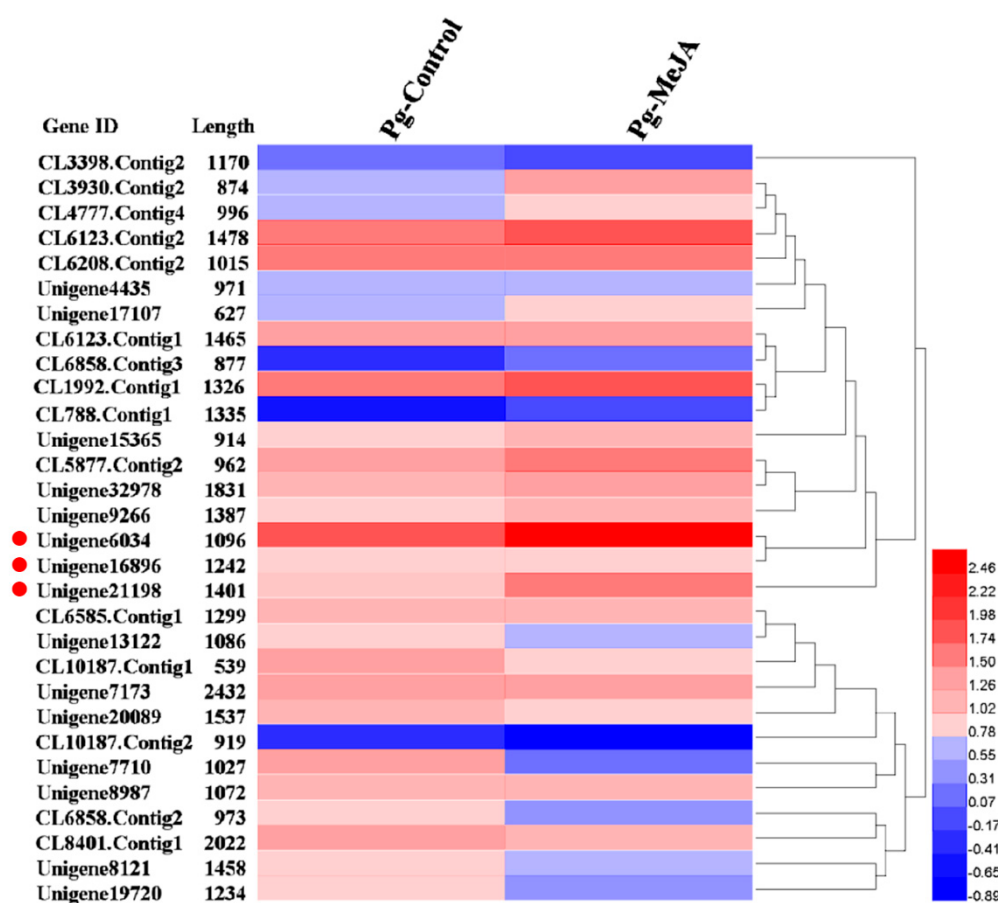


Figure S1. Heatmap of 30 R2R3-MYB unigenes. The levels of expression were determined based on comparison of the intensity of two colors (red and blue) from two samples (Pg-Con and Pg-MeJA). The gradation of color indicated different values which were measured using lg (FPKM). The values

were shown with the 'color-scale' at the right-bottom. The red circles represented three unigenes for subsequent screening. The heatmap was generated by HemI program (<http://hemi.biocuckoo.org/index.php>).

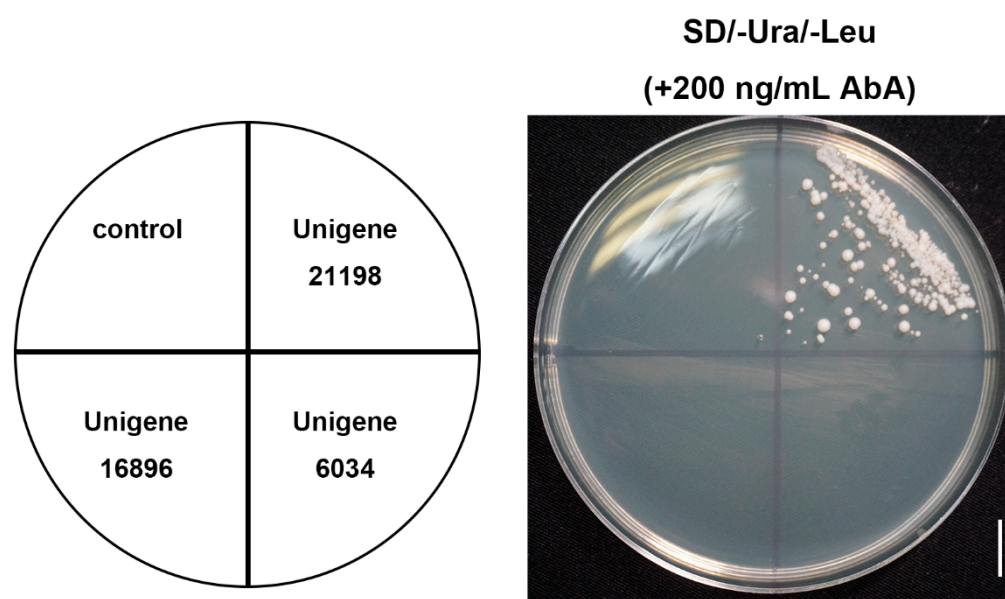


Figure S2. The screening assay of 3 unigenes in Y1H Gold. The pABAi-*DDSpr* were used as the bait and the pGADT7-Rec used as the control. Only the yeast cells with pGADT7-Unigene 21198 (*PgMYB2*) could grow on the SD/-Ura/-Leu selective medium added with 200 ng/mL AbA. Scale bar = 1 cm.

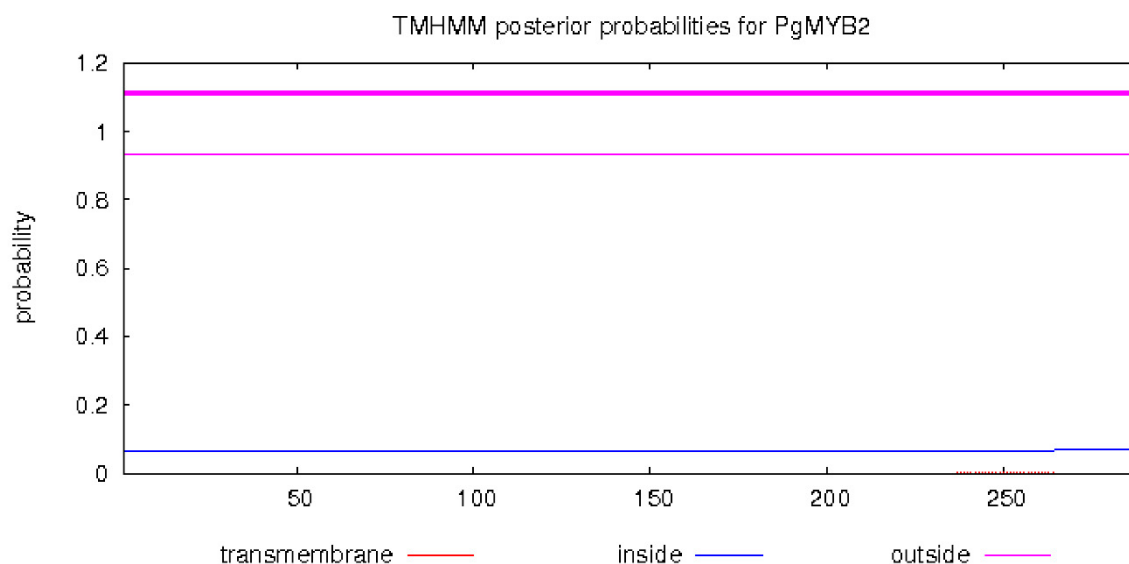


Figure S3. The transmembrane domain of PgMYB2. Predicted by TMHMM Server v. 2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>).

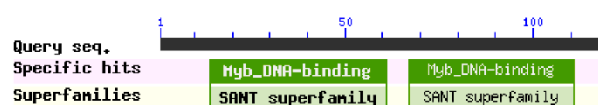


Figure S4. The MYB DNA binding sites of PgMYB2. Predicted by the NCBI conserved domains finder (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>).

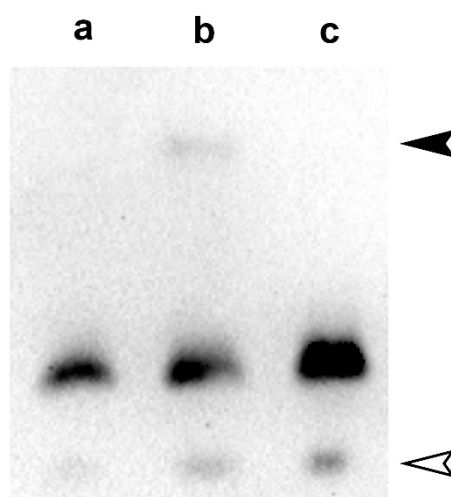


Figure S5. Binding assay of PgMYB2 to MBS site. Contents of reaction: (a) Free probe; (b) labeled probe (containing MBSII site) + PgMYB2: TF protein; (c) labeled probe (containing MBS site) + PgMYB2: TF protein. The protein-probe complexes were indicated with a solid arrow and the free probes were indicated with a hollow arrow.

Table S1. Specific primers used in the study.

Primer Name	Primer sequence (5' to 3')
PgMYB2-F	CCACTCTCAACGCATTCTC
PgMYB2-R	TAGGGGTAGGCACATTCTG
PgDDS-F	ATGTGGAAGCAGAAGGTT
PgDDS-R	AATTTTGAGCTGCTGGTG
β -actin-F	TGCCCCAGAAGAGCACCCCTGT
β -actin-R	AGCATACAGGGAAAGATCGGCTTGA
1302-PgMYB2-F	AGAACACGGGGGACTCTTGACCAAGAAGAAATTGACGACGATG
1302-PgMYB2-R	GTGAAAAGTTCTTCTCCTTTACTATTCCCTTTTCCCAACAGTCC
qPCR-PgMYB2-F	CGGATTATTGAGATGCGG
qPCR-PgMYB2-R	TGATGTGGGTGTTCCAGTAGTTC
qPCR-PgDDS-F	TGAGATTAGATGAAAACGAAC
qPCR-PgDDS-R	GGCAATGATAAGGGGAGGTGT
pABAi-DDSpro-F	AATTCGAGCTCGGTACCCGGGCTTGTAGTTTTGTGATTTTCC
pABAi-DDSpro-R	ATACAGAGCACATGCCTCGAGACTTGTGGTATGTGGTGTA
pGADT7-PgMYB2-F	CATATGGCCATGGAGGCCAGTATGATGGGACGTTACCTTGC
pGADT7-PgMYB2-R	ATCTGCAGCTCGAGCTCGATGTCTATTCCCTTTTCCCAACAGTCC
pCold/TF-PgMYB2-F	ATGGAGCTCGGTACCCTCGAGATGGGACGTTACCTTGC
pCold/TF-PgMYB2-R	AGACTGCAGGTCGACAAGCTTATGTTTTCCCAACAGATGA
EMSA-MBSI-F	ACACGTCTAACACGTCGAATTCCTTT
EMSA-MBSI-R	AAAAGAATTGACGTGTTAGACGTGT
EMSA-MBSII-F	GACTGGCATTGATTTAAAAGGCGGT
EMSA-MBSII-R	ACCGCCTTTTAAATCAATGCCAGTC
EMSA-mutant-MBSII-F	GACTGGCttccgggcacctGGCGGT
EMSA-mutant-MBSII-R	ACCGCCaggtgcccggaaGCCAGTC
pGreenII 0800-DDSpro-F	GACTAGTTTCTTCCAATACTTGTAG
pGreenII 0800-DDSpro-R	CATGCCATGGCATTCTTAAGTCTACTAC
pEGAD-MYC-PgMYB2-F	CCGGAATTCATGGGACGTTACCTTGC
pEGAD-MYC-PgMYB2-R	CGCGGATCCACAATATCTGTAAAACCCA

pGreenII 0800- <i>DDSpr</i> - <i>xMBSII</i> -F	AAATCTGTCTCTGCTGACAAAAATTCCTAGACC
pGreenII 0800- <i>DDSpr</i> - <i>xMBSII</i> -R	GTCAGCAGAGACAGATTTTTGTTAAAGTTAAATAAAGAAAACCTT GAACTA

MBS, MBSII and its mutation sites are underlined in the table. These primers were designed by Premier 5, Oligo 7 and SnapGene software.