

Supplement Materials

Table S1 Screening of differential genes and their corresponding primers

Gene_id	Regulated	Name	Primer	Primer sequence(5'-3')
21390692	up	F-box protein PP2-B15	Forward	CGATCGCGCATATGGATTGG
			Reverse	ACCTTGACCTCAAAGCCTCG
21387802	up	Purple acid phosphatase 17	Forward	ATTGGAGGGGTATTTGCGCCG
			Reverse	TCCTTCGTGTCACCGTGATG
21403433	up	ATP-dependent DNA helicase MER3 homolog	Forward	TCGAGGTTCTGTTC AAGGC
21401914	up	Cytochrome P450 71A1	Reverse	AGGCCATTGCTGACTGAGAC
			Forward	TGTGGCCGTACATCTCAATCC
21402441	up	Zinc transporter 4	Reverse	CTGTTGATGTGCCAGCCTCT
			Forward	GTGAGGGAGCATTACACCGG
21387250	up	Pathogenesis-related protein 1	Reverse	TCTTTCTGCCCCTGAGAAACG
			Forward	CATAGGCCTAGCCATGCTCC
21397686	up	Putative disease resistance protein	Reverse	TTGTCGTCCACGTCATCTG
			Forward	AAGCTTCGGTTAGTGCTCCG
21405925	up	Leucoanthocyanidin dioxygenase	Reverse	TTTGCGCTCTTGCTTCAG
			Forward	CCATGGTATCCCTGACGAGC
21399859	up	Bark storage protein A	Reverse	TTGTTTGCGAGCTTGCTTC
			Forward	GTCTCCAAGGGAAGCCCAAG
21395828	up	Receptor-like protein 12	Reverse	ACAGCCGA ACTCTCCATGTC
			Forward	CCTTTCTCGACCTAGGGCAC
21396136	down	Probable protein phosphatase 2C 75	Reverse	CGACGAGATTGTTGTGAGCG
			Forward	AGTACTGCCGGAGAATGCTG
21405820	down	(R)-mandelonitrile lyase-like	Reverse	CTTGCCGCTTCCA ACTGTTC
			Forward	TGCTACCGGGATTGGAAGTG
21394160	down	Probable protein phosphatase 2C 6	Reverse	CGGCAACGAAAGGAGACTCA
			Forward	GGTCATGACGGAGACAGTCG
21385141	down	Protein CLT3	Reverse	TCCCTCGACAAAGAACTGCC
			Forward	TGAAGTGCTGGAACCCAGTG
21384129	down	Wall-associated receptor kinase 2	Reverse	CTGTATCAGCGGCTTGGAAC
			Forward	GAAACGCTTACCTCCCTGCT
β -actin			Forward	AGCAACTGGGATGACATGGAGA
			Reverse	CGACCACTGGCGTAAAGGGA

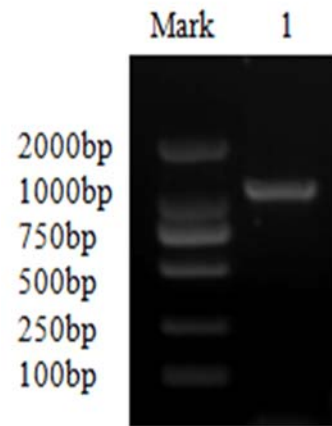


Figure S1. MaZIP4 gene amplification of mulberry: PCR in vitro amplification was performed using the designed primers MaZIP4-F and MaZIP4-R, and gene product band of about 1200 bp in length was obtained. M: DL2000 DNA molecular marker 1: Product of RT-PCR.

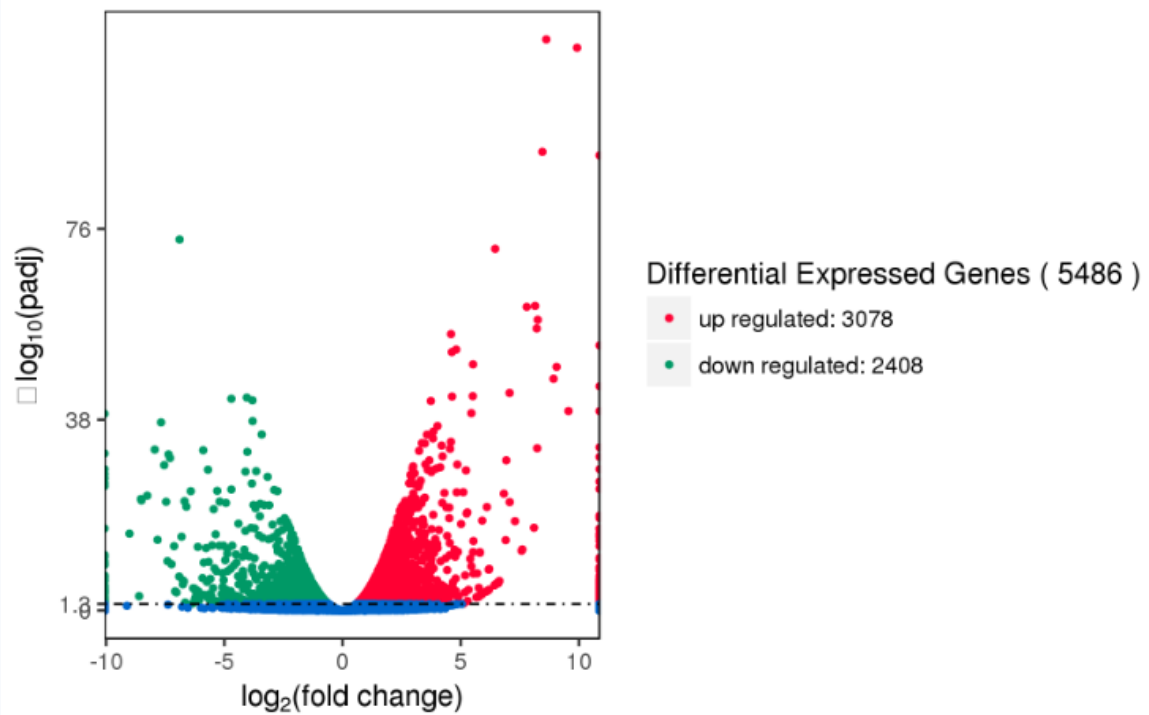


Figure S2. Volcano map of differential genes. The overall distribution of DEGs is visualized with a volcano plot, and genes with significant differential expression are indicated by red dots (up-regulated) and green dots (down-regulated), while genes with insignificant differential expression are indicated by blue dots.

1 **ATG**GGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCAT
 61 M G S S H H H H H S S G L V P R G S H
 121 ATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCATGGCGAATACAAGTTGC
 21 M A S M T G G Q Q M G R G S M A N T S C
 181 CAGAGCGCGGAGCTAGAGGTATGCCGGGATGAATCGGTGGCGCGCGTCCCTCAAATTCGTC
 41 Q S A E L E V C R D E S V A R V L K F V
 241 GCCATCGCCTCGATCCTCCTCTCCGGAGTGGCCGGAGTCGCCATTCCCCCTCATTGGAAAAG
 61 A I A S I L L S G V A G V A I P L I G K
 301 CACCGCCGGTTTCTCCGCACCGATGGCTGCCTCTTTCGTCGCCGCCAAGGCCTTCGCCGCC
 81 H R R F L R T D G C L F V A A K A F A A
 361 GGCGTCATCCTGGCCACCGGGTTCGTCCACATGCTCTCAGGTGGGTTCGGACGCGCTATCC
 101 G V I L A T G F V H M L S G G S D A L S
 421 AATCCTTGCCTGCCCCGAGTACCCGTGGGCTAAGTTTCTTTCTGTGGGTTCCTTCGCGATG
 121 N P C L P E Y P W A K F P F C G F F A M
 481 ATGGCCTCGCTGTTGACCTTACTCGTTGACTTTATGGGCACTCAGTATTACGAGAAGAAG
 141 M A S L L T L L V D F M G T Q Y Y E K K
 541 CAAGGGCTGGCCCCGGTCCAGCAGCGAGGAGCAAGCTCGGGTCGGATCGGAGGATTTCGGGT
 161 Q G L A R S S S E E Q A R V G S E D S G
 601 GGTGAAACCGGAATTGTTGCGGTTTCAGGCCAATGATTGGAATGGGAAGGTGTTTCGGAGAA
 181 G E T G I V A V Q A N D W N G K V F G E
 661 GAAGAGGGTGGTGGTATGCACATTGTGGGAATGCATGCGCACGCTGCTCACCATAGACAC
 201 E E G G G M H I V G M H A H A A H H R H
 721 AGTCATGCTCAAGAGCACGGTGCATGTGATGGGCACGTGAAGGAGCATTACACGGGCAC
 241 S H A Q E H G A C D G H V K E H S H G H
 781 GGGCATGACCACGGGCACTCGCATGGGTTGAGAGATGGGGACGAGGAAGGTGGTGTTAGG
 261 G H D H G H S H G L R D G D E E G G V R
 841 CACGTCGTCGTTTCTCAGGTTTTGGAACCTTGGGATTGTATCACATTTCAGTTATAATCGGG
 901 H V V V S Q V L E L G I V S H S V I I G
 961 CTATCTCTCGGAGTCTCGCAGAGTCCATGTACAATAAGACCCTTAATTGCTGCATTATCC
 1021 L S L G V S Q S P C T I R P L I A A L S
 301 TTCCACCAGTTCTTTCGAAGGATTTGCACTCGGAGGCTGCATCGCCCAAGCCCAATTCAAG
 341 F H Q F F E G F A L G G C I A Q A Q F K
 361 ACCCTTTTCGGCCACAATAATGGCATGCTTTTTTCGCGATAACTACTCCCGCGGGGATTGGC
 1141 T L S A T I M A C F F A I T T P A G I G
 1201 ATTGGGACTGCCATTGCTTCTTTCTACAACCCGTATAGCACTGGAGCATTGGTTACTGAA
 401 I G T A I A S F Y N P Y S T G A L V T E
 GGCATTGTTGGATTCTTTGTCTAGCTGGGATTCTAGTCTACATGGCTTTGGTGGACCTGATT
 G I L D S L S A G I L V Y M A L V D L I
 GCAGCTGATTTTTTGGAGCAAGAGGATGAGCTGCAATTTTAGGCTGCAAGTGGCATCCTAT
 A A D F L S K R M S C N F R L Q V A S Y
 TTAATGCTTTTCTAGGCGCTGGGTTGATGTCTTCATTAGCTATTTGGGCT**TGA**
 L M L F L G A G L M S S L A I W A *

ATG: the start codon; **TGA**: the stop codon

Figure S3. *MaZIP4* gene cDNA sequence and translated amino acid sequence. Sequence analysis of the cloned *MaZIP4* gene using DNAMAN software showed that the sequence length of the *MaZIP4* gene was 1405 bp, containing a complete ORF of 1254 bp, which can encode a protein with 417 amino acids.

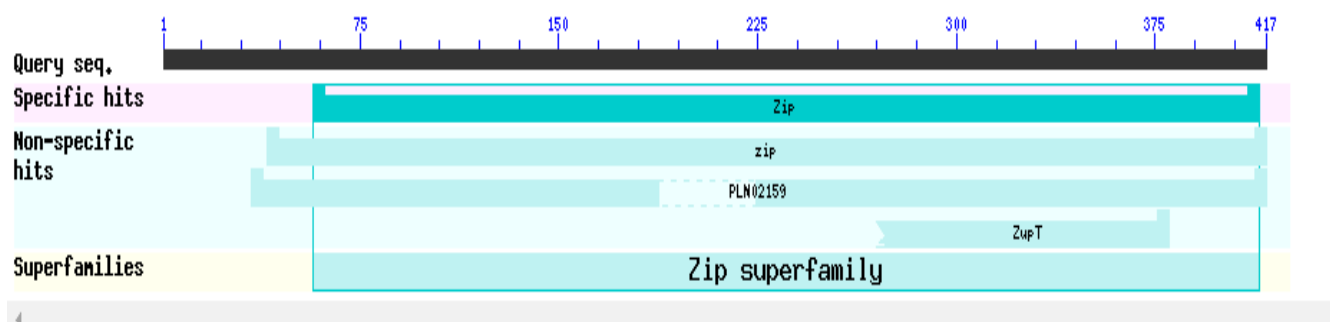


Figure S4. Prediction of amino acid sequence encoded by *MaZIP4* gene. NCBI online Blast results indicate that the amino acid family encoded by the *MaZIP4* gene belongs to the *ZIP4* superfamily.



Figure S5. *MaZIP4* gene protein tertiary structure. Since the tertiary structure of the *MaZIP4*-encoded protein is similar to that of *ZIP* family proteins, it is speculated that: the *MaZIP4*-encoded protein may have a similar function to that of *ZIP* family proteins.

M.atropurpurea	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMANTSC
M.notabilisMANTSC
Trema	MLLLEDIWQFLCLEGFVDKIRLYPGTLLQTVSDSMTNASC
Hevea	MLFFEDLWELIGLNHFTLKTRALSESLFPGISESMNSSC
Ziziphus	MLFIEDLWPLLGRLRGFVGKAQGFSDSILQTIISLMTNASC
Durio	MLFIEDIWPLFSIGCFGSKTKLFSESLQSIITDSMNTSC
Cannabis	MLFLEELWQFLSLGGFVDKTRLSAGFFLQTVSESMANSTC
CorchorusMTNSSC
Consensus	m n c

M.atropurpurea	HRRELRTDGLFVAAKAFAGVILPTGTFVHMLSGGSDALS
M.notabilis	HRRELRTDGLFVAAKAFAGVILPTGTFVHMLSGGSDALS
Trema	RRRELRTDGLFVAAKAFAGVILPTGTFVHMLSGGSEALS
Hevea	QRRELRTDGLFVAAKAFAGVILPTGTFVHMLSGGSNAIR
Ziziphus	QRRELRTDGLFVAAKAFAGVILPTGTFVHMLSGGSDALS
Durio	QRRELRTDGLFVAAKAFAGVILPTGTFVHMLSGGSDALT
Cannabis	HRRELRTDGLFVAAKAFAGVILPTGTFVHMLSGGSEALS
Corchorus	HRRELRTDGLFVAAKAFAGVILPTGTFVHMLSGGSDALT
Consensus	r lrt dg lfvaakafaagvil tgfvhml g al

M.atropurpurea	NPCLEYPWAKFPFGFAMASLLTLLVDFMGTQYYERK
M.notabilis	NPCLEYPWAKFPFGFAMASLLTLLVDFMGTQYYERK
Trema	NPCLEYPWAKFPFGFAMASLLTLLVDFMGTQYYERK
Hevea	NPCLEYPWSKFPFGFAMASLLTLLVDFMGTQYYERK
Ziziphus	NPCLEYPWSKFPFGFAMASLLTLLVDFMGTQYYERK
Durio	LPCLKYPWSKFPFGFAMASLLTLLVDFMGTQYYERK
Cannabis	NPCLEKYPWSKFPFGFAMASLLTLLVDFMGTQYYERK
Corchorus	NPCLEKYPWSKFPFGFAMASLLTLLVDFMGTQYYERK
Consensus	pclp pw kfpf gf am a l tllvdf gtqyye k

M.atropurpurea	QG.LARSSSBEQARVGSSEDSGGGTG.IVA.VQANDWNGKV
M.notabilis	QG.LARPSSBEQARVGSDDSGGTG.IVA.LQANDWNGKV
Trema	QG.LARNSBDQGRVGSSEDSGMESG.IVPVVQAKENGV
Hevea	QG.LNR.ASBEQARVGSVDVGPDS.IVPFAGEKEPNGKV
Ziziphus	QG.LAKP.SBERVRVGFDDSGLEST.IVP.AQAKENGV
Durio	QG.LARGKTBEQGRVESLEADSESA.IVPVVEGKDWNGKV
Cannabis	QGKKTRFNGBEQSRVGSSED..ESG.IVR.VGSKEWNGKV
Corchorus	QG.LARGSTBEQARVLSIEADSESA.GIMPVLEAKDRNGKV
Consensus	qg e rv i ngkv

M.atropurpurea	FGEEEGGGMHIVGMHAHAHHRHSHAQEHGACDGHVKE..
M.notabilis	FGEEEGGGMHIVGMHAHAHHRHSHAQEHGACDGHVRE..
Trema	FGEEEGGGMHIVGMHAHAHHRHNPQEQGACDGHVRE..
Hevea	FGEEEGGGMHIVGMHAHAHHRDHPHGQDQDGHVKE...
Ziziphus	FGEEEGGGMHIVGMHAHAHHRHNPHEQGACDGHVREQS
Durio	FGEEEGGGMHIVGMHAHAHHRHSHPHGQDQDGLLRSG
Cannabis	FGEEEGGGMHIVGMHAHAHHRHSHGQEQGACDGHVTE..
Corchorus	FGEEEGGGMHIVGMHAHAHHRHNPHEGQDQDGLLRSG
Consensus	fgeee ggmhivgmhahaahr h cdg

M.atropurpureaHSHGHG....HDHGSHGLRDGDEEGVVRHVVSQ
M.notabilisHSHGHG....HDHGSHGLGDGNEEGVVRHVVSQ
TremaNSX.....XGSHGFGDGDEEGVVRHVVSQ
HeveaDHGHG.....HNHRFSEGDEESCARHVVSQ
Ziziphus	HGHEHGHGHG....HGHGSHGLGE.DEDSCVVRHVVSQ
DurioHEHDH.....GHEHGFGGDADNCVVRHVVSQ
CannabisVGHGHG....HGHGSHGFGDGDEEGVVRHVVSQ
CorchorusHEHDHGQHGHSHGHGHGFGGSDDDSCVVRHVVSQ
Consensus	h h g rhv vsq

M.atropurpureaVLELGIVSHSVIIGLSL
M.notabilis	WAERSYDFDKETYMRIENMTSVLVLELGIVSHSVIIGLSL
TremaVLELGIVSHSVIIGLSL
HeveaILELGVSHSVIIGLSL
ZiziphusILELGIVSHSVIIGLSL
DurioILELGIVSHSVIIGLSL
CannabisVLELGIVSHSVIIGLSL
CorchorusVLELGIVSHSVIIGLSL
Consensus	lelg vshsviiglsl

M.atropurpurea	GVSQSPCTIRPLIAALSFHQFFEGFALGGCIQAQFKTIS
M.notabilis	GVSQSPCTIRPLIAALSFHQFFEGFALGGCIQAQFKNIS
Trema	GVSQSPCTIRPLIAALSFHQFFEGFALGGCISQAQFKTIS
Hevea	GVSQSPCTIRPLIAALSFHQFFEGFALGGCISQAQFKTIS
Ziziphus	GVSQSPCTIRPLIAALSFHQFFEGFALGGCISQAQFKTIS
Durio	GVSQSPCTIRPLIAALSFHQFFEGFALGGCISQAQFKTIS
Cannabis	GVSQSPCTIRPLIAALSFHQFFEGFALGGCISQAQFKTIS
Corchorus	GVSQSPCTIRPLIAALSFHQFFEGFALGGCISQAQFKTIS
Consensus	gvsqspectirpliaalsfhqffegfalggci qaqfk s

M.atropurpurea	ATIMACFFAITTEPGIGIGTAIASFYNPMSTGALVTEGIL
M.notabilis	ATIMACFFAITTEPGIGIGTAIASFYNPMSTGALVTEGIL
Trema	ATIMACFFAITTEPGIGIGTAIASFYNPMSTGALVTEGIL
Hevea	ATIMACFFAITTEPGIGIGTGISFYNPMSTGALVTEGIL
Ziziphus	ATIMACFFAITTEPGIGIGTAVASFYNPMSTGALVTEGIL
Durio	ATIMACFFAITTEPGIGIGTAVASFYNPMSTGALVTEGIL
Cannabis	TTIMACFFAITTEPGIGIGTAIASFYNPMSTGALVTEGIL
Corchorus	ATIMACFFAITTEPGIGIGTAIASFYNPMSTGALVTEGIL
Consensus	imacffa ttp gigig s ynp s al egil

M.atropurpurea	DSLISAGILVYMALVDLIAADFLSKMSCNFRQLQVASYML
M.notabilis	DSLISAGILVYMALVDLIAADFLSKMSCNFRQLQVASYML
Trema	DSLISAGILVYMALVDLIAADFLSKMSCNFRQLQVASYML
Hevea	DSSSAGILVYMALVDLIAADFLSKMSCNFRQLQVASYML
Ziziphus	DSLISAGILVYMALVDLIAADFLSKMSCNFRQLQVASYML
Durio	DSLISAGILVYMALVDLIAADFLSKMSCNFRQLQVASYML
Cannabis	DSLISAGILVYMALVDLIAADFLSKMSCNFRQLQVASYML
Corchorus	DSLISAGILVYMALVDLIAADFLSKMSCNFRQLQVASYML
Consensus	ds sagilvymalvdliaadflsk mscnfrlq sy ml

M.atropurpurea	FLGAGIMSSLAIWA.....
M.notabilis	FLGAGIMSSLAIWA.....
Trema	FLGAGIMSSLAIWA.....
Hevea	FLGAGMMAALAIWV.....
Ziziphus	FLGAGIMSSLAIWA.....
Durio	FLGAGIMASLAIWA.....
Cannabis	FLGAGIMSSLAIWA.....
Corchorus	FLGAGMMAALAVWA.....
Consensus	flgag m la w

Figure S6. *MaZIP4* encode multiple sequence alignments of amino acids. Sequence alignment of the *mazip4*-encoded protein sequence in *M. atropurpurea* R. was performed using the multiple sequence alignment function in the online site NCBI.