

Figure S1.

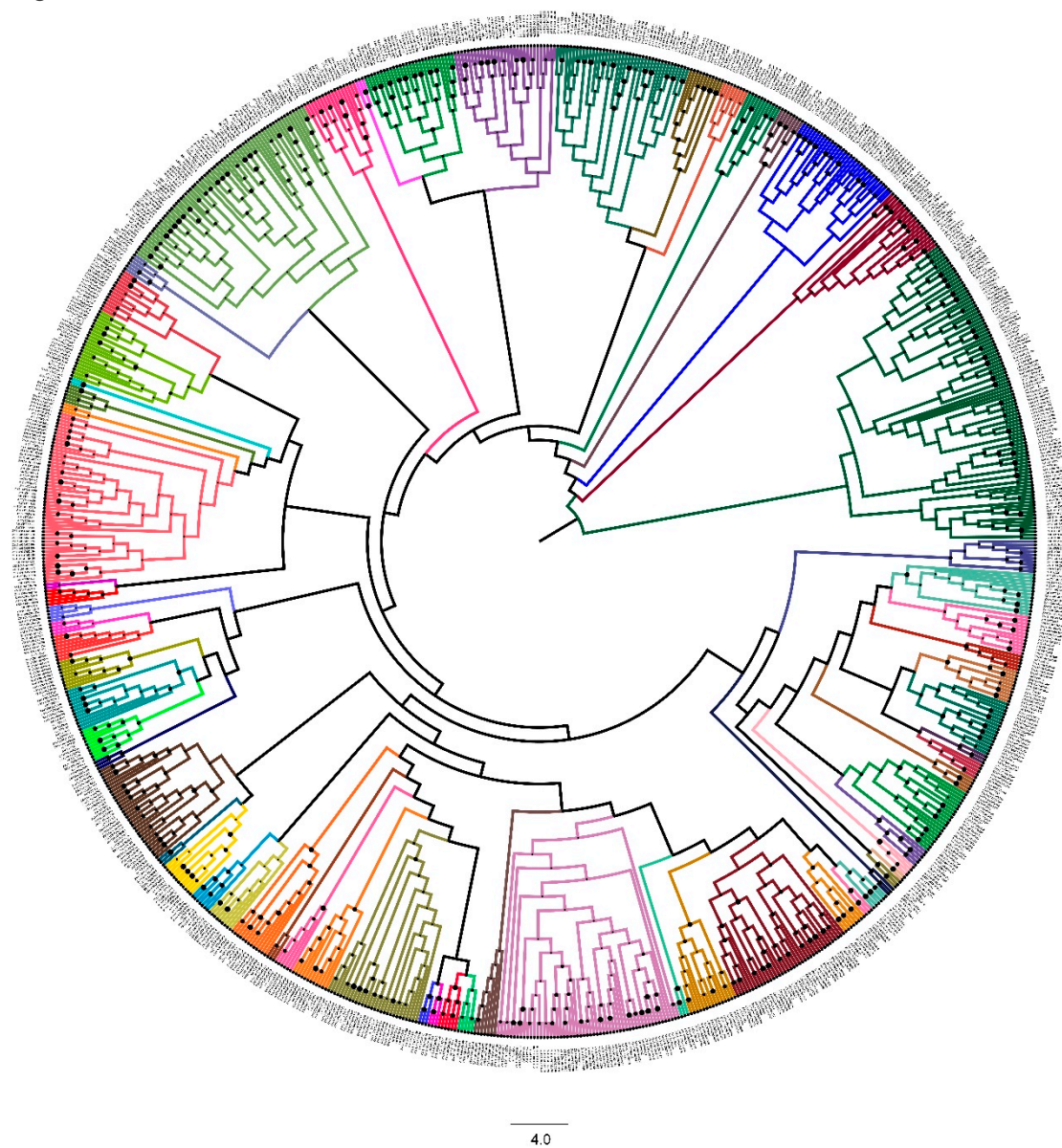


Figure S1. Phylogenetic analysis of AtCYP450s and BnCYP450s.

Figure S2.

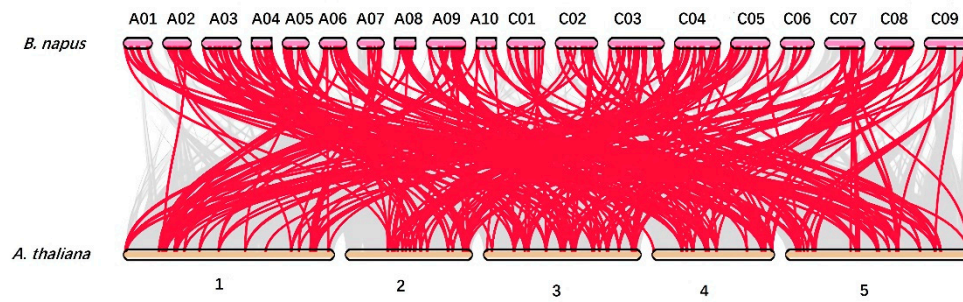


Figure S2. Synteny analysis of *AtCYP450* and *BnCYP450* genes. The gray lines indicate all collinear blocks, and the red lines highlight the orthologous relationships between *BnCYP450*s and *AtCYP450*s respectively.

Figure S3.

BnCYP704B1a	MSMWIVLACM L TSWIFLHRWGQRNKRGPKTWPLVGAAIEQLTNFDRMHDWLVEYLYDSRT	60
BnCYP704B1b	MSMWIVLACM V TSWIFLHRWGQRNKRGPKTWPLVGAAIEQLTNFDRMHDWLVEYLYDSRT	60
*****:*****		
BnCYP704B1a	VVVPMPFTTYTYIADPINVEHVLKTNFSNYPKGETYHSYMEVLLGDGIFNSDGELWRKQR	120
BnCYP704B1b	VVVPMPFTTYTYIADPINVEHVLKTNFSNYPKGETYHSYMEVLLGDGIFNSDGELWRKQR	120

BnCYP704B1a	KTASFEFASKNLRDFSTVVFKEYSLKLFILCQAS F KDQQVDMQELLMRMTLDSICKVGF	180
BnCYP704B1b	KTASFEFASKNLRDFSTVVFKEYSLKLFILCQAS S KDQQVDMQELLMRMTLDSICKVGF	180
***** *****		
BnCYP704B1a	GVEIGTLAP D LPENRFAKAFDTANIIVTLRFIDPLW M KKYLNIGSEALLGKSIKVDDF	240
BnCYP704B1b	GVEIGTLAP E LPENRFAKAFDTANIIVTLRFIDPLW I KKYLNIGSEALLGKSIKVDDF	240
*****:*****:*****		
BnCYP704B1a	TYSMIRRR K TEILEAQKSPSN I KMKHDILSRFIEISDDPSKSTEKSLRDIVLNFVIAG	300
BnCYP704B1b	TYSMIRRR A TEILEAQKSPSN T KMKHDILSRFIEISDDPSKSTEKSLRDIVLNFVIAG	300
*****:***** *****		
BnCYP704B1a	RDTTATTLTWAIIYIMMNEHVAEKLCESELQELEREKAEETNTPLRQYDTEDFKSFNERVT	360
BnCYP704B1b	RDTTATTLTWAIIYIMMNEHVAEKLCESELQELEREKAEETNTPLRQYDTEDFKSFNERVT	360

BnCYP704B1a	QFAGMLSYSLSGLKHLHAVVTETLRLYPVPQDPKGVLEDDILPNGTKVKAGGMVTYVP	420
BnCYP704B1b	QFAGMLSYSLSGLKHLHAVVTETLRLYPVPQDPKGVLEDDILPNGTKVKAGGMVTYVP	420

BnCYP704B1a	YSMGRMEYNWGSDA A TFKPERWLKDGMFQNASPFKFTAFQAGPRICLGKDSAYLQMKMAM	480
BnCYP704B1b	YSMGRMEYNWGSDA M FKPERWLKDGMFQNASPFKFTAFQAGPRICLGKDSAYLQMKMAM	480
***** *****		
BnCYP704B1a	AILCRFY K FHLVPNHPVKYRMMTILSMAHGLKVTVSRRS	519
BnCYP704B1b	AILCRFY R FHLVPNHPVKYRMMTILSMAHGLKVTVSRRS	519
*****:*****		

Figure S3. Alignment the protein sequences of *BnCYP704B1a* and *BnCYP704B1b*. The protein sequences of BnCYP704B1a and BnCYP704B1b are aligned with Clustal Omega. The 8 amino acid-replacements are indicated by yellow box.

Figure S4.

BnCYP704B1a	ATGTCTATGTGGATCGTTCTAGCTTGCATGTTACATCATGGATCTTCTTGACCCGATGG	60
BnCYP704B1b	ATGTCTATGTGGATCGTTCTAGCTTGCATGTTACATCATGGATCTTCTTGACCCGATGG	60
***** * *****		
BnCYP704B1a	GGACAGAGGAACAAGAGAGGTCCCAAGACATGGCCTTTGGTCGGAGCAGCCATTGAGCAG	120
BnCYP704B1b	GGACAGAGGAACAAGAGAGGTCCCAAGACATGGCCTTTGGTCGGAGCAGCCATTGAGCAG	120

BnCYP704B1a	TTGACTAACTTTGACCGAATGCACGACTGGCTCGTTGAGTATCTTTACGACTCAGAACC	180
BnCYP704B1b	TTGACTAACTTTGACCGAATGCACGACTGGCTCGTTGAGTATCTTTACGACTCAGAACC	180

BnCYP704B1a	GTAGTGGTTCCATGCCCTTACCACCTTAACATACATAGCAGATCCCATCAATGTGAA	240
BnCYP704B1b	GTAGTGGTTCCATGCCCTTACCACCTTAACATACATAGCAGATCCCATCAATGTGAA	240
***** *****		
BnCYP704B1a	CACGTCTCAAACCAATTCTCCAACACCCAAAGGGAGAGACGTACCACTCTATATG	300
BnCYP704B1b	CACGTCTCAAACCAATTCTCCAACACCCAAAGGGAGAGACGTACCACTCTATATG	300
***** *****		
BnCYP704B1a	GAAGTTTGTGGGAGATGGGATCTTCAATTCAGATGGAGAGCTCTGGAGGAAACAGAGG	360
BnCYP704B1b	GAAGTTTGTGGGAGATGGGATCTTCAATTCAGATGGAGAGCTCTGGAGGAAACAGAGG	360

BnCYP704B1a	AAAACCGCGAGTTTCGAATTTGCTTCCAAGAATCTTAGAGACTTCAGTACTGTAGTGTTT	420
BnCYP704B1b	AAAACCGCGAGTTTCGAATTTGCTTCCAAGAATCTTAGAGACTTCAGTACTGTAGTGTTT	420

BnCYP704B1a	AAAGAGTATAGCCTCAAGCTCTTCTCCATCCTTTGTCAAGCTCTTCAAAGACCAACAA	480
BnCYP704B1b	AAAGAGTATAGCCTCAAGCTCTTCTCCATCCTTTGTCAAGCTCTTCAAAGACCAACAA	480

BnCYP704B1a	GTAGACATGCAGGAATGTTGATGAGAATGACTCTAGACTCCATATGTAAAGTGGGATTT	540
BnCYP704B1b	GTAGACATGCAGGAATGTTGATGAGAATGACTCTAGACTCCATATGTAAAGTGGGATTT	540

BnCYP704B1a	GGTGTGGAGATAGGAACATTGCTCCAGACTACCAGAGAATCGCTTTGCTAAAGCTTTC	600
BnCYP704B1b	GGTGTGGAGATAGGAACATTGCTCCAGACTACCAGAGAATCGCTTTGCTAAAGCTTTC	600

BnCYP704B1a	GATACCGCAATATAATCGTAACACTTCGTTTCATAGACCTCTTTGGAAGATAAAAAG	660
BnCYP704B1b	GATACCGCAATATAATCGTAACACTTCGTTTCATAGACCTCTTTGGAAGATAAAAAG	660

BnCYP704B1a	TACCTTAACATAGGATCTGAGGCATTACTTGGCAAGAGCATAAAAGTAGTCGATGATTTC	720
BnCYP704B1b	TACCTTAACATAGGATCTGAGGCATTACTTGGCAAGAGCATAAAAGTAGTCGATGATTTC	720

BnCYP704B1a	ACATATTCAATGATAAGAAGAAGGAAA CAGAGATATTAGAGGCACA AAATCTCCTTCC	780
BnCYP704B1b	ACATATTCAATGATAAGAAGAAGGAAA CAGAGATATTAGAGGCACA AAATCTCCTTCC	780
***** *****		
BnCYP704B1a	AACAACA TAAGATGAAGCATGATATACTCTCGAGGTT ATTGAGATCAGCGACGATCCT	840
BnCYP704B1b	AACAACA TAAGATGAAGCATGATATACTCTCGAGGTT ATTGAGATCAGCGACGATCCT	840

BnCYP704B1a	GATAGCAAATCAACTGAGAAAAGCCTAAGAGA ATAGTCCT AACTTTGTTATTGCTGGA	900
BnCYP704B1b	GATAGCAAATCAACTGAGAAAAGCCTAAGAGA ATAGTCCT AACTTTGTTATTGCTGGA	900

BnCYP704B1a	AGAGATACAACAGCAACAACCTCTCACTTGGGCTATATA ATGATAATGATGAATGAACAT	960
BnCYP704B1b	AGAGATACAACAGCAACAACCTCTCACTTGGGCTATATA ATGATAATGATGAATGAACAT	960

BnCYP704B1a	GT GCCGAGAAGCTTTGCTCAGAGCTACAAGAACTCGAAAGAGAAAAGGC GAAGAGACA	1020
BnCYP704B1b	GT GCCGAGAAGCTTTGCTCAGAGCTACAAGAACTCGAAAGAGAAAAGGC GAAGAGACA	1020
** *****		
BnCYP704B1a	AACACACCGTTGCGTCAATACGATACAGAGGACTTCAAGTCCTTCAACGAGAGGGTAACA	1080
BnCYP704B1b	AACACACCGTTGCGTCAATACGATACAGAGGACTTCAAGTCCTTCAACGAGAGGGTAACA	1080

BnCYP704B1a	CAGTTTGCAGGAATGTTGAGTTATGATTCTTTAGGAAAATTACA TACTTACATGC GTG	1140
BnCYP704B1b	CAGTTTGCAGGAATGTTGAGTTATGATTCTTTAGGAAAATTACA TACTTACATGC GTG	1140

BnCYP704B1a	GTAACAGAAACACTTCGTCTCTACCCAGCAGTTCCTCAGGATCCAAAAGGAGTGTTAGAA	1200
BnCYP704B1b	GTAACAGAAACACTTCGTCTCTACCCAGCAGTTCCTCAGGATCCAAAAGGAGTGTTAGAA	1200

BnCYP704B1a	GATGATATATT CTAATGGAACAAAAGTAAAAGCAGGAGG ATGGTAACATATGTTTCCT	1260
BnCYP704B1b	GATGATATATT CTAATGGAACAAAAGTAAAAGCAGGAGG ATGGTAACATATGTTTCCT	1260

BnCYP704B1a	TACTCAATGGGTCGTATGGA TACAAC TGGGGATCAGATGCAGC A GTTTAAACC GA	1320
BnCYP704B1b	TACTCAATGGGTCGTATGGA TACAAC TGGGGATCAGATGCAGC A GTTTAAACC GA	1320
***** * *****		

BnCYP704B1a	AGATGGCTTAAAGATGGGATGTTTCAGAACGCTTCCCCATTCAAGTTCACAGCATTTTCAG	1380
BnCYP704B1b	AGATGGCTTAAAGATGGGATGTTTCAGAACGCTTCCCCATTCAAGTTCACAGCATTTTCAG	1380

BnCYP704B1a	GCTGGACCTAGATATGCTTGGGAAAGATTTCAGCTTATCTACAAATGAAGATGGCATG	1440
BnCYP704B1b	GCTGGACCTAGATATGCTTGGGAAAGATTTCAGCTTATCTACAAATGAAGATGGCATG	1440
	***** ***** *****	
BnCYP704B1a	GCAATTCTTTGCAGATTATATGTTCCATTGGTACCAAATCATCCTGTCAAGTACCGG	1500
BnCYP704B1b	GCAATTCTTTGCAGATTATATGTTCCATTGGTACCAAATCATCCTGTCAAGTACCGG	1500
	***** *****	
BnCYP704B1a	ATGATGACAATTCTATCTATGGCGCATGGTTTGAAAGTTACTGTATCCAGACGTTTCATAG	1560
BnCYP704B1b	ATGATGACAATTCTATCTATGGCGCATGGTTTGAAAGTTACTGTATCCAGACGTTTCATAG	1560

Figure S4. Alignment the CDS sequences of *BnCYP704B1a* and *BnCYP704B1b*. The CDS sequences of *BnCYP704B1a* and *BnCYP704B1b* are aligned with Clustal Omega. The 41 nucleotide-replacements are indicated by green box.

Figure S5.

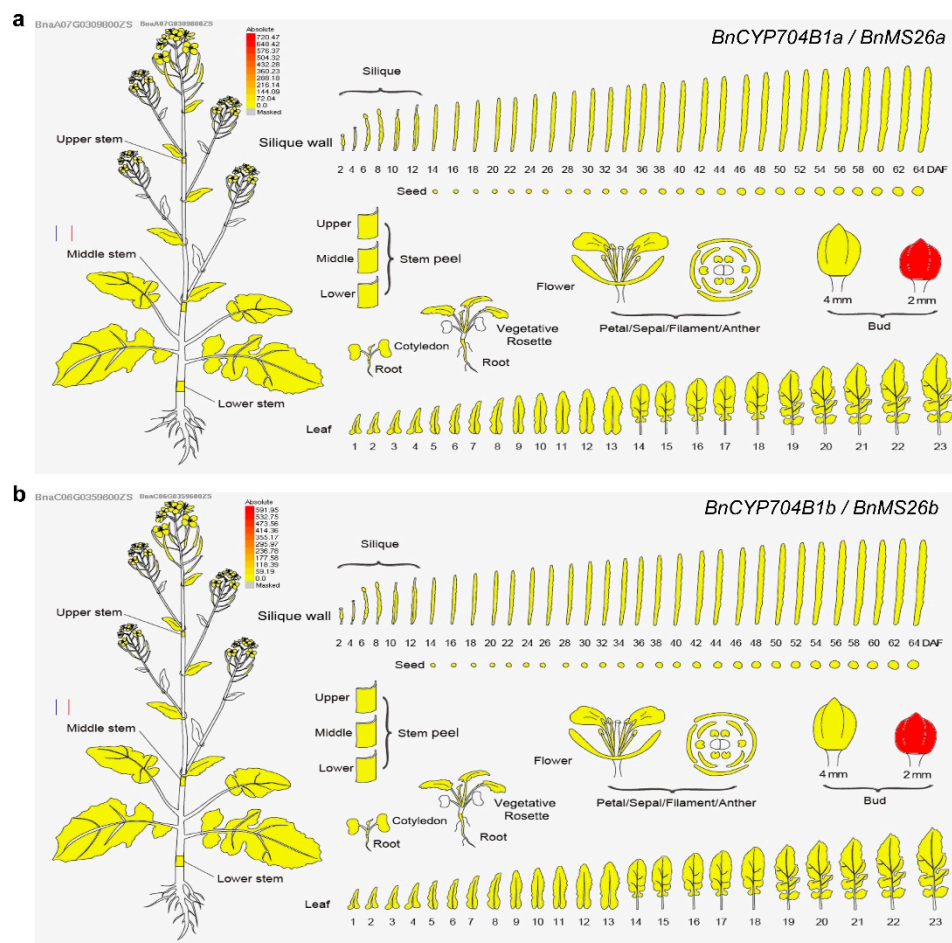


Figure S5. The expression profiles of *BnCYP704B1a* and *BnCYP704B1b*. The expression levels of *BnCYP704B1a* and *BnCYP704B1b* were obtained from the *Brassica napus* RNA-seq data (<http://yanglab.hzau.edu.cn/BnTIR>).

Figure S6.

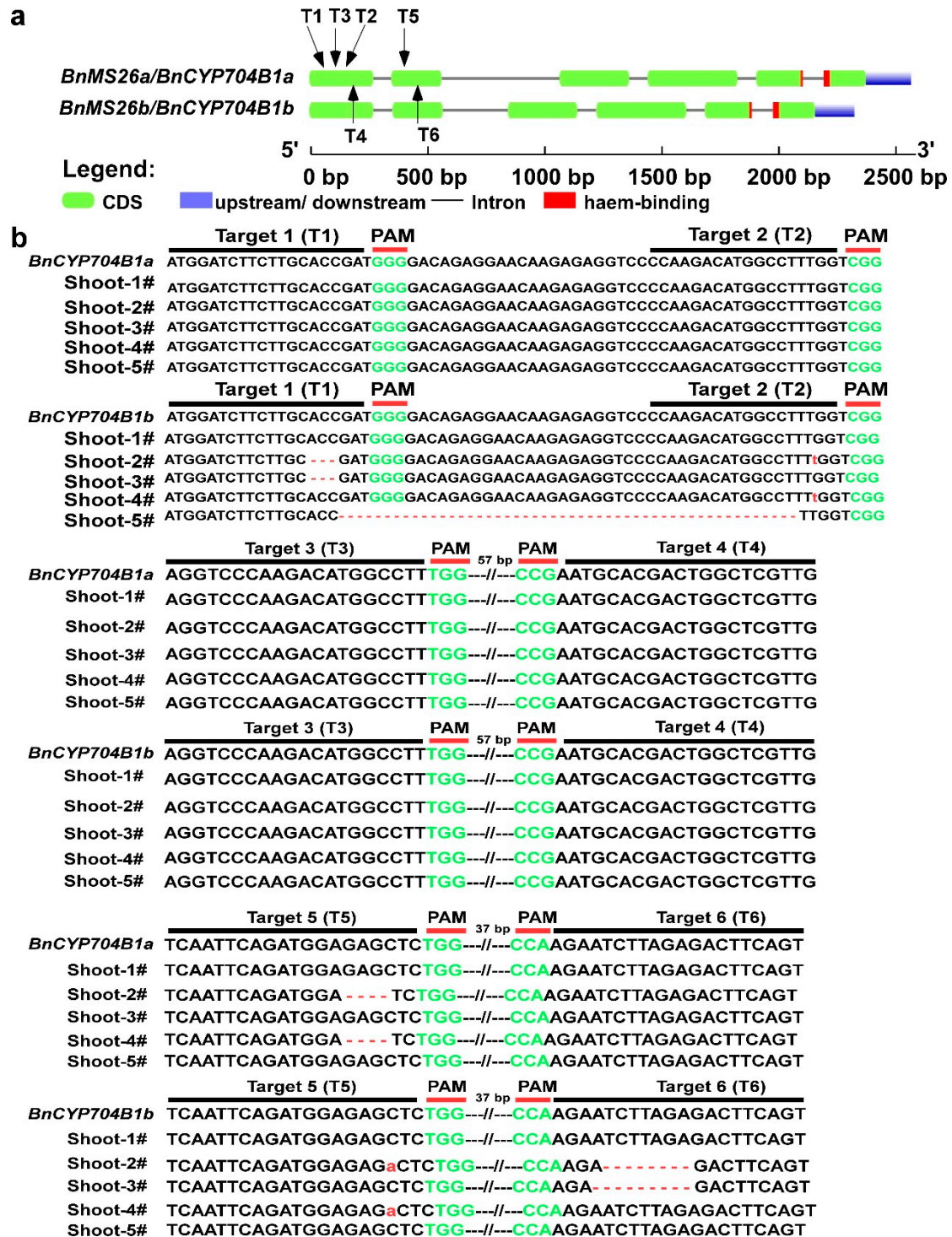


Figure S6. The sgRNA target sites and induced mutations upon *BnCYP704B1a* and

BnCYP704B1b in regenerated shoots. (a) Gene structures of *BnCYP704B1a* and

BnCYP704B1b and the corresponding CRISPR/Cas9 guide RNA target sites marked by

black arrows. (b) Mutated genotypes of *BnCYP704B1a* and *BnCYP704B1b* in

regenerated shoots derived from infected hypocotyl explants. The target sites sequences are marked in *BnCYP704B1a* and *BnCYP704B1b* sequences with PAM green highlighted.