Supporting information (Tables and figures)

Co-regulation of biosynthetic genes and transcription factors for aporphine-type alkaloid production in wounded lotus provides insight into the biosynthetic pathway of nuciferine

Thitirat Meelaph[†,§], Khwanlada Kobtrakul[†,▽], N.Nopchai Chansilpa[⊥], Yuepeng Han[‡], Dolly Rani[†,▽], Wanchai De-Eknamkul[†,▽], Sornkanok Vimolmangkang[*,†,▽]

[†]Research Unit for Natural Product Biotechnology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

[‡]Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden of the Chinese Academy of Sciences, Wuhan, 430074, P.R. China

[§]Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

[⊥]Faculty of Agriculture and Natural Resources, Rajamangala University of Technology Tawan-ok, Chonburi 20110, Thailand

[▽]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

*Corresponding author:

Sornkanok Vimolmangkang; E-mail: Sornkanok.v@pharm.chula.ac.th

Postal address: Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

Table S1. Primers for amplification of cDNA fragments of *NnCNMT* and *NnOMT* genes in lotus

Target genes	Forward Primer (5'-3')	Reverse Primer (5'-3')	**Template**
CNMT	ATGGATGCGTTGATCCAGGTACC	TTAATTTATTTTTTTCTTGAATAGGAACA	NNU_11880
6OMT	ATGGAAATTCAGAAGGAAGTTCAAGCAGCCGAC	CTAATAAGGATAGGCCTCAATTACAGATTGAACGGCAGC	NNU_19035
	GAAGGAAGTTCAAGCAGCCGAC	GATGTGAGGAGCCTGGTCGT	NNU_03165
	ATGGAAATTCCGAAGGAAGTTCAAGCT	GAGAGAAGGCTAATAAGGATAGGCCAT	NNU_03166
	GAGGAGAATGGAAATTCAGAAGGAAGG	CTAATAAGGATAGGCCACAATTACAG	NNU_23168
CYP80G2	CTCATGTGGCTGCTGTGGAA	GCTTGAATATCCAACATCTTGGTAGAG	XM_010255688.1

Table S2. A list of putative BIA-related gene retrieved from coding sequences of *Nelumbo nucifera*

Gene code	Sequences (5'-3')
NnCNMT1	ATGGATGCGTTGATCCAGGTACCATACGATGCAACTGTACGTTTAATGCTG TCGTCTCTCGAGCGTAACCTCCTCCCCGACGTCGTCATAAGGAGGCTCAC GCGGCTGCTGTTGGCTAGCCGTCTTCGTTGGGGATACAAGCCGTCCTCTC AACTCCAACTTTCTGATCTTCTCCAATTTGTTCACTCGCTAAAAGATATGCC CATTGCCATCAAGACCGACTTACCAAAGTCCCAACATTATGAATTACCCACT TCCTTCTTCAAGCTGGTTTTAGGGAAGAATCTCAAATACAGCTGCTGTTACT TCCTTGACAAGTCAAGCACCTTAGAGGATGCAGAGAAAGCTATGCTGGAG CTGTACTGTGAGAGGGCACAGATCAAAGATGGCCAATCTGTGCTTGATGTT GGTTGTGGCTGGGGATCATTGTCCTTGTATATTGCACAAAAGTTTTCTAGCT GCAGGATAACAGGGATTTGCAATTCAAAGACACAGAAAGCATATATAGAGG AGCAATGTAGGGAACTGAAGCTGCAAAATGTGGAGATCATTGTTGCAGATA TCAGCACTTTTGAAATGGAGGCATCATTTGATAGGATTTTATCCATAGAAAT GTTTGAACACATGAAGAACTACAAGGCACTTCTTAATAAGATATCAAAATGG ATGAAGGAGGATAGCCTCCTTTTATTTAACTACTTCTGCCATAAAGCATTTG CTTACCACTTTGAGGACAAGAATGAAGATGACTGGATTACCAGGTACTTCT TCACTGGAGGGACAATGCCTGCTGCAAACCTTCTCCTCTATTTCCAGGATG ATGTTTCTGTTGTCAACCATTGGCTTGTAAATGGGAACCATTATGCAAGAAC AAGTGAGGAGTGGCTTAAAAGAATGGACCAGAACATGGCTTCTATTAAGCC AATAATGGAGTCAACTTATGGCAAGGATTCCGCTGTTAAGTGGACTGCCTA TTGGCGTACATTCTTCATCTCAGTGGCAGAACTGTTTGGCTATAACAATGG AGAAGAATGGATGGTTGCACTGTTCCTATTCAAGAAAAAAATAAATTAA
NnCNMT2	ATGGATGCGTTGATCCAGGTACCATACGATGCAACTGTACGTTTAATGTTG TCGTCTCTCGAGCGTAACCTCCTCCCCGACGTCGTCATAAGGAGGCTCAC GCGGCTGCTGTTGGCTAGCCGTCTTCGTTGGGGATACAAGCCGTCCTCTC AACTCCAACTTTCTGATCTTCTCCAATTTGTTCACTCGCTAAAAGATATGCC CATTGCCATCAAGACCGACTTACCAAAGTCCCAACATTATGAATTACCCACT TCCTTCTTCAAGCTGGTTTTAGGGAAGAATCTCAAATACAGCTGCTGTTACT TCCTTGACAAGTCAAGCACCTTAGAGGATGCAGAGAAAGCTATGCTGGAG CTGTACTGTGAGAGGGCACAGATCAAAGATGGCCAATCTGTGCTTGATGTT GGTTGTGGCTGGGGATCATTGTCCTTGTATATTGCACAAAAGTTTTCTAGCT GCAGGATAACAGGGATTTGCAATTCAAAGACACAGAAAGCATATATAGAGG AGCAATGTAGGTAAGTTGCATGAAATACACAATATGTTCTGTTAGATCCCTT ATTTGATCTTCTGGACCAGTAACAAAAGGGTTACAAGGCGCTGAAAACAGT TACGGATGGCAAAGTTGCATCTACAAGAACCACAGCTTCAAACCTCTACTT TTTCTGTTTGAATCAACATATCACAGCACTTCCTACTTCTACAAGATGATTG AAGTTCCATTAGCTCATTTCTGTTTGGTCCATATTTATAACTACAGGTGCAT GCTTTCTGAAAATGGTGAATTCATCTCTGCAACCTAAGATAACCCACACATT TTCATCTTATACAGGGAACTGAAGCTGCAAAATGTGGAGATCATTGTTGCA GATATCAGCACTTTTGAAATGGAGGCATCATTTGATAGGATTTTATCCATAG AAATGTTTGAACACATGAAGAACTACAAGGCACTTCTTAATAAGATATCAAA ATGGATGAAGGAGGATAGCCTCCTTTTAGTTAACTACTTCTGCCATAAAGC ATTTGCTTACCACTTTGAGGACAAGAATGAAGATGACTGGATTACCAGGTA CTTCTTCACTGGAGGGACAATGCCTGCTGCAAACCTTCTCCTCTATTTCCA GGATGATGTTTCTGTTGTCAACCATTGGCTTGTAAATGGGAACCATTATGCA AGAACAAGTGAGGAGTGGCTTAAAAGAATGGACCAGAACATGGCTTCTATT

	AAGCCAATAATGGAGTCAACTTATGGCAAGGATTCCGCTGTTAAGTGGACT GCCTATTGGCGTACATTCTTCATCTCAGTGGCAGAACTGTTTGGCTATAAC AATGGAGAAGAATGGATGGTTGCACTGTTCCTATTCAAGAAAAAAATAAATT AA
NnCNMT3	ATGGATGCGTTGATCCAGGTACCATACGATGCAACTGTACGTTTAATGCTG TCGTCTCTCGAGCGTAACCTCCTCCCCGACGTCGTCATAAGGAGGCTCAC GCGGCTGCTGTTGGCTAGCCGTCTTCGTTGGGGATACAAGCCGTCCTCTC AACTCCAACTTTCTGATCTTCTCCAATTTGTTCACTCGCTAAAAGATATGCC CATTGCCATCAAGACCGACTTACCAAAGTCCCAACATTATGAATTACCCACT TCCTTCTTCAAGCTGGTTTTAGGGAAGAATCTCAAATACAGCTGCTGTTACT TCCTTGACAAGTCAAGCACCTTAGAGGATGCAGAGAAAGCTATGCTGGAG CTGTACTGTGAGAGGGCACAGATCAAAGATGGCCAATCTGTGCTTGATGTT GGTTGTGGCTGGGGATCATTGTCCTTGTATATTGCACAAAAGTTTTCTAGCT GCAGGATAACAGGGATTTGCAATTCAAAGACACAGAAAGCATATATAGAGG AGCAATGTAGTTACGGATGGCAAAGTTGCATCTACAAGAACCACAGCTTCA AACCTCTACTTTTTCTGTTTGAATCAACATATCACAGCACTTCCTACTTCTAC AAGATGATTGAAGTTCCATTAGCTCATTTCTGTTTGGTCCATATTTATAACTA CAGGGAACTGAAGCTGCAAAATGTGGAGATCATTGTTGCAGATATCAGCAC TTTTGAAATGGAGGCATCATTTGATAGGATTTTATCCATAGAAATGTTTGAA CACATGAAGAACTACAAGGCACTTCTTAATAAGATATCAAAATGGATGAAG GAGGATAGCCTCCTTTTAGTTAACTACTTCTGCCATAAAGCATTTGCTTACC ACTTTGAGGACAAGAATGAAGATGACTGGATTACCAGGTACTTCTTCACTG GAGGGACAATGCCTGCTGCAAACCTTCTCCTCTATTTCCAGGATGATGTTT CTGTTGTCAACCATTGGCTTGTAAATGGGAACCATTATGCAAGAACAAGTG AGGAGTGGCTTAAAAGAATGGACCAGAACATGGCTTCTATTAAGCCAATAA TGGAGTCAACTTATGGCAAGGATTCCGCTGTTAAGTGGACTGCCTATTGGC GTACATTCTTCATCTCAGTGGCAGAACTGTTTGGCTATAACAATGGAGAAG AATGGATGGTTGCACTGTTCCTATTCAAGAAAAAAATAAATTAA
Nn6OMT35	ATGGAAAATCAGAAGGAAGTTCAAGCAGCCGAGGCTAAAATCTGGAATTTC GTCTATGGCTTTGCCGACACTTTAGTCCTCCGATGTGCCATTGAGCTGGGT ATTGCAGACATAATCCATAAGCAGGGAGAACCCTTGACGCTCTCTGAACTG GGGGCTCAAATTCCTCTGAAGTCGGTCAACACCGACCACTTGCACAGGTTA ATGCGTTACTTGGTGCACATGAAGCTCTTCACCAAGGAAACCCTAGATGGC GAAGCTCGATATGGGCTGGCTCCACCGGCTAAGTTGCTTGTAAAATGGTG GGAGGACAAGGGCTTGGCGTCAATCATATTTGGGATCACTGACAAGGATTT CATAGCACCCTGGCACCATCTCAAGGATAGCTTGGCCGGCGATGGCGAGG AGACAACTTTTGAGAAGGTGTTAGGGAAGAGCATATCGACATACATGGCTG ATCATCTGGAGAAGAGTATGTTGTTCAATGAATCAATGGTTCATGATACCAG GCTCTTCACATCAGTCTTGATTCAAGACTTCAAGGATGTATTCCAAGGAATT AAGTCGTTGGTGGATGTTGGTGGAGGCTCTGGAACTGACATGGGAGCCAT TGCCAAGGCCTTTCCCCACCTAAAATGTACAATTTATGGTCTACCTCATGTC ATTGCCGACTCCCCTGATTACCCTGAGGTCGACCGGATTTCAGGCGACAT GTTCAAACACATTCCCAGTGCCGATGCCATCTTATTGAAGTGCATCCTCCA TTACTGGGGTGATGGTCAATGCATTGAAATTCTAAAGAGATGCAAAGAATC AGTGCCTAGAGAGGGTGGAATAGTTATCATCGCCGACGCAGTAGTAGATTT GGAATCTAAGCATCCCTACTTAACAAAAACTTTACTAAGCACGGATTTGGAC ATGATGCTCAACACTGGAGGAAAAGAGAGGACTGAGGCAGAATGGAAGAA GCTTTTTAATGCTGCAGGGTTCCCTGCATATAAGATTACACATGTAGCTGAC GTTGAGTACTCTGTAATTGAGGCCTATCCTTATTAG

Nn6OMT65	ATGGAAATTCAGAAGGAAGTTCAAGCAGCCGACGTTGAAATCAGGAAATTC GGTTATGGCTTTGCCGACATTTTAGTCATCCGATGTGCCATTCAGCTCGGA ATTGCAGACATAATCCATAAGCAGGGGGAACCCTTGACGCTCTCTGAACTG GAGGCTCAAATTCCTGTGAAACCGGTCAACACCGATCACTTGCACAGGTTA ATGCGTTACATGGTGCACATGAAGATCTTCACCAAGGAAACCCCTGATGGC GAAGAACGATATGGGCTGGCTCCACTGGGTAAGTTCCTTGTAAATGGGTG GGACAGGAACATGGTGTCAGCCATATTAGCGGTCACTGACAAGGATTTCAT GGTACCCTGGTACCGTCTCAAGGATAGCTTGGTCGGCGAGGGGACAGCTT TTGAGAAGGCGTTAGGGAAGACCATATGCGAATGCATGGCTGATCATCCG GAGAAGAAAAAGCCCTTCAATGAAGCAATGGCTTGTGATACGACCAGGCTC CTCACATCAGCCTTGATTCAAGACTGCAAGGATTTATTCCAAGGAATAATGT CGTTGGTGGATGTTGGTGGAGGCACTGGAACTGCCATGAGAGACATTGCC AAGACCTTTCCCCACCTAAAATGTACAATTTATGATCTACCTCATGTCATTG CCGACTCCCCGGATTACCCTGAGGTCGACCGGATTGCAGGCAACATGTTC AAACACATTCCTAGTGCCGATGGCATCTTGTTGAAGTGCATCCTCCATGAC TTGGGTGACCGTCAATGCATTGAAATTCTACAGCGATGCAAAGAATCAGTG CCTAGAGAGGGTGGAAAAGTTATCATCGTCGACATAGTACTAGATCCGGAA TCTACGGATCCCTTAACAAAGGCCAGATTAAGGTTGGATTTGGACATGATG GTCTACACTGGAGGAAAAGAGAGGAGTGAGGCAGAATGGAAGAAGCTTTT GAATGCTGCAGGGTTCCCTCGATATAAGATTTTACATATAGCTGCCGTTCA ATCTGTAATTGAGGCCTATCCTTATTAG
Nn6OMT66	ATGGAAATTCCGAAGGAAGTTCAAGCTGACGAGGTTGAAATCTGGAAATTC GGATATGACTTTGCCGACACTTTAGTCCTCCGATGTGCCATTGAGTTCGGT ATTGCAGACATAATCCATAAGCAGGGAGAACCCTTGACGCTCTTTGAACTG GGGGCTCAAATTCCTGTGCAACCAGTCAACACCGATCACTTGCACAGGTTA ATGCGTTACATGGTGCACATGAAGATCTTCACCAAGGAAACCCTAGGTGGC GAAGAACAATATGGGCTATCTCCACACGGTAAGTTCCTTGTAAAAGGGTGG GACAAGAGCATGGCGTCAGCCATATTAGCGATCACTGACGAGGATTTCTTT GCACCCTGGCACTGTCTCAAGGATGTCTTGGCCGGCGAGGGGACAGCTTT TGAGAAGGCGTTAGGCAAGAGCATATGGGCATACGTGGCTGATCATCCGG AGAAGAATAAACTCTTCAATGAAGTAATGGCTTGTGATACCAGTTTCATCAC ATCAGTCTTGATTCAAGACTGTAAGGATGTATTCCAAGGAATAAAGTCGGT GGTGGATGTTGGTGGAGGCACTGGAACTGCCATGAGAGACATTGCCAAGG CCTTTCCCCACCTAAAATGTACAATTTATGATCTACCTCATGTCATTGCCGA CTCACCTGATTACCCTGAGGTCGACCGGATTGCAGGCGACATGTTCAAACA CATTCCTAGTGCCGATGCCATCTTATTGAAGTGGATCCTCCATGATTGGGA TGATGGTGAATGTATTGAAATTCTAAAGCGATGCAAGGAATCAGTGCCTAG AGAGGGTGGAAAAGTTATCATCGTCGACATAGTACTAGATCCGGAATCTAA GGATCCCTTAACAAAGGCTAGATTAAGGTTGGATTTGGACATGATGGTCTA CACTGGAGGAAAAGAGAGGAGTGAGGCAGAATGGAAGAAGCTTTTGAATG CTGCAGGGTTCCCTGGATATAAGATTTTACATGTAGCTGCCGTTCAATCTG TAATTATGGCCTATCCTTATTAG
Nn6OMT68	ATGGAAATTCAGAAGGAAGGTCAAGCAGCGGCGGCTAAAATCTGGAAATT CGTTTATGGCTTTGCCGACTGTTTAGTCCTCCGATGTGCCATTGACCTCGG AATTGCAGACATAATCCATAAGCAGGGAGAACCCTTGACGCTCTCTGAACT GGGGGCTCAAATTCCTGTGCAACCGGTCAACACCGATCACTTGCACAGGT TAATGCGTTACTTGGTGCACATGAAGATCTTCACCAAGGAAACCCTAGATG GCGAAGCACGATATGGGCTGGCTCCACCGGCTAAGTTCATTGTAAAAGGG TGGGACAAGAGCATAGTGTCAATCATATTAGTGGTCACCGACAAGGATTTC ATGGCACCCTGGCACTGCCTCAAGGATAGCTTGTGCGGCGAGGGGACAG CTTTTGAGAAGGCGTTAGGGAGGAGCATATGGACATACATGGCTGATCATC

	CGGAGAAGAATAAGCTCTTCAATGAAGGAATGGCTTGTGATACCAAACTCC TCATATCAGCCTTGGTTCAAGACTGCAAGGATTTATTCCAAGGAATAATGTC GTTGGTGGATGTTGGTGGAGGCACTGGAACTGCCATGAGAGCCATTGCCA AGGCCTTTCCCCACCTAAAATGTACAATTTATGATCTACCTCATGTCATTGC CGACTCCCCTGATTACCCTGAGGTCGACCGGATTGCAGGCGACATGTTCA AACACATTCCTAGTGCCGATGCCATCTTATTGAAGTGCATCCTCCATGACT GGGATGATGGTGAATGCATTGAAATTCTAAAGCGATGCAAGGAATCAGTGC CTAGAGAGGGTGGAAAAGTTATCATCGTCGACATAGTAGTAGATTTGGAAT CTAAGCATCCCTTAACAAAGACTAGACTAAGCTTGGATTTGGACATGATGG TCACCACTGGAGGAAAAGAGAGGACTGAGGCAGAATGGAAGAAGCTTTTG AATGCTGCAGGGTTCCCTGTATTTAAGATTACACATATATCTGCCGTTCAAT CTGTAATTGTGGCCTATCCTTATTAG
WRKY1	ATGACTCCGGCAATAGCAGCAGCACTCAAGCTGAAGGGACTAGCAGGAGT AACATGCAAGCGACGGGGAACTAATAAGGCTGTTGGAGCAAGAGTTGTATT TAGAACAAAAACCGAGCTAGATATTATGGACGACGGCTTCAAGTGGAAGAA GTATGGGAAGAAGATGGTGAAGAACAGACCATTTCCAAGGAACTACTACAG GTGTTCGGTGGAAGGATGCCCAGTGAAGAAGAGAATAGAAAGAGATGCAG ACGATCCACAGCACGTGATAACGACATACGAAGGCACCCACAACCACGAA AGCCCCTCGGCCTGA
WRKY 2	ATGAATATTGCCCAGAATTTGTCTATCGATATCGAGATGTCTAATCGTCATC CGACTCCAGCCGGAATTTCACCGGAAAAAATCTCCCATCATCTCCTAAACT TCGAACTTCCCGACTACCTGGACTTGAACCAATGGTTTGAAGGAGACACAG CATCTGTCACTCATGGATCTTCTAATCAGGATTTGATCCTTCCAATGCCCAA CATCGTCGGTTCCGGCAAGAATGGCAGTCAAGCTGAAGTCTCTAGCCCGA GCAACATGCAATGTTACTTTCTTCTGTGTGCAGCTTTGGAATTTTCTTTCTTT TCTGTATCAATTAAAACTGGTATTTGTTTTCATGTTGTCAGGGGACGTCGTA GTCGAACCAGAAATTCCGTTCTGGCTAGAGTTGCATTCAGAATAAAAACCG AGAAAGATATCTTAGACGATGGATTCAAGTGGAAGAAGTATGGAAAGAAGA TGGTGAAGAACAAACCATATCCAAGGAACTACTTCCGGTGTTCAGTTGAAG GATGTCCAGTTAAGAAGAGAATAGAAAGAGATGCAGACGACCCACGCCAT GTGATAACTACATATGAAGGCACCCATAACCACGAAAGCCCCTTTTCCTGA
WRKY 3	ATGGATGTAGGCTCCAGAGTTGCATTCAGAACCAAATCTGAGCTTGAGGTC ATCGACGATGGATTTAAATGGAGAAAGTACGGGAAGAAGACGGTGAAGAA CAGTCCAAACCCGAGGAATTACTATCGCTGCTCAAGTGGAGGATGCAATGT GAAGAAGAGAGTGGAAAGAGACCGTGAGGACTCGAGGTATGTGATAACGA CGTATGAGGGTGTGCACAATCATGAAAGCCCGTGTGTGGTTTATTACAACG AAATGCCATTAATGGTTCCTAGTGGATGGACTTTGCAAGCTTCACATTCACA CTCATCATCCTCTTGA
WRKY 4	ATGGAAGGAGAAGCCCCACCACTACTGCCACCATTGTCGTCCCACAATAAC CCATCTTACATCTTGACACCCTCACTTGCATCCACGTCATTGCACCCTCCTC TTCTTTATCAACCTTATAACCTGCTGCAAGGTTCCAATATCCTACCAGACAT CGACTGGGTTAGCCTCCTCTCTTCGCCATTTGGATTTGGCGATCTGCAGAA GCCACTCTTGTCGAATGCAGATGTGACAACTAGAACTGGAAATAAAGCCGA AGATGAAAAGAGTGGTAAAGATAAGGTAAAATCGAGCAGGATGAAGAAGG CAAGTCGGCCGAGGTTTGCGTTCCAGACGCGGAGCACAGAAGATATTCTC GATGATGGTTACCGCTGGAGGAAATACGGGCAGAAAGCTGTGAAGAACAG CAACTTTCCCAGGAGTTATTATCGCTGCACGCATCATACATGCAATGTGAA GAAGCAGGTTCAACGACTGTCAAAGGACACAAGCATCGTCGTGACAACATA TGAAGGCATACACAACCATCCATGTGAGAAACTAATGGAGAGTTTGAGTCC

	TCTTCTGAAGCAAATACAGTTCCTCTCT
WRKY 5	ATGGACATGGAGAACTACCCAATACTCCTCTCCTCTTCATCATCATCATCGT TAGCAACCGCTATTCCATTCTCATCTAACATGGTGACTTCTCATGTTATTAA CCATCTTCATGGAAACAATCCACCTGGGTTCTTAGGATTGAAGTCGGAGAT GGATACCCCACTTAGCTCCGACGACTTTACAACCACCCTTCCTCAGATTCA GAGCTTTGGTGGGCCTAAAAATGAGATGAAACTAGGTATCAAAAAGGGGG AGAAGAAGATTAGAAAGCCCAGATATGCTTTTCAAACAAGAAGCCACGTCG ATATACTTGATGATGGATATCGATGGAGGAAATATGGCCAAAAGGCTGTGA AGAACAACAAATTTCCTCGAAGCTACTATCGGTGTACGCACCAAGGATGCA ACGTGAAGAAGCAAGTTCAACGGCTATCCAAAGATGAAGGAATTGTGGTGA CAACCTACGAAGGGATGCATACCCATCCTATTGAGAAGTCTACCGACAACT TTGAACACATCTTGAATCAGATGCAAATCTATTCTGCCTTTTAG
WRKY 6	ATGGAGAACTATTCAATACTCTTCCCGTGTTCATCATCATCGTCGGCAGCA GTAGCTGTTCCATTCTCCTCAAACATGGCAAATTCTCGTATTTTTGCTGATC TTAATGACACCAGTCCAGCTGGGTTCTTAGGATTGAAGACGGAGACGGAT GCACATGCACCACGTTCAGATGTTAAAACCCTTCTTCAGAATGAAAGCTTT GGCCGGCCTAAAAGTGAAACGAAGCTCGGTATCAATAAGAAGGGTGATCA GAAGAAAATCAGAAAACCCAGATATGCTTTTCAAACAAGAAGCCAGGTCGA TATACTTGATGATGGATATCGATGGAGGAAATATGGGCAAAAGGCTGTGAA GAACAACAAATTTCCTCGGAGCTATTATCGATGTACGCATCAAGGATGCAA TGTCAAGAAGCAAGTTCAACGCCTATGCAAAGATGAAGGAATCGTCGTGAC AACCTACGAAGGGATGCATACTCATCCAATTGAGAAATCTACGGACAACTT CGAACACATTCTGAGTCAGATGCAAATCTATGCTTCCTTTTAG
WRKY 7	ATGGAGAAGAAAGAGACAACAATGGAGACAGATAATTCGATCGGAGCTAC GACATTTTCCGATCAGATTCCAACCACTTTCTCTTTATCCAGCATCTTTGAC ATGTCCTGTGAAGGTGAAAAAGGCTCTTTAGGCATCATGGATTTATTGGGC ATCCAAGATTTCACTCCTTCTATATTCGATTTGCTACAGCAACCGTCGACGC TACTACCACCATCACCACCACCGCCACTACCACCGACGTCATCACTTCCGG AGTCGTCTGAGGTGTTGAATTTGCCAGCAACACCCAACTCTTCTTCGATTT CATCATCATCGACTGAAGCAGCAAATGATGAACAGACCAAAGCAGTGGAAG AGGAGGAGCAGGAGAAGACTAAGAAGCAGCTGAAACCCAAAAAAAAGAAC CAGAAACGGCAGAGAGAACCGAGATTTGCTTTCATGACAAAGAGCGAGGT CGATCATCTGGAAGACGGGTACAGATGGAGAAAGTATGGACAAAAAGCTG TGAAAAATAGCCCTTTTCCAAGGAGCTACTATCGTTGCACCAGTGCCACAT GCGGTGTGAAGAAGCGAGTGGAGAGATCATCAGATGATCCTTCCATTGTC GTGACAACGTACGAAGGCCAGCACACACATCCAAGCCCAGTAATGCCTCG TGGAAGCTCCACCGGAATCTCTTCGGATTCCGGCAGCTACGGTGCGGCCT TTGCCATGCCAATGCAATTGACGCAGTCTCACTTCCAACAACAGCAACAAC AACAACAACCCCATTTCCACAACTTACCACCTTTGAATTTTAATTCTAATATT TCTTCGTCTCCTACTTTTGTACAAGAGAGACGATTTTGCACTTCAGCAGCTT CCTTCCTTAGAGATCATGGCCTTCTTCAAGATGTCGTTCCTTCGGATATGAT GATCAAAAAGGAGTAG
WRKY 8	ATGGAGACAGAGAATTCCATTGGAGCAGCAGTTACGGCGTTTTCGGATCA GATTCCCACCAACTTCGCTTTATCCAGCATCTTCGACACGCCTTTCGGAGG TGAAAAATGGTCTCTAGGATTCATGGATTTGTTGGGAGTCCAAGATTTTACC CCATCCATGTTCGATTTACTACAGCAACCTTCGATGCCATCACCGCCACCC ATAGTGTCAGTCGGGGAGTACTCCTCCGATATATTGAATTTGCCTGCAACG CCCAACTCTTCTTCCATTTCGTCATCATCGACTGAAGCAGCAAATGATGAAC AGTCTAAAGCAGTGGAAGAGGAGGAGCAGGAGAAGACTAAGAACCAACTG

	AAATCCAACAAGAAGAATAAAAAACGGCAGAAAGAGCCGAGATTTGCTTTC ATGACGAAGAGCGAGGTTGATCATCTGGAAGACGGGTATAGATGGAGAAA GTACGGGCAAAAAGCAGTAAAGAACAGCCCCTTTCCGAGGAGCTACTACC GTTGCACCACTGCCACATGTGGTGTGAAGAAGAGAGTGGAGAGATCGTCG GATGATCCGACCATTGTCGTGACAACGTACGAAGGTCAGCACACACATCC GAGCCCTGTAATGCCTCGTGGAATCTCCACCGGAATCTCTCCGGATTCCA GCAGCTACGCTGCGGCCTTCGCCATGGCGCCGATGCAATTGACGCAACCT CATCATGACTTTCAAGAACAGCAGCAACCCTATTTCCATAACTTATTACCAC CTTCTCTCAACTATAATTCTAGTGTTTCTTTGGCTCCTACTTTCGTACCAGA GAGACGTTTTTTCACTTCTGCAACTTCTTTTATCAGAGACCACGGCCTTCTT CAAGACATAGTCCCTTCGGATATGAGAAGTGCTTAG
WRKY 9	ATGGGGACAGTGGAATCTCCGTGGCCGGAAAATTCGCCAATCAACCGAAG AAAGGCGATCAACGAGCTTGTCCGTGGCCGTGAATTTACGACCCAGCTTC AAATTATCCTCCGGAATCCCCTCGGAGGTCATGGATCCGTGTCGGCGGAA GACCTCCTCCCAAAAATCTTAACATCGTTCACTGAGGCTATTGCGGCACTC AACACCACTGTCGAATCCGGGGAGGTTTCCCAGAATCCGGCGAGTACCCA TGTAAGTTCGCCCAGTTGCGGTGACCGGGCGACCGAGGATTCCGGTGAGA GCAAAAAGTCTACGGTTCTCAAGGATCGCAGAGGAAGTTATAAGCGAAGAA AGTTGTTGCAGACATGGACAAAGCTCAGCGCTACTCCGATCGATGACGGC CGTGCGTGGCGGAAATACGGCCAGAAAGTGATCCTCAACTCCAAATACCC AAGGAACTACTACAGGTGTACCCACAAGAACGATCAAGGCTGCCAAGCAA CCAAACAGGTCCAACAAACCGAAGACAACCCGCCCATGTATCGGACCACA TACATAGGCGATCACACATGCATAGACATGTCAAAGGCTCCCCGATTCCTC CTGGATTCTATCCACAACAACGCTTTTGTGCTCAGCTTTGAACCCGAAGCT CCGAGGAAGCAAGACCAACCCCACCCCTTTTTCTCCTGCTTTTCTCCCTCA ATAAAACAGGAATGCAAAGAGGTGGAGATCCCGAGTGACCCGACCCACAA GCAATGTTCATCGGAATACCATCTTTCCCACGATGAAATCACATTCGGATC GTCCTGCCCCACAACAGTATTGCCGTCGACACCCGGGTCGGATCACGGGG ATGTGATCTCGGGCGTGTACTCAGGTAGCACCAGCTCTCGCAGTCTGGAC AATTATTTTGTGGGGATGAGTGACTTTGATGACGTATTCCACTTTGAAGAGG ACATCTTTCAGGTTTCAATGTAA
WRKY 10	ATGGACACAACTGTTGAATCTCCGTGGCCGGGAAATTTCTCTATTGATCGG AAAAGGCTGATCAACAGTCTCGTGCGAGGCCGTGAACTCACAACCCAACTT CAAACTATCCTCTGCAATCGCCTCGGACATGAGGATGGGTCCTTGTCCACC GAAGATCTCCTCCCAAGAATCTTACGATCCTTCACCGAGGCTATTTCTGCA CTCAAGTCCGCTGACTCCGGAGAGGTCTGTCAGAACCCGTCGAGTACGAA TGTGAGTTCGCCCAGTTGTGATGGCCCAAGGACGGAAGATTCCGGCGAGA GCAGGAAGTCTCCAGCAGTCAAGGATCGTAGAGGAGATTATAAGAGAAGA AAGGTTTCTGAGACATGGACAAAGATCACTCCCACTCCCATTGACGACGGC CGTGCGTGGCGAAAATACGGCCAAAAAGTGATCCTCAATTGCAAATACCCA AGGAACTATTATAGGTGCACTCACAAAAACGACCAAGGATGTGCAGCAACC AAACAAGTACAGCAAATTGAAGATGACCCACCAAAGTATCGAACTATATACA AGGGCCAGCACACATGCAAAGATATATCAAAGCCCCCCCAATTCATCATGG ACTCTACCCATACAGACTCCTCCTCCTTCGTTCTAAGCTTTCAGTCAGACTC AGACGCTCCAATAACTAACCAGCAGCAGCTCCAGGAGCACCATCCCTTCTT CTCTTCCTCTTTCCCCCCAATAATAAAACAGGAATGCCAGGAAGAAATCCC AAACCATGAGATGGAGGAGCTCACCCACAACCAGCAATCTTCATCACAATA TTTTCTGCCATCTGTCCCTACATCAGCGTTGCCATCCACACCCGGGTCTGA TCATGGGGATGTGATTTCGGGCGTCTACTCGTGTAGCACCAGCTCTCACA GTCTGGACATGAGCTTCGTCGGTGACTTTGATGATGTTTTCCATTTTGATGA TGATGACTTTTTTCCGGTTTAA

CYP80G2	ATGGCTCTACTAGTCTCGTTTATTTTCTTCTTACTGTCCATTCTCTCAGTAGT ACTCTTCCTTAAACCATCTCCTAATAACCTTCCCCCAGGACCCTTTTCATGG CCAATTATAGGGACCCTGTTGCCCAAGCTGAAGAAGCAACCCCATGTCGA GCTAAGCAAATTGGCACAGAGATTTGGTCCACTAATGCTCCTAAAATTTGG GGTTGAGCCCGTTGTTGTGGCTTCGAGCCATGTTGCAGCCGTGGAGGTGC TCAAGAATCAGGATCGATTGCTTTCAGGCCGCTTTGCACCACACAGCGTCC GAATTAAGGGCTATATTGAACACTCCATGGTGTGGGCTGATTGCACTGATT ACTGGAAGATGGTCAGGAAGGTATGGAGGACTGAATTGTTCTCTACTAAGA TGTTGGACATTCAGGCACACGCCAGAGAGGAGAAAGTATCGGAACTGATG AAATTTCTCATACGGAAGGAAGGAGAGAAGGTGAATTTTGCTGATGTGATA TTCGGTTCCATTTTGAACATATTAGGTGCACTTATTTTCTCCAAAGACGTGT ATGATTTTGAAGACAGGACGGATAATAACTTGGGTATGAAGGGCATGATTC GGCAGCTGATGATATTGGCAGCCATCCCAAATCTAGCCGACCTCTACCCAA TTCTTGGCGGATCAGACTTTCAGGGCTTGAGGAAGGCATCGGCAGCGTGT GTCAAGCGGATGAATGAGTCATGGGCCGCCATTGTTAAACAAAGGAGGAA GAATGATGACCACTCCAAGAATGATTTCTTGCAAGTTTTGCTCGATTCTGG GTTCAGTGATCCCCAGATTGACGCCATGCTTCTGGAAACGTTTGGACCTGG TTCAGACACTAGTACCTCCACGATTGAATGGGCAATGGCAGAATTGCTGCG GAACCCAGAGAAGCTGGTAAAGGTCCGCGAGGAACTCGACAGGGTGATCA GAAGAAGCAACAATGTGAAGGAGTCTGATCTGCCAAACCTACCTTATCTCC ATGCCTGTGTCAAAGAGACCCTCAGGTTACACCCTCCGGTCACCTTCCTTC TTCCACACCGAGCAATGGAAACTTGTCAAATGATGAATTACACGATTCCAAA GGGATGCCAACTGATGGTAAACACATATGCAATTGGAAGAGATTCCAAGAC ATGGGAGAAACCCTTGTCTTTCTTGCCAGAACGATTTCTGAACTCAGAACTT GATTACCAAGGTAACGATTTCCAGTACATACCATTTGGTGCCGGCAGAAGA ATCTGTCCAGGGTTGTCATTGGCAACTCGAGTTGTTCGACTGATACTCGCT TCTCTTCTCCACACTTTTGATTGGAGCCTTCCTGATGGAATGCACCCAGAT GAGCTAGACATGAACGATAAGTTTGGGCTGGCTCTCCAGAAGGACATCCC TCTGGTAGTCATTCCCAAGTTGAGGAAGTAA

Table S3. The list of primers for qRT-PCR

Target genes	Forward Primer (5'-3')	Reverse Primer (5'-3')	PCR product size (bp)	Template
NCS	TGCCTGCTGACGATATTTGGG	GTGCCGACCGTTCCATCAC	121	Consensus sequence from 7 isoforms of NCS (Vimolmangkang, Deng et al. 2016)[1]
CNMT	TGCCATCAAGACCGACTTACCAAAG	GATCTGTGCCCTCTCACAGTACAG	175	Consensus sequence from 2 isoforms of isolated CNMT
6OMT	TCCGATGTGCCATTGAGCTGG	GTAACGCATTAACCTGTGCAAGTG	134	Consensus sequence from 4 isoforms of isolated 6OMT
ACTIN	GGTGCTGAGTTCGTCGTAGA	TGGGAATGATGTTGAAGGAA	120	XM_010267617
WRKY1	ATAAGGCTGTTGGAGCAAGAGT	CTGTGGATCGTCTGCATCTCT	191	NNU_11881RA
WRKY2	GACACAGCATCTGTCACTCATG	GACGTCCCCTGACAACATGAA	211	NNU_05136RA
WRKY3	CGGTGAAGAACAGTCCAAACC	GTTGTAATAAACCACACACGGGC	161	NNU_09891RA
WRKY4	TCTGCAGAAGCCACTCTTGT	GAATATCTTCTGTGCTCCGCGT	190	NNU_01372RA
WRKY5	GAAACAATCCACCTGGGTTCTTAGG	ACGTGGCTTCTTGTTTGAAAGC	190	NNU_22208RA
WRKY6	ATCTTAATGACACCAGTCCAGCT	CTGATTTTCTTCTGATCACCCTTCTTA	166	NNU_02028RA
WRKY7	AGTCTCACTTCCAACAACAGC	GATCATCATATCCGAAGGAACGACA	188	NNU_05834RA
WRKY8	GGCGCCGATGCAATTGAC	CGTGGTCTCTGATAAAAGAAGTTGC	173	NNU_13849RA
WRKY9	GCCGGAAAATTCGCCAATCAA	GACAGTGGTGTTGAGTGCC3	190	NNU_24385RA
WRKY10	CATGAGATGGAGGAGCTCACC	CGACGAAGCTCATGTCCAGAC	169	NNU_12194RA
CYP80G2	CTCATGTGGCTGCTGTGGAA	GCTTGAATATCCAACATCTTGGTAGAG	193	XM_010255688.1

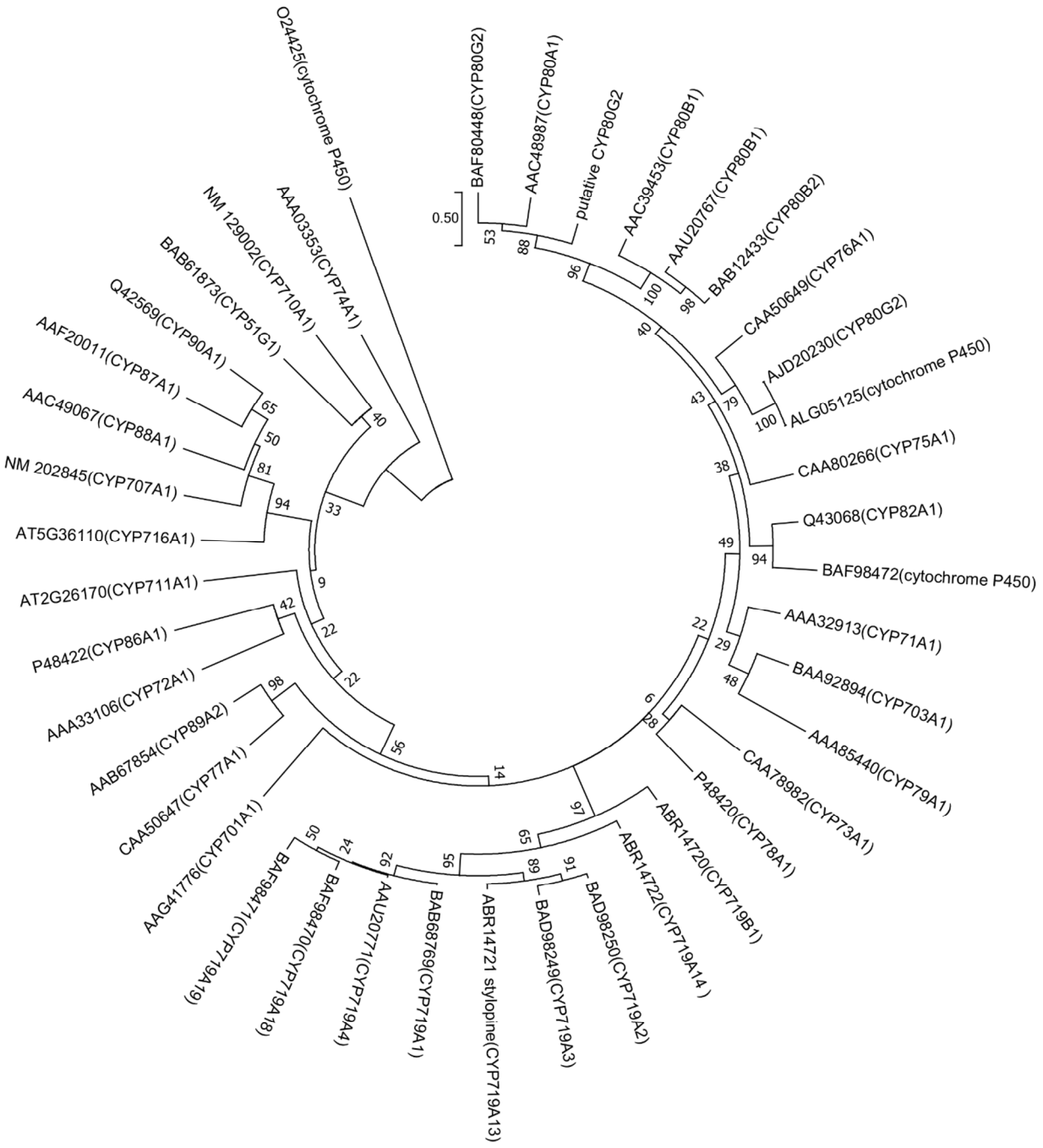

Figure S1. Phylogenetic tree of deduced amino acid sequences of 60 WRKY TF sequences retrieved from lotus genome analyzed with secondary metabolite-related WRKY from GenBank. Molecular phylogenetic analysis was constructed by Maximum Likelihood method based on the JTT matrix-based model conducted in MEGA7. The bootstrap consensus tree inferred from 1000 replicates.

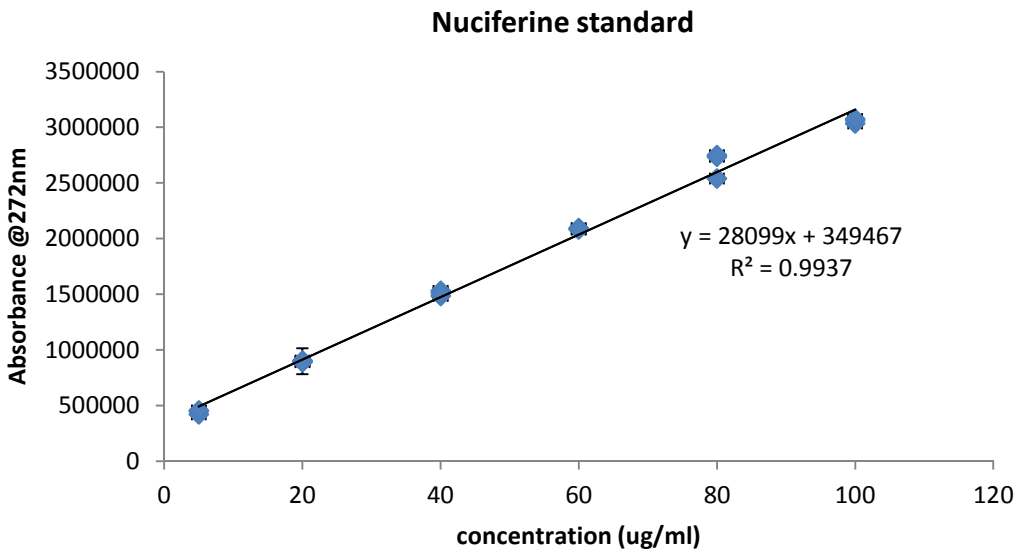

Figure S2. Linearity of calibration curve of nuciferine standard. The absorbance was recorded at 272nm and column temperature was controlled at 30□C. Each standard concentration was conducted in triplicate.

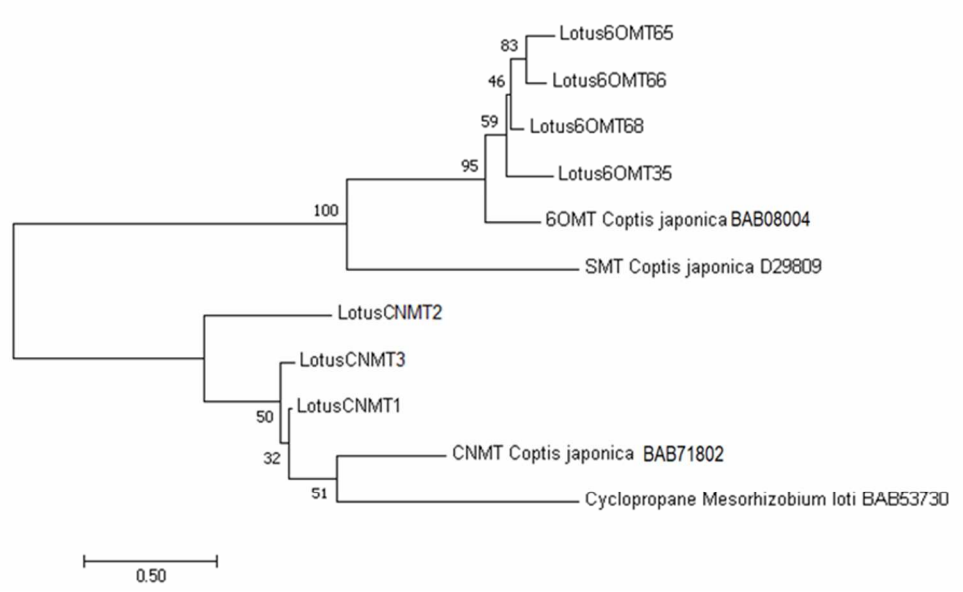

Figure S3. Phylogenetic tree of deduced amino acid sequences of isolated 6OMT and CNMT analyzed with plant *S*-adenosyl-*L*-methionine-dependent methyltransferases (SAM). Molecular phylogenetic analysis was constructed by Maximum Likelihood method based on the JTT matrix-based model conducted in MEGA7. The bootstrap consensus tree inferred from 1000 replicates.