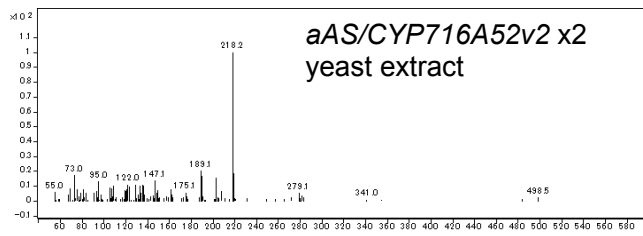
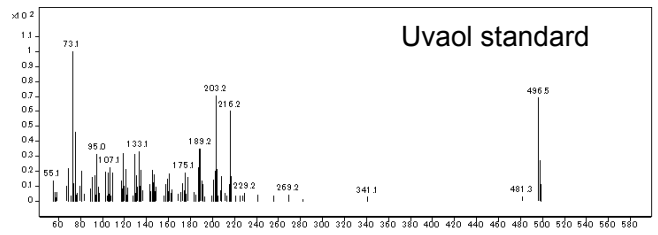
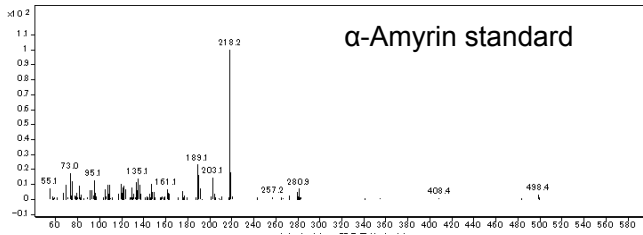
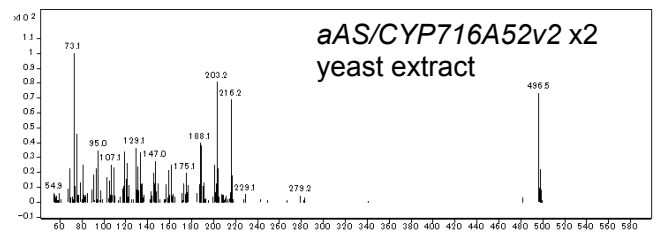
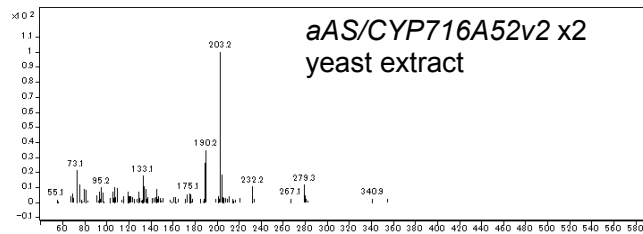
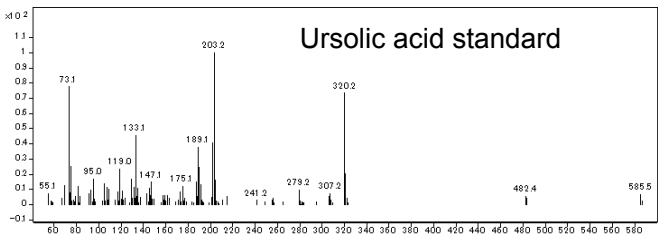
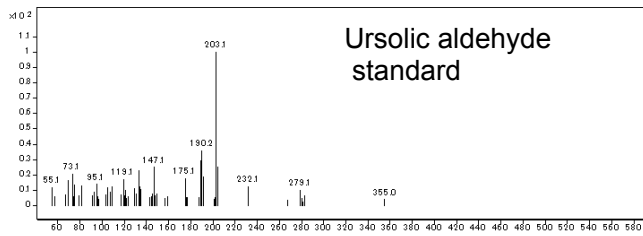
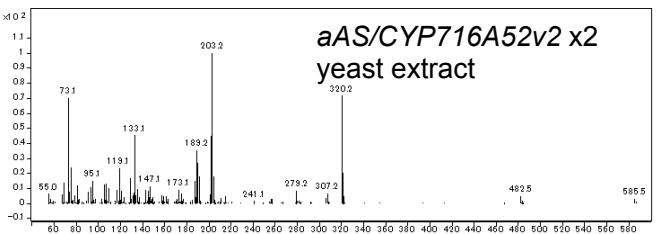
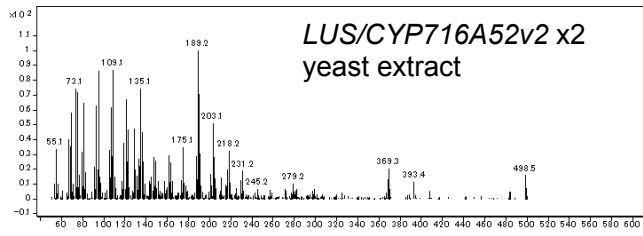
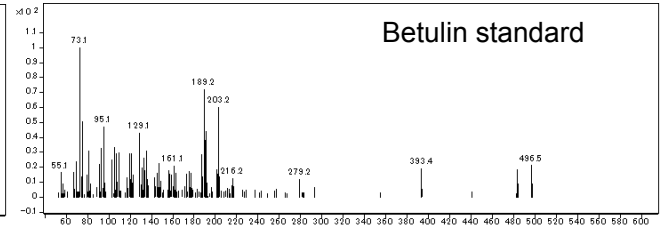
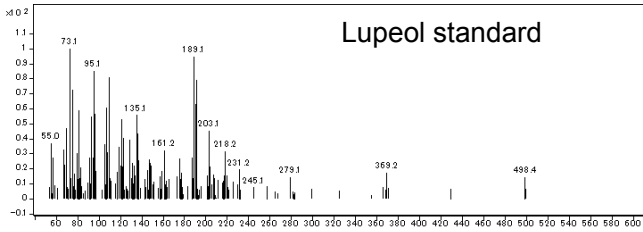
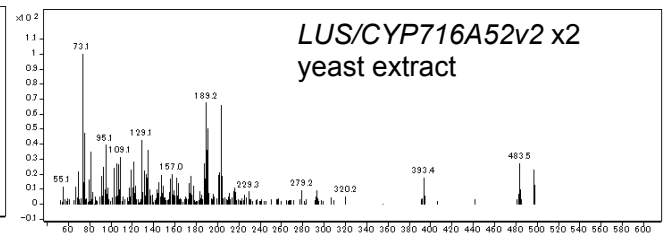
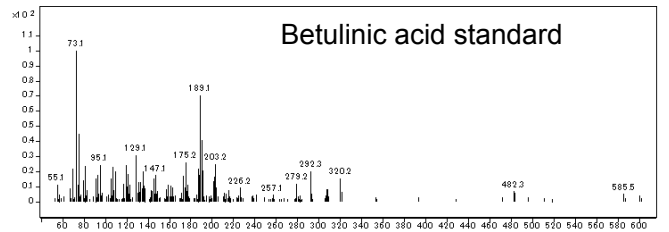
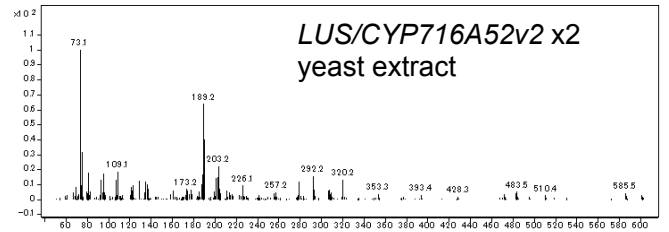
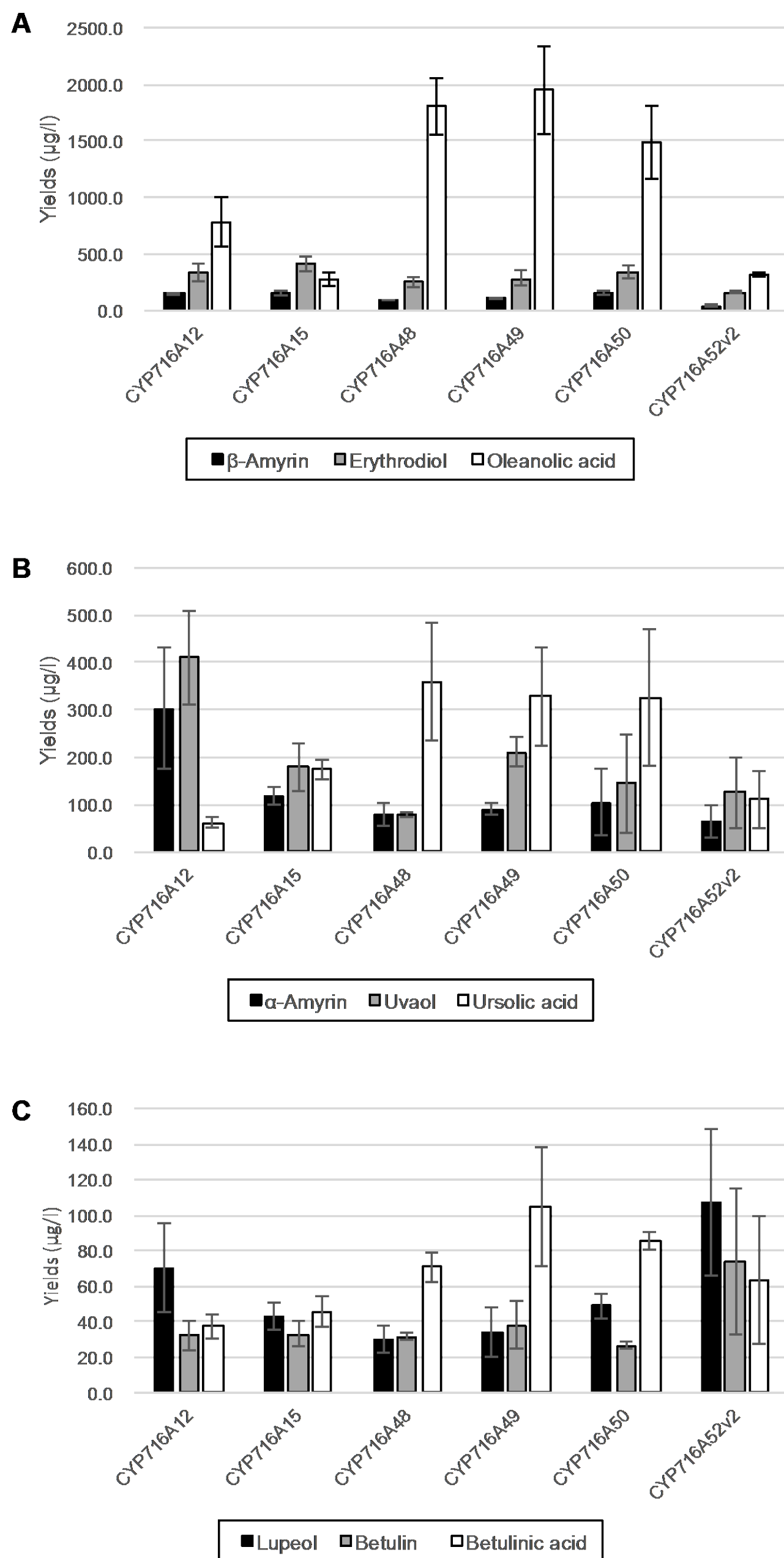


Figure S1. Mass spectra of target compounds and authentic standards shown in Figure 2.
A) Mass spectra of β -amyrin derivatives. **B)** Mass spectra of α -amyrin derivatives. **C)** Mass spectra of lupelol derivatives.

B**Peak 5 (Rt=29.93 min)****Peak 6 (Rt=35.63min)****Peak 7 (Rt=37.32 min)****Peak 8 (Rt=38.40 min)****Figure S1. Cont.**

C**Peak 9 (Rt=30.21 min)****Peak 10 (Rt=36.33 min)****Peak 12 (Rt=37.29 min)****Figure S1. Cont.**



Supplemental Figure S2. Absolute amounts of detected triterpenoids.

The amounts of (A) β -amyrin derivatives in *bAS/CPR/CYP716A*-expressing yeast extracts, (B) α -amyrin derivatives in *aAS/CPR/CYP716A*-expressing yeast extracts, and (C) lupeol derivatives in *LUS/CPR/CYP716A*-expressing yeast extracts are given. Quantitation and error bars correspond to the mean and standard deviation, respectively (n = 3).

CYP716A12	1	MEPFFYLSLLLFVFSFISLSLFFIYKQKSPLN----LPPGKGVPIIGESLEFLSTGWKGFPEKFIIDRIRKYSSE-LFKTISIVGESTVVCGAAANKFLFSNENKLV	105
CYP716A15	1	-MEVFFLSLLLFVFSVSIQLHLLFYKHSHFITG-PNLPPGKIGVPMVGESLEFLSTGWKGFPEKFIIDRISKYSSE-VFKTISLLGEPAAVFGAAGNKFLFSNENKLVH	107
CYP716A48	1	-MEFFVYVLLCLFVFLTISLSLHFLFYKNSFSFG--QIIPPGKIGVPIIGESLEFLSNGWKGPEKFIIDRITAKYSY-VFKTILFGEPAAVFCGANGKFLFSNENKLVQ	106
CYP716A49	1	-MELFFLCGLILF-LSLSLASLFLYLNHNSI-KG-YRVPVPGIGVPIIGESLEFLSTGWKGYPEKFIIDRISKYAPNQIFKTSILGKVAVITCGAAGNKFLYSNENKLVQ	106
CYP716A50	1	-MEFFVYVLLCLFLLVTLSLHFLFYKSRASLGG--PLPPGKIGVPMVGESLEFLSCGWKGFPEKFIIDRIRKYSSE-VFKTHLLGKAAVFCGAPGNKFLFSNENKLVQ	106
CYP716A52v2	1	-MELFFVPLLSLFLFISLSFHFLFYKSPSSSGGFPPTIGESVFEFLSTGWKGYPEKFIIDRITKYSN-VFKTISIFGEPAAVFCGAAANKFLFSNENKLVQ	108
Proins			
CYP716A12	106	AWWPDSVNRIFPTTSLDNLKEESTIKMRKLLPQFFKPEALQRYVGVMDVIAQRHFVTHWDNKNEITVYPLAKRYTFLACRIFVSEIDENVAKFSDFPQLIAAGTISLP	215
CYP716A15	108	AWWPSSVNRVFPSSQTQSS--KEEAIKMRKLLPQFFKPEALQRYVGMIDIAQRHFADSDNRDEIVVPLAKRYTFLWACRIFLSTIEDPAVAKFEKPFHVLASGLITVP	216
CYP716A48	107	AWWPASVNRVFPSSNQTS--KEEAIKMRKLLPQFFKPEALQRYVGMIDIAQRHFSDGDNKNEVWVPLAKRYTFLWACRIFVSEIDPAVAKFADPFNELASGLITIP	215
CYP716A49	107	AWWPSSVNRIFPSSQTQSS--KEESIKMRKLLPQFFKPEALQRYVPMIDTIAIRHMESQDGGKDKVEVPLAKRYTFLWACRIFLSTIEDPAVAKFAEPFNDIAAGTISLP	215
CYP716A50	107	AWWPASVNRVFPSSQTQSS--KEEAIKMRKLLPQFFKPEALQRYVGMIDIAQRHFSSQWENKDOQVWVPLCKNYTFWIASRLFVSEIDPEVAKLLEPFLVNLASGLISVP	215
CYP716A52v2	109	AWWPDSVNRVFPSSQTQSS--KEEAIKMRKLLPQFFKPEALQRYVGMIDIAQRHFSSQWENKNEVWVPLAKSYTFWITACKVFSVSEIDPAVAELLEPFSATASGLISVP	217
CYP716A12	216	IDLPGTPFNRAIKASNFIRKELIKTIKORRIDLAEGKASPTQDILSHMLLTSDENGKSNELNIADKILGLLIGGHDTASAACTFVVKYLAELEPEIYEGVWQEQMEIAKS	325
CYP716A15	217	IDLPGTPFNRAIKASNFIRKELRAITIKORRIDLAEGKASQDQDILSHMLLATIDEDGCHNEMXIADKILGLLIGGHDTASAAITFLIKYMAELPHIYKWEQEQMEIANS	326
CYP716A48	216	IDLPGTPFNRAIKASNFIRKELVSIKORRIDLAEGKASPTQDILSHMLLTSDESGKFMHLDIADKILGLLIGGHDTASSACTFVVKYLAELPEIYEGVWQEQMEIAKS	325
CYP716A49	216	VNLPGTPFNRAIKASNFIRKELRAITIKORRIDLAEGKASPTQDILSHMLLTADEIDGRVTEMDIADKILGLLIGGHDTASAACTFVVKYLAELPHIYKWEQEQMEIAKS	325
CYP716A50	216	IDLPGTPFNRAIKASNFIRKELVSIKORRIDLAEGKASPTQDILSHMLLTSDENGKFMHLDVADKILGLLIGGHDTASSACTFVVKYLAELPEIYEGVWQEQMEIANS	325
CYP716A52v2	218	IDLPGTPFNRAIKASNFIRKELVSIKORRIDLAEGKASPTQDILSHMLLTSDESGKFMHLDIADKILGLLIGGHDTASSACTFVVKYLAELPEIYEGVWQEQMEIANS	327
Oxygen activation			
CYP716A12	326	KAPGELLNWDITQMKYSWNVACEVRLAPPLQGAFFREALTDFVFNFGSTIPKGWLYWSANSTHKNAGCFPEKFDPIRFEFGVGPAPYTFVFPFGGPRMCPGKEYARL	434
CYP716A15	327	KAPGE--NDDVQXMRYSWNVACEVRLAPPLQGAFFREALTDFVFNFGSTIPKGWLYWSANSTHKSPECFDPIRFEFGVGPAPYTFVFPFGGPRMCPGKEYARL	434
CYP716A48	326	KAPGELLNWDITQMKYSWNVACEVRLAPPLQGAFFREALTDFVFNFGSTIPKGWLYWSANSTHRSSEFFPEPLKFDPSRFEFGVGPAPYTFVFPFGGPRMCPGKEYARL	434
CYP716A49	326	KAEGELLNWDITQMKYSWNVACEVRLAPPLQGAFFREALTDFVYGGQVPGKWLYWSANSTHNPCEGFPPEKFDPSRFEFGVGPAPYTFVFPFGGPRMCPGKEYARL	434
CYP716A50	326	KAPGELLNWDITQMKYSWNVACEVRLAPPLQGAFFREALADFYNGSTIPKGWLYWSANSTHNPCEGFPPEKFDPSRFEFGVGPAPYTFVFPFGGPRMCPGKEYARL	434
CYP716A52v2	328	KKAGELLNWDITQMKYSWNVACEVRLAPPLQGAFFREALSDFTYNGSTIPKGWLYWSANSTHINSEVFPPELKFDPSPRFEGAGPPFSEVFPFGGPRMCPGKEYARL	436
Exxx			
CYP716A12	435	EILVFNHVLVKKRFWEKVIPEKTIIVDPMPITPAKGLPIRLVPHKA----	479
CYP716A15	435	EILVFNHVVKKRFWEKVIPEKTIIVDPMPITPAKGLPIRLVPHKPK----	479
CYP716A48	435	EILVFNHVLVKKRFWEKVIPEKTIIVDPMPITPAKGLPIRLVPLNA----	479
CYP716A49	435	EILVFNHVVKKRFWEKVIPEKTIIVDPMPITPENGLPIRLVPHQIVAA	483
CYP716A50	435	EILVFIHVVKKRFWEKVIPEKTIIVDPMPITPAKGLPIRLVPHKA----	479
CYP716A52v2	437	EILVFNHVLVKKRFWEKVIPEKTIIVDPMPITPANGLPVRLVPHKA----	481

Supplemental Figure S3. Protein alignment of CYP716A enzymes used in this study. Full primary sequences of CYP716s were aligned using GENETYX Mac (ver. 12.0). Common features found in plant P450s are highlighted.

Supplemental Table 1. List of primers used in this study

No.	Target seq	Sequence (5' to 3')
1	CYP716A48 F	<u>CACCATGGAGTTCTTCTATGTCTCTCTTC</u>
2	CYP716A48 R	TTAAGCATTAAAGGGATAAAGAC
3	CYP716A49 F	<u>CACCATGGAGCTCTTCTTCCTTT</u>
4	CYP716A49 R	TTAAGCAGCAACAATTTGAGG
5	CYP716A50 F1	CGCTCACAAACAATCTGGAA
6	CYP716A50 F2	<u>CACCATGGAGTTTTTCTATGTCTCTTTG</u>
7	CYP716A50 R	TTAGGCCTTGTGTGGAAAA
8	CYP716A52v2 F1	<u>CACCATGGAACTCTTCTATGTCCCTCT</u>
9	CYP716A52v2 R	TTAGGCCTTGTGTGGAAATAGGC

The underlined sequences were added to facilitate the unidirectional cloning of the product into pENTR/D-TOPO (Invitrogen)

pplemental Table 2. Amino acid identities of the enzymes analyzed in this stu

	CYP716A12	CYP716A15	CYP716A48	CYP716A49	CYP716A50	CYP716A52v2
CYP716A12						
CYP716A15	74					
CYP716A48	77	79				
CYP716A49	71	74	74			
CYP716A50	71	75	83	73		
CYP716A52v2	73	70	81	73	79	