Supporting Information

Article title: **The *TuMYB46L-TuACO3* module regulates ethylene biosynthesis in einkorn wheat defense to powdery mildew**

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Table S3 Significantly enriched biological processes identified by GO analysis

Table S4 Expression changes of the four chitinase genes up-regulated by both ethylene treatment and *Bgt* infection

Table S5 Presence of putative MYB46 recognition sites in the 2 kb promoter region of the five genes related to cellulose or isoprenoid synthesis

Methods S1 Additional description of methods

Dataset S1 Sequence information and raw data (see separate file)

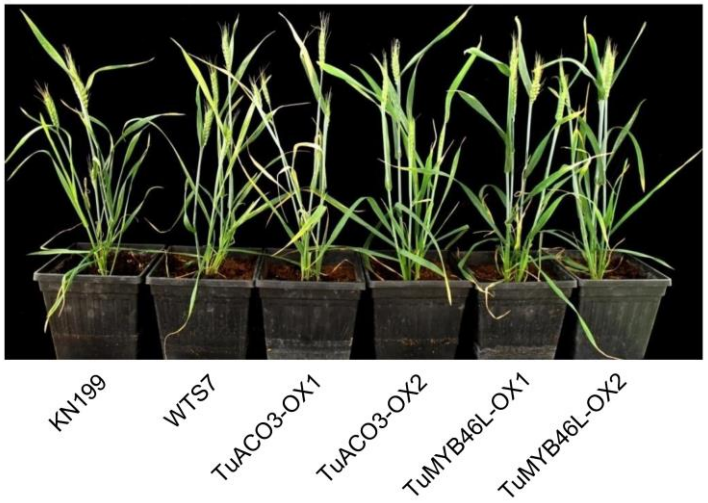


Fig. S1 Morphology of transgenic wheat lines overexpressing *TuACO3* or *TuMYB46L*. Kenong 199 (KN199) was the common wheat cultivar used as the recipient for genetic transformation. WTS7 was a wild type segregate. The transgenic lines overexpressing *TuACO3* (TuACO3-OX1 and -OX2) or *TuMYB46L* (TuMYB46L-OX1 and -OX2) resembled KN199 and WTS7 in their growth and development in the greenhouse.

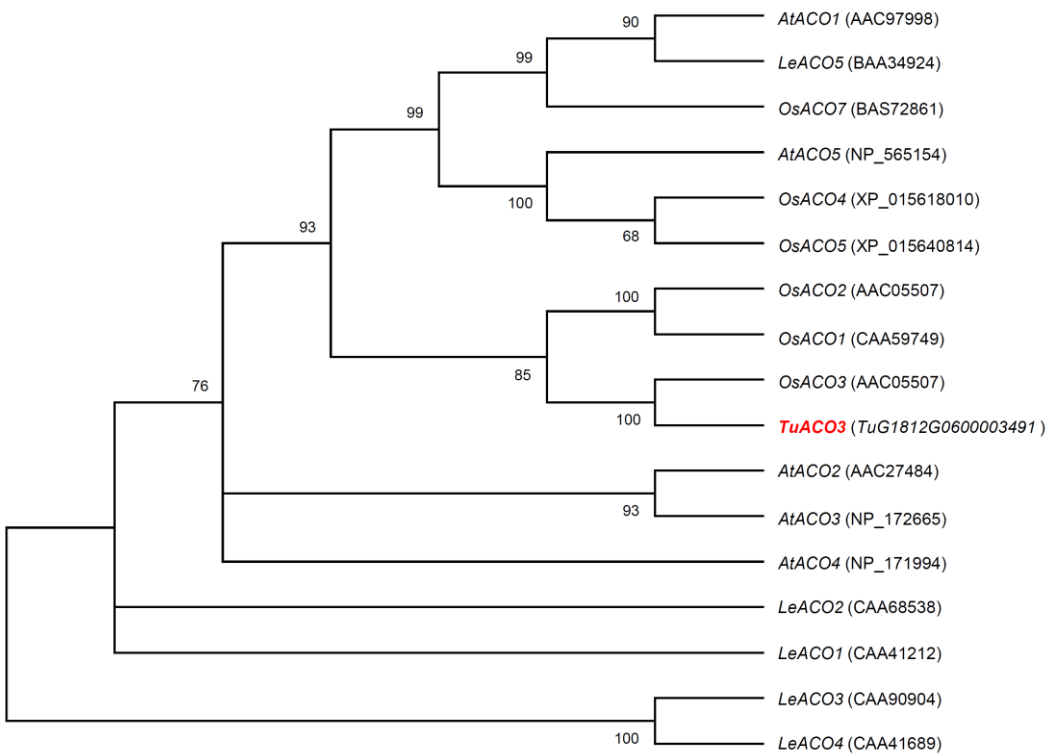


Fig. S2 Phylogenetic analysis of *ACO* genes from different plant species. The tree shown was constructed using the deduced protein sequences of 17 plant *ACO* genes whose GenBank accession number or chromosomal locus designation (for *TuACO3*) were provided in the brackets. The neighbor joining algorithm (installed at the MEGA website https://www.megasoftware.net/) and the complete deletion distance option were used for tree construction. The bootstrap values were obtained using 1000 replications.

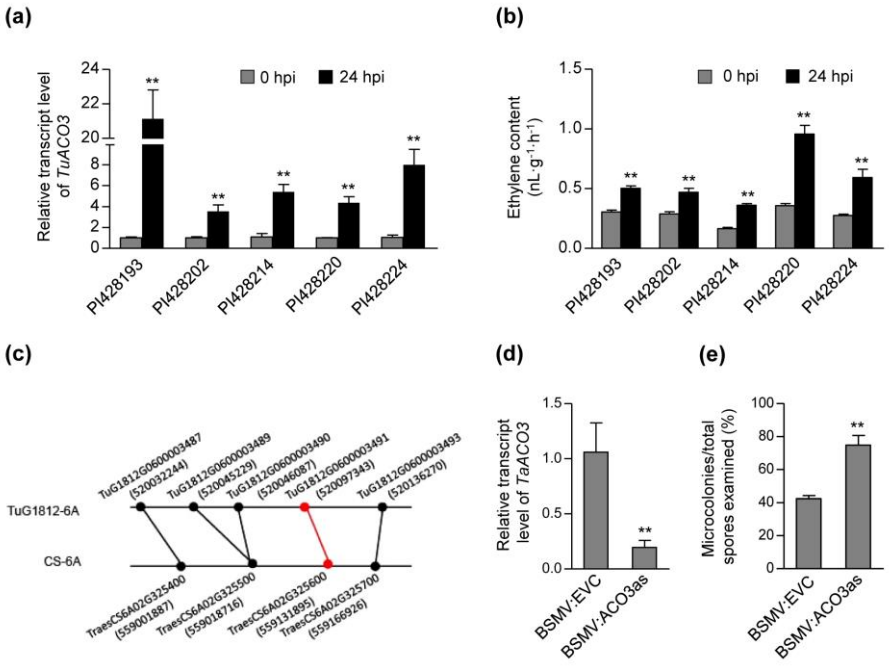


Fig. S3 Elevation of *TuACO3* expression and ethylene production by *Bgt* infection in five different *T. urartu* accessions and analysis of *TaACO3* function in common wheat using BSMV-mediated VIGS. (a) Elevation of *TuACO3* transcript level by *Bgt* infection revealed by qRT-PCR. The samples analyzed were taken at 0 and 24 hpi, respectively. (b) Up-regulation of ethylene production by *Bgt* infection determined using gas chromatography. (c) Identification of the ortholog of *TuACO3* (TuG1812G0600003491 located on the 6A chromosome of G1812) in the common wheat cultivar Chinese Spring (CS) through analyzing syntenic collinear genes. The common wheat ortholog (*TaACO3*, TraesCS6A02G325600) was located on the 6A chromosome of CS. The number in the brackets indicate the position of the gene along the assembled chromosome of G1812 or CS. (d, e) Efficient decrease of *TaACO3* transcript level in common wheat by the recombinant virus BSMV:ACO3as (d) and significant increase of *Bgt* microcolonies in the plants in which *TaACO3* was silenced by BSMV:ACO3as. The numerical data shown were reproducible in three independent experiments. Each mean (± SE) was determined using five (a, b) or three (d, e) biological replicates. **, $P < 0.01$ (Student's *t*-test).

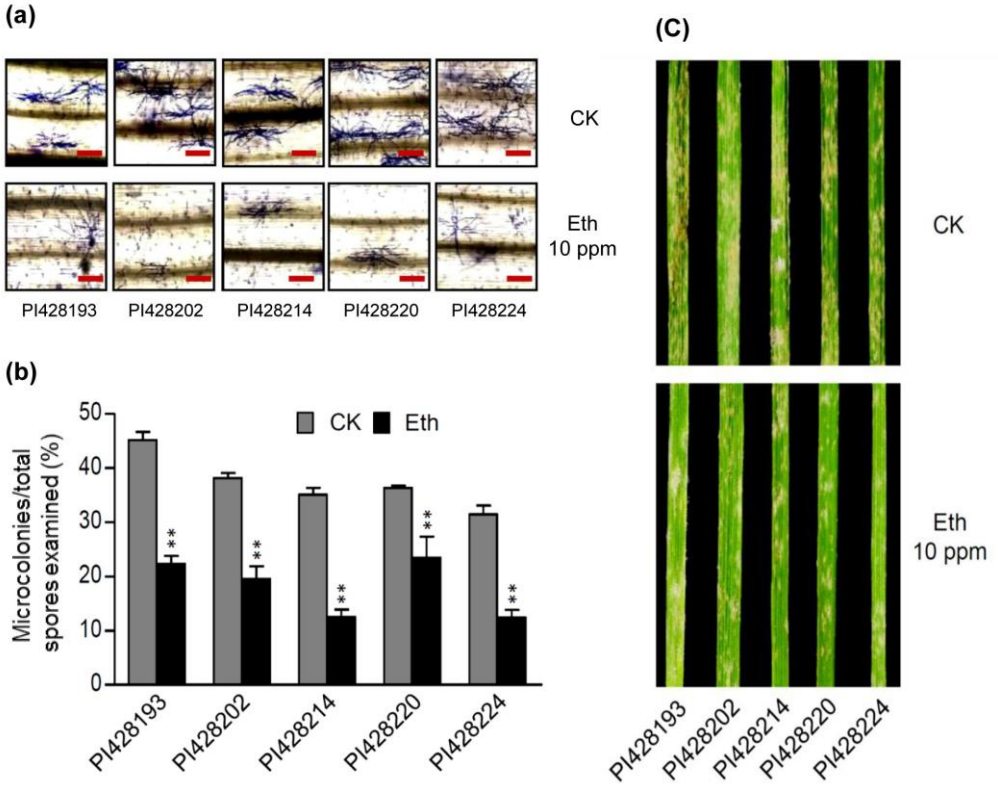


Fig. S4 Inhibition of *Bgt* colony development and growth by ethylene treatment in five different *T. urartu* accessions. The *Bgt* isolate used in the inoculation was E09. (a, b) Differences in *Bgt* microcolony development between ethylene treated plants and untreated controls (CK) at 72 hpi, as shown by Coomassie blue staining of fungal structures (al) and quantitative comparison (b). (c) Comparison of *Bgt* colonies in ethylene treated plants and untreated controls (CK) at 8 dpi. The data shown were representative of three independent experiments. Each mean (±SE) was calculated using five biological replicates. **, $P < 0.01$ (Student's *t*-test). Bars, 200 μm.

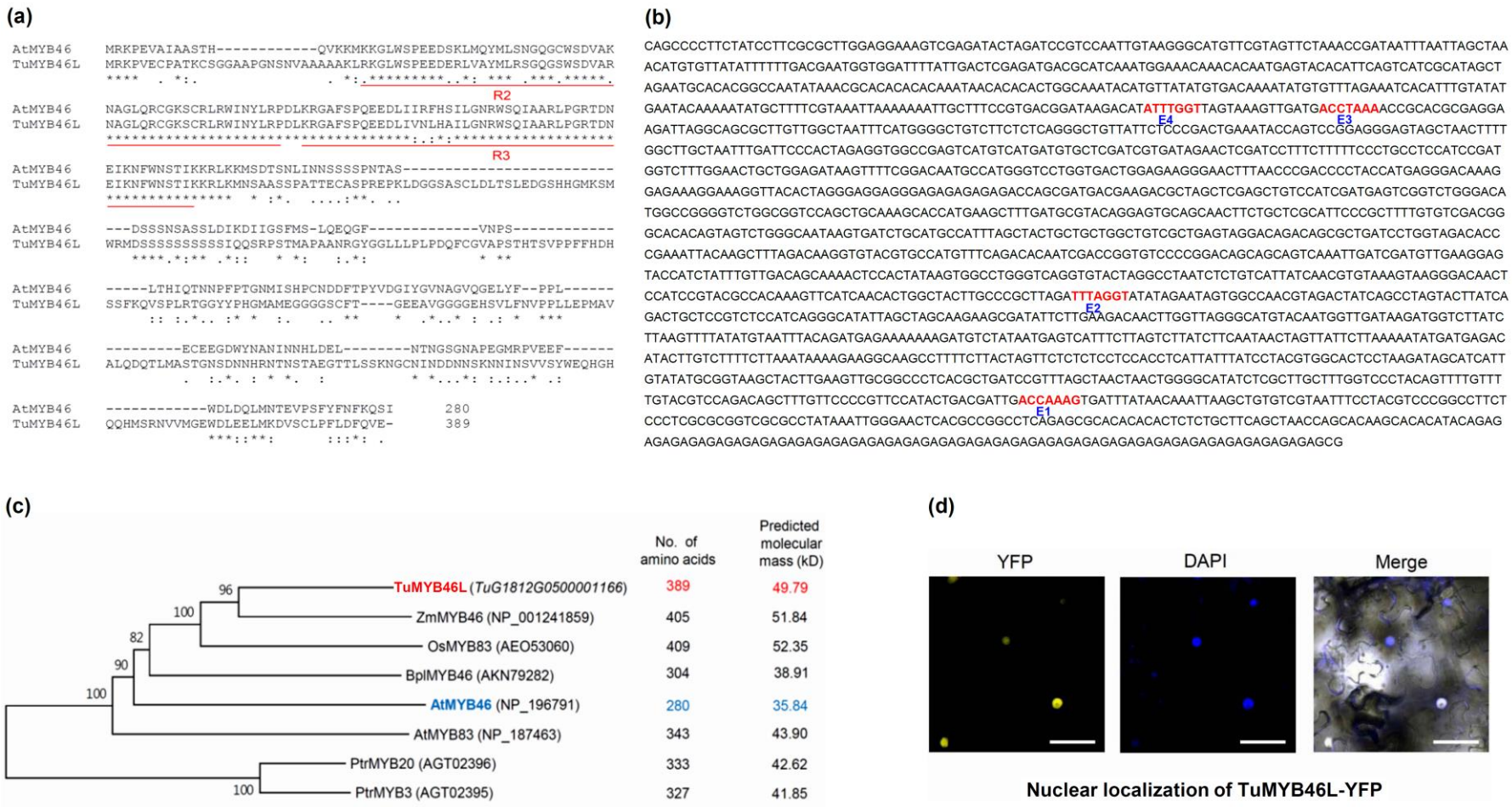


Fig. S5 Primary structure, phylogeny and nuclear localization of TuMYB46L and the predicted MYB46 binding sites in the 2 kb promoter region of *TuACO3*. (a) Comparison of amino acid sequence between AtMYB46 and TuMYB46L. The conserved R2R3 MYB domain is underlined. (b) Presence of four putative MYB46 bindings sites (E1 - E4) in the 2 kb promoter region of *TuACO3*. (c) Phylogenetic analysis of MYB46 homologs from different plant species. The tree shown was

constructed with the deduced amino acid sequences of eight MYB46 homologs whose GenBank accession number or chromosomal locus designation (for TuMYB46L) are provided in the brackets. The number of amino acid residues and the molecular mass of the predicted proteins are also presented. (d) Nuclear localization of TuMYB46L-YFP fusion protein in the leaf cells of *N. benthamiana*. The TuMYB46L-YFP fusion protein was expressed from the T-DNA construct p35S:TuMYB46L-YFP by agroinfiltration (Methods S1). The infiltrated leaf was counter-stained with the nucleophilic compound 4',6-diamidino-2-phenylindole (DAPI), followed by microscopic examination under YFP and DAPI channels, respectively. The TuMYB46L-YFP signal (arrowed) was confirmed to be in the nucleus after merging the images taken under YFP and DAPI channels, respectively. The data shown was typical of three separate experiments. Bars, 25 μm.

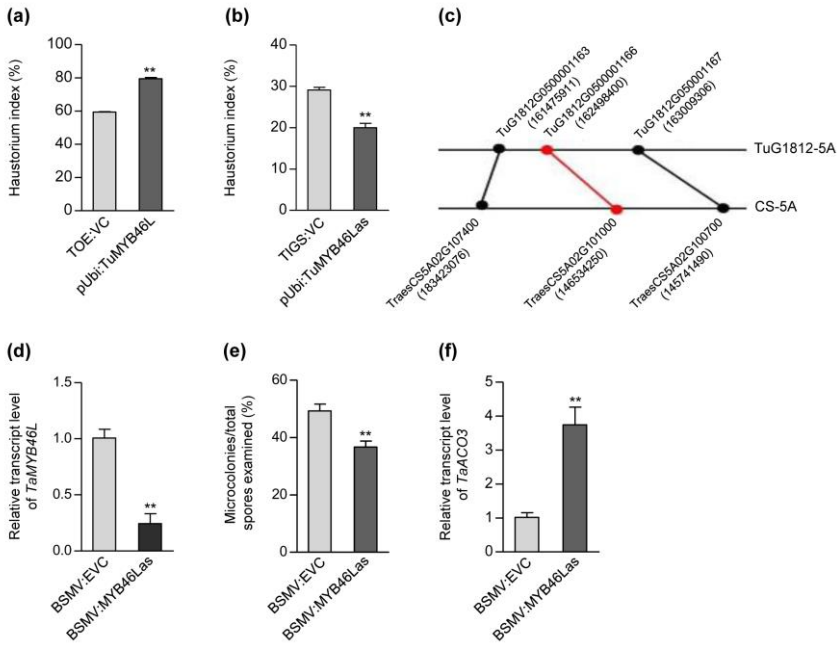


Fig. S6 Effects of transiently overexpressing or silencing *TuMYB46L* on *Bgt* haustorium growth and analysis of *TaMYB46L* function by VIGS in common wheat. (a, b) *Bgt* haustorium growth in G1812 leaf cells was stimulated by transiently expressing *TuMYB46L* from the construct pUbi:MYB46L, but suppressed by transiently silencing *TuMYB46L* with the construct pUbi:MYB46Las. TOE:VC and TIGS:VC, empty vector controls. (c) Identification of the ortholog of *TuMYB46L* (TuG1812G0500001166 located on the 5A chromosome of G1812) in the common wheat cultivar Chinese Spring (CS) through analyzing syntenic collinear genes. The common wheat ortholog (*TaMYB46L*, TraesCS5A02G101000) was located on the 5A chromosome of CS. The number in the brackets indicate the position of the gene along the assembled chromosome of G1812 or CS. (d-f) Significant decrease of *TaMYB46L* transcript level in common wheat by the recombinant virus BSMV:MYB46Las (d), which led to substantial reduction of *Bgt* microcolonies (e) and clear increase of *TaACO3* transcript level (f) in the plants in which *TaMYB46L* was silenced by BSMV:MYB46Las. The results in (a) and (b) were typical of two independent experiments, with each mean (± SE) calculated from the data of three technical repeats (more than 200 transfected cells examined in each repeat). The data in (d) to (f) were represent of three independent experiments, with each mean (± SE) calculated from three biological replicates. **, $P < 0.01$ (Student's *t*-test).

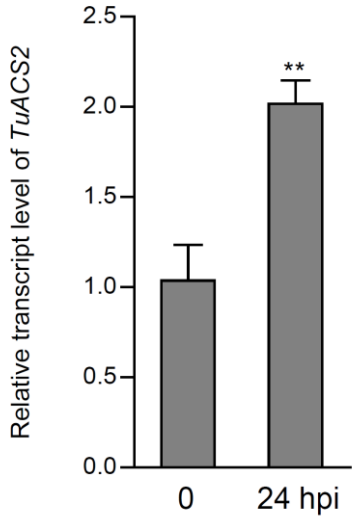
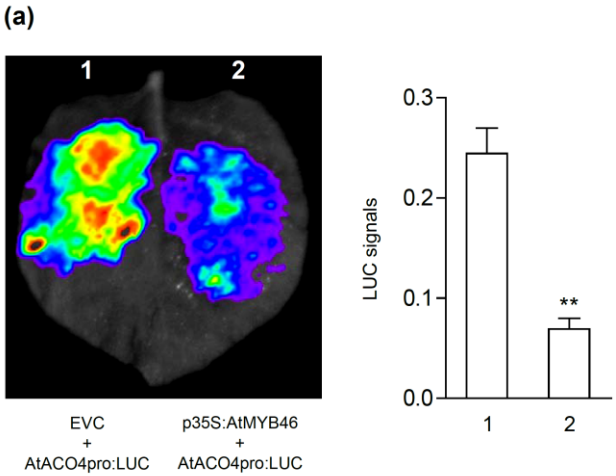


Fig. S7 Elevation of *TuACS2* transcript level by *Bgt* infection at 24 hpi. Three *ACS* genes, *TuACS1* (TuG1812G0300001193), *TuACS2* (TuG1812G0400000544) and *TuACS3* (TuG1812G0700000711), were annotated in the genomic sequence of the *T. urartu* accession G1812 (Ling *et al.*, 2018). By qRT-PCR assay with gene specific primers (Table S1), only the transcript level of *TuACS2* was found to be significantly up-regulated by *Bgt* at 24 hpi. The result displayed was typical of three independent experiments. Each mean (± SE) was determined from three biological replicates. **, $P < 0.01$ (Student's *t*-test).



(b)

AGGAGGTCCACT**AGTAGGT**CAAGTTGGCTTTGTGGTGTCGATAACATGAGATTGACCAAAGCTGTAGCTTGACCCCAAGGCCACGAGGG
AGAAAGATCT**ACCAAAC**CAAAATCATCATAACACATCTCTTTCTAGTATATAGCCCACCACAAATATTAATTCTTTTAATAGTTATACACCAC
AAATTTTATCCCCCATTGGCCATTGACTAGAGTCGTTTTCGTCTTTGACAGTGGTAAAAGCTTCTGTATATGTGAATTTAGGATTCCACCTT
TCTTAGGTTTCTCATTACAAGTTGATTTAACTTCTTGACTTCTCAAGTTGTTGTTTTGTAATTTAGTAAAAATTTGCTTACAAGTCATACATGA
TTGAATATAACACCGGCTGCTAAGCCTATTTTTCTATCATAAGTTTCGCTTTCGAGTCAAAATGTTAGTCAACATGAAATAAAACACGTTTC
TTTATTTGTTTTGCTTATTGATCAACTTTAAAATAGTCCCATAAAATAGCAAATATTAAGAAATTTCAAATCAATACATTAGTTGCCAAAACCC
GTCTGTGATATTATACTTTTTGAGGCTCCATAATAATGACGTGAGATTTGTTTGTGACTGAGGAAGTGGAAAATAAAGTTAAGCATGTCTTA
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AGGAAGTAAGGCAATGAATGTTTTTTTTTTTTTTTTCCATTGCATTTGCTTTTAAATCCTTATAAGAATTTGAAAGAAAAATATCTTATCAACT
CTTAGAGGTGATCTTTGGGCCAAAGAGGGAACCACGTAATCGAATCTGAACTTCTCCGCTTAAATGGGAAATTTTATTAGATCTGATAAAC
GGCCAAAAGTGATAGCTCATGATTCTTAATATAGACGTGCATACAGAGATGTTGTTACCGGCCGAAGAGCAGCTTCGTAATCAACGGACA
CAAATAAACCACTATTGGTCCATCAAAATTAATACAAAACGAATGAAAACTGCTGTGTCATAAGACTAAATAATTTTGTATATTATTATGATT
TAGATGGATGCGGTCTACAAATTTTTTTAACAAAA**ACCAACC**ATTAGTTTGTTTAAGTTACTTGAAAACAAGAAAAGGTTCAGTGAAAGGTG
ATATATAAGCTATTATTATTATACACAGAGTAAAAGGAAGAAATGTGAAAAGGAAGATTAATCCACACTAAAAGTAAAAGGAAGAAAACGG
GTCAAACAATGAATCGTCTGACTGAGTAATAATATACGTCGCTGTCATATATTTTGTATAACCATAAATTTTTTATATTTTTTCCGACCCAAA
TTATTTTTAGTTTTCTATAGCTAGTTACTAAATTAAAGGTGATGAATAGTAATCATGACTTTATGTCACACGATAAGGTTATTATC**ACCTACC**
TTTGAGTATATTTAGGATTTTCCGAAGACTTGGACGACCCAACTCAGTTTGGATAAAACCTTGTGGTCTATTCTAAATTTAATTCTAATTTAC
TTCTGATTATGTGGATTTATACACGGTTAAACATCAGAGGCTGAATAATAATTAGTTTGATAGATCTGATAGTTCCTCTGTTTTCTTTTTCTT
TTTTGAGTTGATTATACTGTTTGCTTTTGTTGATTATTAGTCCAAAACATTAAAAGAACAAAAACAAAAAAAAGTGTTCACGCTTTTTGGTCG
ACCGTAACATACTTTATTGAATTTTGTTAAAAAATCATTACACAAATGATATTATGGATGTGTGTGGTCCAAAA**AACAAAT**ATTGACTGTAG
AATGATAACTCGTAGAAGTAACTCAAAGCTCCTCTCTGCGCTTCTTTTCTCTCTATAAAACCAAGTCTTTGATCATCAGATTATATCAAACTT
CAAAAGCGTCAAACATAAAGTTAGGCCAAGAAACCCATTTAAAAAAAAAGAGAGAGAG

Fig. S8 Suppression of *AtACO4* promoter activity by *AtMYB46*. (a) Suppression of *AtACO4* promoter activity by AtMYB46 transiently expressed from the construct p35S:AtMYB46 in *N. benthamiana* leaf cells, as indicated by luminescence imaging (left panel) and quantitative comparison of luciferase signals (right panel). (b) Presence of five putative MYB46 binding sites (highlighted in red) in the 2 kb promoter region of *AtACO4*. The data shown in (a) were representative of three independent experiments. Each mean (± SE) was determined from three biological replicates. **, $P < 0.01$ (Student's *t*-test).

Table S1 PCR primers and oligonucleotides used in this study

Primer name	Use	Forward primer (5'-3')	Reverse primer (5'-3')
Wheat actin gene	qRT-PCR	CAACGAGCTCCGTGTCGCA	GAGGAAGCGTGTATCCCTCATAG
TuACO3	qRT-PCR	CTGTGCGAGAACCTGGGC	GCGACCAGGTCGGGGCGC
TuMYB46L	qRT-PCR	TGCCCGGCGACGAAGTGCAG	TCCGCAGCATGTACGCCACC
TaACO3	qRT-PCR	CTGTGCGAGAACCTGGG	GGCGTCGGTGTGCGCGCG
TaMYB46L	qRT-PCR	TGCCCGGCGACGAAGTGCAG	TCCGCAGCATGTACGCCACC
TuACS2	qRT-PCR	ACGCGCTCGGCCTCCGCCG	CGCCACCCTGAAGTCTCTCT
TuG1812G0200004053	qRT-PCR	CATGGCGATGCTCCCGTTC	CAGTAGGCGTCGGTAGTGCC
TuG1812G0200004054	qRT-PCR	CATGGCGATCTTGGCTCTCG	CATGGCGATCTTGGCTCTCG
TuG1812G0300003072	qRT-PCR	GCAGCGGCGGCACCCCGGT	CCTTGGCCAGGCACGCCGCG
TuG1812G0100002418	qRT-PCR	CTGCCCTCGCCGTGTGCGCC	GCATGCTCGCGTACACCGACC
TuACO3-CDS	CDS cloning	ATGGCAATTCCTGCTAATGC	TCACGCAGTGGCGATGGGC
TuMYB46L-CDS	CDS cloning	ATTTAATTAGCTAAACATGTG	CGCTCTCTCTCTCTCTCTCTC
TuACO3-PRO	Promoter cloning	CAGCCCCTTCTATCCTTCG	TCACTCAACTTGGAAATCAAGG
pDONR201-TuACO3 (TIGS)	Constructing pUbi:TuACO3as for transiently silencing *TuACO3*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCTGTGCGAGAACCTGGGC	GGGGACCACTTTGTACAAGAAAGCTGGGTCGCGACCAGGTCGGGGCGC
pDONR201-TuMYB46L (TIGS)	Constructing pUbi:TuMYB46Las for transiently silencing *TuMYB46L*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTGCCCGGCGACGAAGTGCAG	GGGGACCACTTTGTACAAGAAAGCTGGGTCGTCCGCAGCATGTACGCCAC
pDONR201-TuMYB46L-YFP	Constructing p35S:TuMYB46L-YFP for nuclear localization of TuMYB46L	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAGGAAGCCCGTGGAGTG	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTCAACTTGGAAATCAAGG
pDONR201-TuACO3	Constructing pUbi:TuACO3 for transiently overexpressing *TuACO3*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCAATTCCTGCTAATGC	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCACGCAGTGGCGATGGGC
pDONR201-TuMYB46L	Constructing pUbi:TuMYB46L for transiently overexpressing *TuMYB46L*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAGGAAGCCCGTGGAGTG	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCACTCAACTTGGAAATCAAGG
VIGS-ACO3	Constructing BSMV-γ vector for silencing *TuACO3*	AAGGAAGTTTAACTGTGCGAGAACCTGGGC	AACCACCACCACCGTGCGACCAGGTCGGGGCGC
VIGS-MYB46L	Constructing BSMV-γ vector for silencing *TuMYB46L*	AAGGAAGTTTAATGCCCGGCGACGAAGTGCAG	AACCACCACCACCGTTCCGCAGCATGTACGCCACC
pHZ206-TuACO3	Constructing overexpressing vector for *TuACO3*	AGATCCAGTGGGATCCATGGCAATTCCTGCTAATGC	TTATAGTCAAGCTTGGTACCCGCAGTGGCGATGGGC
pHZ206-TuMYB46L	Constructing overexpressing vector for *TuMYB46L*	AGATCCAGTGGGATCCATGAGGAAGCCCGTGGAGTG	TTATAGTCAAGCTTGGTACCCTCAACTTGGAAATCAAGG
pABAi-TuACO3-PRO	Constructing Y1H BD vector	TTGAATTCGAGCTCGGTACCCAGCCCCTTCTATCCTTCG	ATGCCTCGAGGTCGACCGCTCTCTCTCTCTCTCTCTC
pGADT7-TuMYB46L	Constructing Y1H AD vector	CAGATTACGCTCATATGATGAGGAAGCCCGTGGAGTG	CACCCGGGTGGAATTCTCACTCAACTTGGAAATCAAGG
pGADT7-GFP	Constructing Y1H AD vector	CAGATTACGCTCATATGATGGTGAGCAAGGGCGAGGA	CACCCGGGTGGAATTCTTACTTGTACAGCTCGTCCA
pLL00r-TuACO3-PRO	Constructing reporter vector	GGCCAGTGCCAAGCTTCAGCCCCTTCTATCCTTCG	CCTCTAGAGTCGACCTGCAGCGCTCTCTCTCTCTCTCTCT

pRI101-TuMYB46L	Constructing effector vector	ACATATGCCCGTCGACATGAGGAAGCCCGTGGAGTG	CAGAATTCGGATCCGGTACCCTCAACTTGGAAATCAAGG
pRI101-GFP	Constructing effector vector	ACATATGCCCGTCGACATGGTGAGCAAGGGCGAGGA	CAGAATTCGGATCCGGTACCTTACTTGTACAGCTCGTCCA
pET32a-TuACO3	Constructing *E. coli* expression vector for expressing TuACO3 HIS tag protein	CGGTTCTGGTTCTGGCCATATGGCAATTCCTGCTAATGC	CCGCAAGCTTGTCGACCGCAGTGGCGATGGGCGC
pET32a-TuMYB46L	Constructing *E. coli* expression vector for expressing TuMYB46L HIS tag protein	CGGTTCTGGTTCTGGCCATATGAGGAAGCCCGTGGAGTG	CCGCAAGCTTGTCGACCTCAACTTGGAAATCAAGG
ChIP-TuACO3-1	ChIP-qPCR	TTCTTCTTCTTTTCTTGAGG	GATCTAGTATCTCGACTTTC
ChIP-TuACO3-2	ChIP-qPCR	CTAGATCCGTCCAATTGTAAG	GCGTCATCTCGAGTCAATAA
ChIP-TuACO3-3	ChIP-qPCR	GACTCGAGATGACGCATCAA	CCAGTGTGTGTTATTTGTGTG
ChIP-TuACO3-4	ChIP-qPCR	ACACACACTGGCAAATACAT	GTCTTATCCGTCACGGAAAG
ChIP-TuACO3-5	ChIP-qPCR	GCTTTCCGTGACGGATAAGA	GAGAGAAGACAGCCCCATG
ChIP-TuACO3-6	ChIP-qPCR	CATGGGGCTGTCTTCTCTC	GACTCGGCCACCTCTAGTGG
ChIP-TuACO3-7	ChIP-qPCR	GAGGTGGCCGAGTCATGTCA	CATTGTCCGAAAACTTATCTC
ChIP-TuACO3-8	ChIP-qPCR	CGGACAATGCCATGGGTCCT	GGTCTCTCTCTCTCCCTCC
ChIP-TuACO3-9	ChIP-qPCR	GAGACCAGCGATGACGAAGAC	ACGCATCAAAGCTTCATGGTG
ChIP-TuACO3-10	ChIP-qPCR	GATGCGTACAGGAGTGCAGC	CGACAGCCAGCAGCAGTAGC
ChIP-TuACO3-11	ChIP-qPCR	GCTGGCTGTCGCTGAGTAGG	GGGACACCGGTCGATTGTGT
ChIP-TuACO3-12	ChIP-qPCR	CGGTGTCCCCGGACAGCAGC	CAGAGATTAGGCCTAGTAC
ChIP-TuACO3-13	ChIP-qPCR	CCTAATCTCTGTCATTATCA	CACTATTCTATATACCTAAAT
ChIP-TuACO3-14	ChIP-qPCR	AGAATAGTGGCCAACGTAGAC	CCTAACCAAGTTGTCTTCAAG
ChIP-TuACO3-15	ChIP-qPCR	TAGGGCATGTACAATGGTTG	TATTGAAGATAAGACTAAG
ChIP-TuACO3-16	ChIP-qPCR	CTTCAATAACTAGTTATTCT	TAATGAGGTGGAGGAGAGAG
ChIP-TuACO3-17	ChIP-qPCR	CCTCCACCTCATTATTTATCC	CCAGTTAGTTAGCTAAACGG
ChIP-TuACO3-18	ChIP-qPCR	GCTAACTAACTGGGGCATAT	TACGACACAGCTTAATTTGT
ChIP-TuACO3-19	ChIP-qPCR	ACAAATTAAGCTGTGTCGTAAT	GCTGAAGCAGAGAGTGTGTG
ChIP-TuACO3-20	ChIP-qPCR	GCTTCAGCTAACCAGCACAAG	CGCTCTCTCTCTCTCTCTCTC
EMSA-TuACO3-E1	EMSA probe	CCCCGTTCCATACTGACGATTGACCAAAGTGATTTATAA	TTATAAATCACTTTGGTCAATCGTCAGTATGGAACGGGG
EMSA-TuACO3-E3	EMSA probe	ATATTTGGTTAGTAAAGTTGATGACCTAAAACCGCACGC	GCGTGCGGTTTTAGGTCATCAACTTTACTAACCAAATAT
EMSA-TuACO3-E4	EMSA probe	GCTTTCCGTGACGGATAAGACATATTTGGTTAGTAAAGT	ACTTTACTAACCAAATATGTCTTATCCGTCACGGAAAGC
EMSA-TuACO3-mE1	EMSA probe	CCCCGTTCCATACTGACGATTGAAAAAAATGATTTATAA	TTATAAATCATTTTTTTCAATCGTCAGTATGGAACGGGG
EMSA-TuACO3-mE3	EMSA probe	ATATTTGGTTAGTAAAGTTGATGAAAAAAAACCGCACGC	GCGTGCGGTTTTTTTTCATCAACTTTACTAACCAAATAT
EMSA-TuACO3-mE4	EMSA probe	GCTTTCCGTGACGGATAAGACATTTTTTTTTAGTAAAGT	ACTTTACTAAAAAAAAATGTCTTATCCGTCACGGAAAGC

Table S2 List of six *T. urartu* genes found in two independent Y1H screens*

Gene	Protein encoded
TuG1812G0200003631	Calmodulin-binding protein 60 D-like isoform X2
TuG1812G0300001837	DDB1- and CUL4-associated factor 13
TuG1812G0300004040	Phosphatidylinositol:ceramide inositolphosphotransferase
TuG1812G0500001166	Transcription factor MYB46
TuG1812G0500002238	Transducin/WD40 repeat-like superfamily protein
TuG1812G0700002677	TBC1 domain family member 5 homolog A

*The CDS and deduced amino acid sequences of the six genes are provided in Dataset S1.

Table S3 Significantly enriched biological processes identified by GO analysis*

GO term	Description	*P* -value	Regulation	Representative *T. urartu* genes in the enriched process
GO: 0006032	Chitin catabolic process	6.60E-06	Up	*Homolog of carrot EP3-3 chitinase* (*TuG1812G0200004053*) *Homolog of carrot EP3-3 chitinase* (*TuG1812G0200004054*) *Basic chitinase* (*TuG1812G0100002418*) *Basic chitinase* (*TuG1812G0300003072*)
GO: 0030244	Cellulose biosynthetic process	0.00065	Up	*Cellulose synthase-like D3* (*TuG1812G0700003174*) *Cellulose synthase-like D5* (*TuG1812S0000765300*) *Cellulose synthase A4* (*TuG1812S0001895600*) *Cellulose synthase family protein* (*TuG1812G0500002544*)
GO: 0008299	Isoprenoid biosynthetic process	0.0012	Up	*Hydroxyl methylglutaryl CoA reductase 1* (*TuG1812G0700002624*) *Farnesyl diphosphate synthase 1* (*TuG1812G0100004098*) *GHMP kinase family protein* (*TuG1812G0500004644*) *Deoxyxylulose-5-phosphate synthase* (*TuG1812G0100002961*)
GO: 0055114	Oxidation reduction	2.40E-07	Down	*Cytochrome P450* (*TuG1812G0500001523*) *Cytochrome P450* (*TuG1812G0400002828*) *Polyamine oxidase 5* (*TuG1812G0300002967*) *Alcohol dehydrogenase 1* (*TuG1812G0400001050*)
GO: 0030001	Metal ion transport	0.00017	Down	*Heavy metal transport/detoxification superfamily protein* (*TuG1812G0500005269*) *Heavy metal transport/detoxification superfamily protein* (*TuG1812S0000313700*) *Tonoplast dicarboxylate transporter* (*TuG1812G0300002099*) *Magnesium transporter 4* (*TuG1812G0300004483*)

*GO enrichment analysis of the up- or down-regulated DEGs shared by the two treatments was accomplished using the software agriGO (http://bioinfo.cau.edu.cn/agriGO/). The CDS and deduced amino acid sequences of the six genes are provided in Dataset S1.

Table S4 Expression changes of the four chitinase genes up-regulated by both ethylene treatment and *Bgt* infection

Gene	Treatment	0 h (control)	24 h (post treatment)	Log2 (Fold_change)
TuG1812G0100002418	Ethylene	5.9832*	64.2132	3.4238
	Bgt infection	5.5522	2851.4400	9.0044
TuG1812G0200004053	Ethylene	11.1727	84.0994	2.9121
	Bgt infection	10.4543	82.1065	2.9734
TuG1812G0200004054	Ethylene	0.4373	5.4552	3.6407
	Bgt infection	0.4073	96.9208	7.8944
TuG1812G0300003072	Ethylene	0.9015	4.3136	2.2584
	Bgt infection	0.8319	474.3240	9.1551

*Mean value of transcript level (represented by fragments per kilobase of exon per million transcriptomic reads) calculated from three biological replicates.

Table S5 Presence of putative MYB46 recognition sites in the 2 kb promoter region of the five genes related to cellulose or isoprenoid synthesis*

Gene	Protein encoded	Potential TuMYB46L binding sites	Treatment	0 h (control)	24 h (post treatment)	Log2 (Fold_change)
TuG1812G0200004302	Phenylalanine ammonia lyase	ACCAAAT (-707 to -714) ACCAACC (-382 to -375) ACCAACC (-229 to -222)	*Bgt* infection	3.12	19.18	2.62
			Ethylene	3.33	7.41	1.15
TuG1812G0100000551	Phenylalanine ammonia lyase	ACCAAAT (-1433 to -1426) ACCAACC (-254 to -247) ACCAACC (-85 to -78)	*Bgt* infection	0.82	18.46	4.47
			Ethylene	0.89	9.15	3.36
TuG1812G0500002544	Cellulose synthase A9	ACCAACT (-1451 to -1444) ACCAAAT (-805 to -798) ACCAACC (-325 to -318)	*Bgt* infection	3.14	24.22	2.94
			Ethylene	3.36	25.35	2.91
TuG1812S0001895600	Cellulose synthase A4	ATTTGGT (-1974 to -1967) GGTTGGT (-186 to -179)	*Bgt* infection	7.33	36.17	2.31
			Ethylene	7.87	23.11	1.55
TuG1812G0700003174	Cellulose synthase-like D3	ATTAGGT (-1987 to -1980) GGTAGGT (-477 to -470) GGTAGGT (-473 to -466)	*Bgt* infection	2.68	18.55	2.79
			Ethylene	2.85	13.83	2.27

*The five genes were among the up-regulated DEGs shared by *Bgt* infection and ethylene treatment (see also Table S2).

Methods S1 Additional description of methods

Treatment with ACC, aminoethoxyvinylglycine or ethylene

The seedlings of *T. urartu* and KN199 (at one-leaf stage) were treated with 10 ppm ethylene in sealed 50 ml Falcon tubes for 24 h at 22°C (Yin *et al.*, 2015), and used in subsequent experiments. Treatment of *T. urartu* and KN199 seedlings by ACC (20 μM, Sigma, A3903) or aminoethoxyvinylglycine (AVG, 10 μM, Sigma, A6685) was accomplished by spraying, with the sprayed seedlings used for the desired analysis after 24 h at 22°C.

Gene expression analysis by qRT-PCR

Total RNA was extracted using the TriPure Isolation reagent (Roche, 11667165001). The cDNAs were synthesized using the GoScript Reverse Transcription System (Promega, A5000), followed by quantification in the LightCycler 480 II Real-time System (Roche Diagnostics, Penzberg, Germany) with TB Green Premix ExTaq (TaKaRa, RR420A). An *Actin* gene (GenBank accession AB181991), conserved in both common wheat and *T. urartu* (Zhang *et al.*, 2016), was used as internal control for qRT-PCR. Each assay was repeated at least 3 times using independent biological replicates.

Single-cell functional assay

At 4 h after transfections with pUbi:TuACO3 or pUbi:TuMYB46L, the leaves were inoculated with *Bgt*, with haustorium index determined using the leaf segments collected at 48 hpi. The transfections with pUbi:TuACO3as or pUbi:TuMYB46Las were accomplished similarly except that *Bgt* inoculation was performed at 48 h of the bombardment. Each transient expression or silencing assay had 3 technical repeats, with at least 200 cells with GUS expression and *Bgt* mycelia examined for each repeat. Haustorium index was calculated as the percentage of examined cells with *Bgt* haustorium (Zhang *et al.*, 2016).

Immunoblotting assay

Total leaf proteins, extracted from about 100 mg tissues, were separated in 12%

SDS-PAGE, followed by transferring to nitrocellulose membrane (Amersham Corp., 10600002). After antibody-antigen reaction, the signals were detected using the SuperSignal West Pico Chemiluminescent Substrate Kit (Thermo Fisher Scientific Inc., 34078). A parallel assay, conducted with a polyclonal antibody specific for plant actin proteins (Bioeasy Corp., BE0027), was performed as control for equal protein loading. Ponceau S staining was also conducted for checking equal loading.

Virus induced gene silencing

BSMV:ACO3as, BSMV:MYB46Las and the empty vector control (BSMV:EVC) were each introduced into at least 50 G1812 seedlings by mechanical inoculation. At 10 days after BSMV infection, the seedlings with clear BSMV symptoms ($n \geq 40$) were used for further analysis. After confirming the silencing of *TuACO3* or *TuMYB46L* expression by qRT-PCR, the seedlings were inoculated with *Bgt* as outlined above, with the inoculated leaves collected at 72 hpi for checking *Bgt* microcolonies. Ethylene content was measured either before *Bgt* inoculation or at 24 hpi of *Bgt* by placing 4 seedlings into a 300 ml glass bottle.

The common wheat orthologs of *TuACO3* and *TuMYB46L*, i.e., *TaACO3* and *TaMY46L*, were identified through Blast search of the genomic sequence of Chinese Spring at the website http://plants.ensembl.org/Triticum_aestivum/Info/Index. The VIGS inducing fragment in BSMV:ACO3as was highly conserved in *TaACO3* (93.4% identical). Likewisely, the VIGS inducing fragment in BSMV:MYB46Las was strongly conserved in *TaMYB46L* (99.2% identical). Consequently, the two viruses were also employed to silence *TaACO3* and *TaMY46L* in the common wheat cultivar KN199, respectively, with downstream analyses conducted as described above.

Yeast one-hybrid assay

The control construct pABAi:TuACO3mpro was prepared as described for pABAi:TuACO3pro, except that the promoter region of *TuACO3* was mutated by changing all four putative MYB46 binding sites (Fig. S5b) into AAAAAAA. The mutant promoter region was synthesized commercially (Genewiz, Nanjing, China, https://climsprod.genewiz.com.cn/). Another control construct, pGADT7:GFP, was

developed by cloning the GFP coding sequence into the vector pGADT7 using the primers listed in Table S1.

Effector-promoter reporter assay

The promoter region (2 kb) of *TuACO3* was cloned into the pLL00r:LUC vector carrying the coding sequence of luciferase (Zhang *et al.,* 2015), producing the reporter construct pTuACO3pro:LUC. The cDNA coding region of *TuMYB46L* was cloned into the pRI101 vector, generating the effector plasmid p35S:TuMYB46L. The *Agrobacterium* strains carrying p35S:TuMYB46L and pTuACO3pro:LUC were employed for effector-promoter reporter assay, which was performed in the leaves of 5-week-old *N. benthamiana* plants (Zhang *et al.,* 2015). The construct pTuACO3mpro:LUC, carrying the mutated promoter region of *TuACO3*, was also prepared and used as a negative control. The mutant promoter region was synthesized commercially (see above). The T-DNA construct p35S:GFP, expressing free green fluorescence protein, was used as an additional control for the assay. This construct was developed by cloning the coding sequence of GFP into the pRI101 vector. The assay was repeated 3 times, with each performed using three biological replicates.

Nuclear localization assay

A T-DNA construct, p35S:TuMYB46L-YFP, was prepared by in frame fusion of the coding sequence of *TuMYB46L* to the 5′ end of YFP coding region in the vector CTAPi-GW-mYFP (Bai *et al.,* 2012). Expression of the TuMYB46L-YFP fusion protein in *Nicotiana benthamiana* leaf cells and examination of its nuclear localization by Confocal microscopy were executed as reported by Bai *et al.* (2012).

RNA sequencing and GO analysis of differentially regulated genes

The high-quality reads were mapped to the draft genome sequence of *T. urartu* (Ling *et al.,* 2018) with the software RSEM (Li & Dewey, 2011). Two criteria were used to identify differentially expressed genes, 1) a Log2 ratio >1.0 and 2) the expression difference was consistently observed in between different biological replicates. Gene ontology (GO) enrichment analysis was accomplished using the software agriGO (http://bioinfo.cau.edu.cn/agriGO/).

Analysis of chitinase genes and chitinase enzyme assay

In the first set of chitinase assays, total chitinase activities were measured for the G1812 seedlings treated by 10 ppm ethylene (for 24 h) or infected by *Bgt* (for 24 h) and the controls. The second set of chitinase assays used the transgenic lines overexpressing *TuACO3* (TuACO3-OX1 and -OX2) or *TuMYB46L* (TuMYB46L-OX1 and -OX2) and their corresponding WTS controls. Each assay was repeated 3 times using independent biological replicates.

Bioinformatic analysis

The search of MYB46 binding sites in gene promoters was performed using the DNAMAN program (https://www.lynnon.com/dnaman.html). Alignment of amino acid sequences used the software Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Phylogenetic tree was built with the neighbor joining algorithm (installed at the MEGA website https://www.megasoftware.net/) and the complete deletion distance option were employed for tree construction. The bootstrap values were obtained using 1000 replications.

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