

Supporting Information

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SI Text

Cloning of Transcription Factor *BoMYC* as an Example in the Study. A partial sequence (575 bp) of the cabbage transcription factor (*MYC*) was constructed after sequencing fragments were obtained by RT-PCR. In the RT-PCR we used degenerate oligonucleotide primers that were derived from *MYC* sequences of other plant species (Tables S6 and S7). A full-length cDNA of 2,071-bp nucleotides was isolated using rapid amplification of cDNA ends (5'-RACE and 3'-RACE). This cDNA clone contained an ORF starting with an ATG codon preceded by an in-frame stop codon, indicating that it contains the complete coding region. The ORF of 1,833 nucleotides (from 61 to 1893) encoded a predicted protein of 610 amino acids. This cDNA clone was designated as the

Brassica oleracea MYC gene, and we use *BoMYC* as the abbreviated term referring to this gene.

BoMYC contains a helix-loop-helix domain found in specific DNA-binding proteins that act as transcription factors. In the GenBank database, the deduced protein matches well with other bHLH-like proteins. The full-length cDNA sequence of *BoMYC* has been deposited to the GenBank database under accession no. EF423803. The deduced protein sequence of *BoMYC* contains a bHLH-type DNA-binding/dimerization domain in the C-terminal region. Sequence alignment and phylogenetic tree analysis showed that *BoMYC* shared an overall identity of 87% at the amino acid level with the *Arabidopsis AtMYC2* protein (Figs. S1 and S2). We used the same method to clone the other genes used in this study (for accession numbers see Table S6).

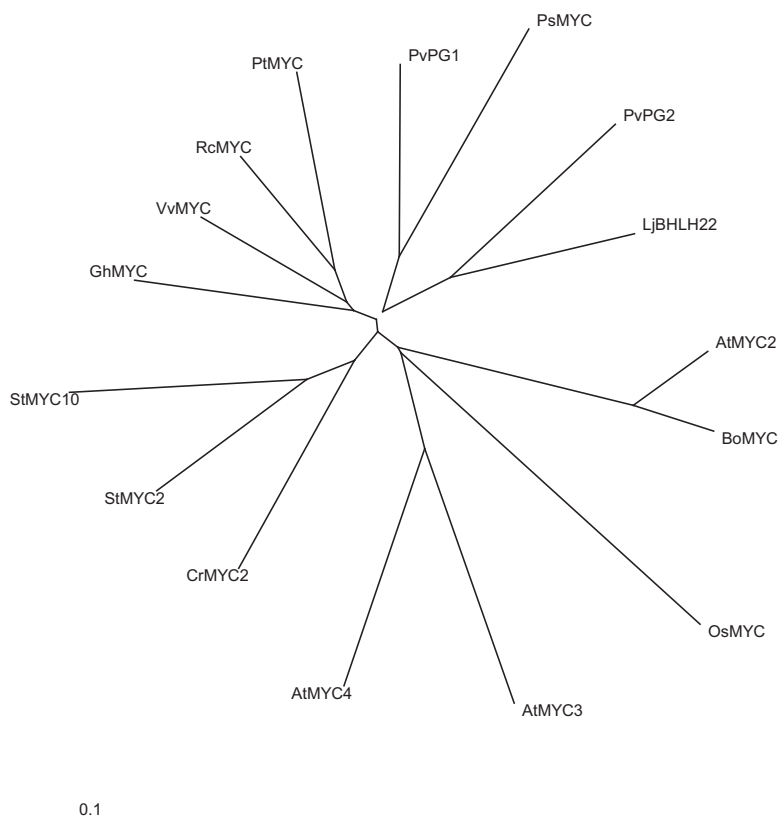


Fig. S1. Phylogenetic tree of *MYC* genes from different plant species. Accessions for *MYC* amino sequences represented in this tree are *Arabidopsis thaliana AtMYC2* (At1g32640); *A. thaliana AtMYC3* (At5g46760); *A. thaliana AtMYC4* (At4g17880); *B. oleracea BoMYC* (EF423803); *Oryza sativa OsMYC* (AY536428); *Catharanthus roseus CrMYC2* (AF283507); *Solanum tuberosum StMYC2* (AJ630505); *S. tuberosum StMYC10* (AJ630506); *Gossypium hirsutum GhMYC* (FJ358540); *Vitis vinifera MYC* (XM_002280217); *Ricinus communis RcMYC* (XM_002519768); *Populus trichocarpa PtMYC* (XM_002326688); *Phaseolus vulgaris PvMYC* (U18348); *P. vulgaris PvMYC* (U18349); *Pisum sativum PsMYC* (DQ399528); and *Lotus japonicus LjBHLH22* (FJ379754).

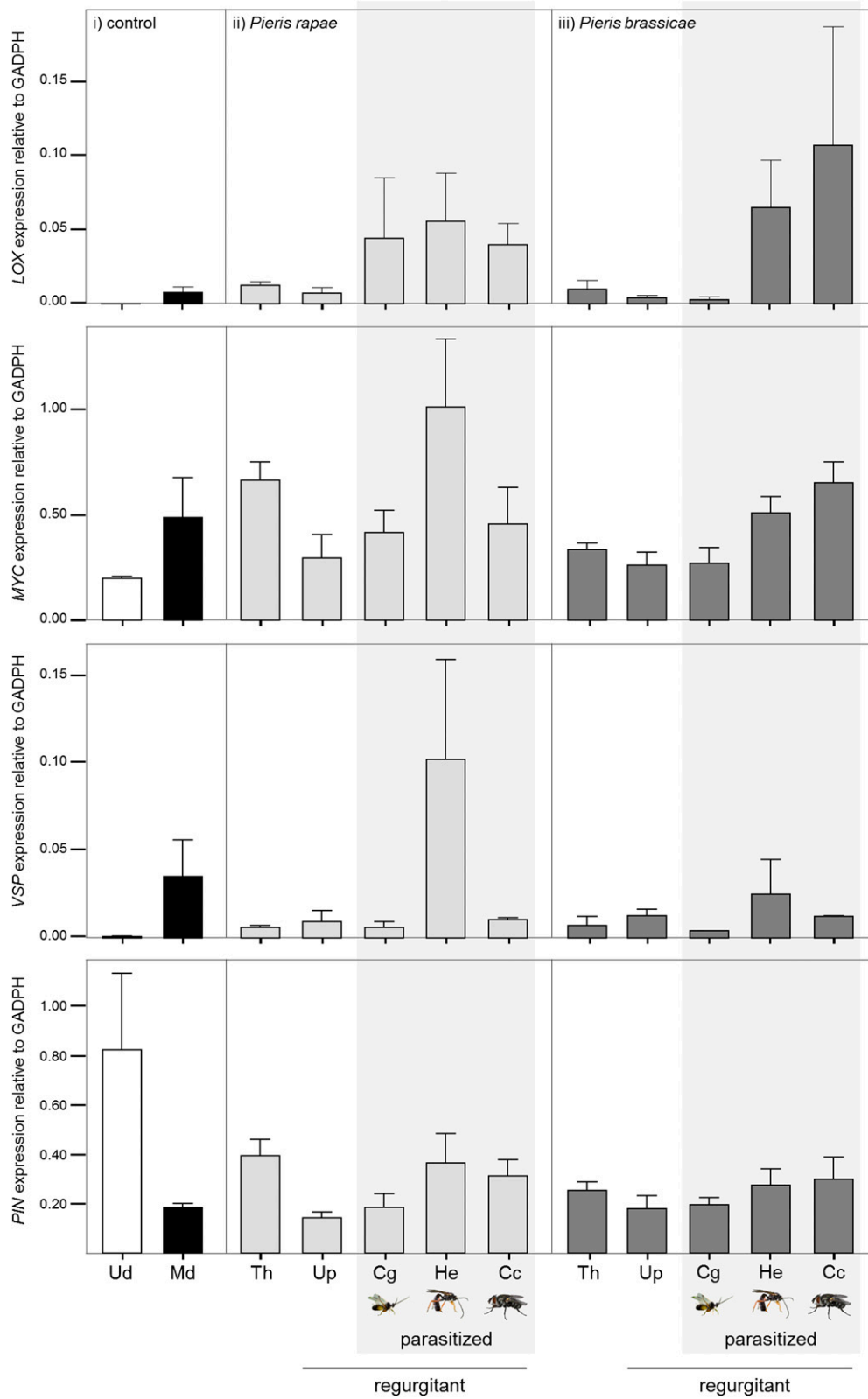


Fig. S3. Expression levels of *BoLOX*, *BoMYC*, *BoVSP*, and *BoPIN* 6 h after treatments were applied to *B. oleracea* plants. Treatments: undamaged plants (Ud, white bars); mechanical damage consisting of three needle punctures (Md, black bars); herbivore treatments with *Pieris rapae* (light gray) or *Pieris brassicae* (dark gray): true herbivory (Th), mechanical damage plus regurgitant from unparasitized caterpillars (Up) or caterpillars parasitized by *Cotesia glomerata* (Cg), *Hyposoter ebeninus* (He), or *Compsilura concinnata* (Cc).

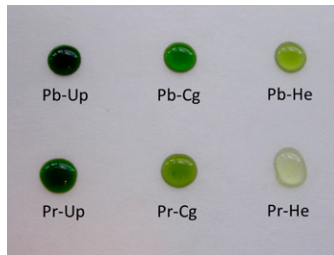


Fig. S5. Regurgitant of *Pieris brassicae* (Pb) and *Pieris rapae* (Pr) collected from unparasitized (Up), *Cotesia glomerata*-parasitized (Cg), and *Hyposoter ebeninus*-parasitized (He) caterpillars.

Table S1. Lists of different treatments in plant leaves for gene expression studies

Parameter	Treatment																								
	WT	MD		Pb		U-Pb		Cg-Pb		He-Pb		Cc-Pb		Pr		U-Pr		Cg-Pr		He-Pr		Cc-Pr			
Description	Undamaged	Mechanical damage by needle punching		First-instar <i>P. brassicae</i> feeding		Regurgitant from unparasitized <i>P. brassicae</i>		Regurgitant from <i>C. glomerata</i> parasitized <i>P. brassicae</i>		Regurgitant from <i>H. ebeninus</i> parasitized <i>P. brassicae</i>		Regurgitant from <i>C. concinnata</i> parasitized <i>P. brassicae</i>		First-instar <i>P. rapae</i> feeding		Regurgitant from unparasitized <i>P. rapae</i>		Regurgitant from <i>C. glomerata</i> parasitized <i>P. rapae</i>		Regurgitant from <i>H. ebeninus</i> parasitized <i>P. rapae</i>		Regurgitant from <i>C. concinnata</i> parasitized <i>P. rapae</i>			
Time point (h)		2	6	24	2	6	24	2	6	24	2	6	24	2	6	24	2	6	24	2	6	24	2	6	24
Symbol																									

Table S2. Multiple ANOVA analyses of gene expression over time (2, 6, 24 h) after plants were induced with different types of damage (undamaged, mechanical damage, mechanical damage plus regurgitant, true herbivory)

Variable	Time (df = 2)		Treatment (df = 3)		Time × Treatment (df = 6)		Post hoc treatment*
	F	P	F	P	F	P	
Pillai's trace	3.285	<0.001	6.202	<0.001	2.546	<0.001	
Gene							
<i>BoLOX</i> (JA)	16.859	<0.001	15.553	<0.001	15.439	<0.001	(a = 1, 2, 3) (b = 4)
<i>BoMYC</i> (JA)	0.091	0.913	5.466	0.002	7.481	<0.001	(a = 1, 2) (ab = 3) (b = 4)
<i>BoVSP</i> (JA)	15.057	<0.001	16.005	<0.001	16.599	<0.001	(a = 1, 2, 3) (b = 4)
<i>BoDEF</i> (JA)	0.900	0.412	14.023	<0.001	1.297	0.273	(a = 2, 3, 4) (b = 1)
<i>BoPAL</i> (SA)	4.277	0.018	3.624	0.018	7.425	<0.001	(a = 1) (ab = 2, 4) (b = 3)
<i>BoPR1</i> (SA)	2.127	0.128	4.793	0.005	4.341	0.001	(a = 1, 2) (ab = 4) (b = 3)
<i>BoACS</i> (ET)	1.327	0.273	2.216	0.096	3.425	0.006	—
<i>BoMYR</i>	0.047	0.954	0.694	0.559	3.160	0.009	—
<i>BoPIN</i>	3.366	0.041	22.202	<0.001	1.838	0.107	(a = 2, 3) (b = 4) (c = 1)

Values in bold indicate significant effects at $P < 0.05$.

*Post hoc classes for the factor Treatment based on Tukey: (1) undamaged plants, (2) mechanical damage, (3) mechanical damage plus regurgitant, (4) true herbivory. Classes are indicated with a, b, and c.

Table S3. MANOVA analysis of gene expression over time (2, 6, 24 h) after plants were manually damaged and regurgitant of either *Pieris rapae* or *Pieris brassicae* caterpillars was applied, which originated from unparasitized caterpillars or caterpillars parasitized by different parasitoids (*Cotesia glomerata*, *Hyposoter ebeninus*, *Compsilura concinnata*)

Variable	Time (df = 2)		Herbivore (df = 1)		Parasitoid (df = 3)		Time × herbivore (df = 2)		Time × parasitoid (df = 6)		Herbivore × parasitoid (df = 3)		Time × herbivore × parasitoid (df = 6)	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Pillai's trace	11.907	<0.001	1.686	0.125	2.764	<0.001	1.759	0.045	1.935	<0.001	1.482	0.077	1.077	0.344
Gene														
<i>BoLOX</i> (JA)	9.279	<0.001	0.004	0.947	4.318	0.009*	0.135	0.874	0.354	0.904	2.211	0.099	1.205	0.320
<i>BoMYC</i> (JA)	40.231	<0.001	1.523	0.223	11.604	<0.001 [†]	0.321	0.727	2.389	0.042	1.752	0.169	0.849	0.539
<i>BoVSP</i> (JA)	3.354	0.043	0.785	0.380	3.868	0.015 [‡]	1.923	0.157	2.672	0.026	2.055	0.119	1.336	0.260
<i>BoDEF</i> (JA)	23.552	<0.001	1.726	0.195	1.920	0.139	0.026	0.975	5.501	<0.001	5.771	0.002	0.816	0.563
<i>BoPAL</i> (SA)	12.936	<0.001	0.050	0.825	1.446	0.241	0.938	0.398	0.989	0.443	0.821	0.489	0.521	0.789
<i>BoPRT</i> (SA)	0.412	0.665	0.805	0.374	0.296	0.828	0.900	0.413	0.528	0.784	0.479	0.698	0.539	0.776
<i>BoACS</i> (ET)	5.490	0.007	1.735	0.194	0.491	0.690	2.142	0.129	0.225	0.967	0.914	0.441	0.629	0.706
<i>BoMYR</i>	0.907	0.411	0.894	0.349	2.648	0.059	2.546	0.089	0.713	0.641	1.534	0.218	0.469	0.828
<i>BoPIN</i>	26.174	<0.001	1.591	0.213	27.871	<0.001 [§]	3.521	0.037	3.509	0.006	0.965	0.417	1.372	0.345

Values in bold indicate significant effects at $P < 0.05$.

*Post hoc classes based on Tukey (a = unparasitized) (ab = *C. glomerata*, *C. concinnata*) (b = *H. ebeninus*).

[†]Post hoc classes based on Tukey (a = unparasitized) (ab = *C. glomerata*) (b = *C. concinnata*) (c = *H. ebeninus*).

[‡]Post hoc classes based on Tukey (a = unparasitized, *C. glomerata*) (ab = *C. concinnata*) (b = *H. ebeninus*).

[§]Post hoc classes based on Tukey (a = unparasitized, *C. glomerata*) (b = *C. concinnata*, *H. ebeninus*).

Table S4. Amount of feeding damage on leaves of the different induction treatments that entered the oviposition experiments

Herbivore*	Parasitism [†]	No. of leaves	Mean damage (mm ²)	SD
<i>Pieris rapae</i> (Pr)	Unparasitized (Up)	135	64.96	28.02
<i>Pieris rapae</i> (Pr)	<i>Cotesia glomerata</i> (Cg)	145	40.15	19.90
<i>Pieris rapae</i> (Pr)	<i>Hyposoter ebeninus</i> (He)	125	23.38	9.78
<i>Pieris brassicae</i> (Pb)	Unparasitized (Up)	135	74.77	30.11
<i>Pieris brassicae</i> (Pb)	<i>Cotesia glomerata</i> (Cg)	123	49.42	24.22
<i>Pieris brassicae</i> (Pb)	<i>Hyposoter ebeninus</i> (He)	137	30.44	15.29

*Herbivore species significantly differed in the amount of damage inflicted to the plants (ANOVA, $F = 30.11$, $P < 0.001$).

[†]Parasitoid species significantly affected the amount of leaf damage (ANOVA, $F = 246.66$, $P < 0.001$). The interaction between the factors "herbivore" and "parasitism" was not significant (ANOVA, $F = 0.28$, $P = 0.76$).

Table S5. Pearson's correlations between relative leaf damage and relative number of eggs oviposited on treatment A for each of the two choice tests offered to diamondback moth to study oviposition preference

Treatment A*	Treatment B	Pearson's correlation	n	P
Up Pr	He Pr	-0.06	28	0.76
Up Pr	Cg Pr	-0.23	43	0.14
He Pr	Cg Pr	-0.08	30	0.69
Up Pb	He Pb	-0.09	34	0.59
Up Pb	Cg Pb	-0.22	31	0.23
He Pb	Cg Pb	-0.07	31	0.71
Up Pr	Up Pb	-0.16	30	0.93
He Pr	He Pb	0.28	31	0.13
Cg Pr	Cg Pb	-0.13	30	0.49

*Unparasitized (Up), *Hyposoter ebeninus*-parasitized (He), and *Cotesia glomerata*-parasitized (Cg) caterpillars of *Pieris rapae* (Pr) and *Pieris brassicae* (Pb).

Table S6. Cloning specific genes from different signal pathways in cabbage plants (*B. oleracea*) by using degenerate primers that were derived from sequences of other plant species

GenBank accession no.	Gene	Function	GenBank accessions of different plant species whose sequences were used for designing degenerate primers						
			<i>Arabidopsis</i>	<i>Brassica</i>	Rice	Tomato	Potato	Tobacco	Soybean
EU921648	<i>BoACS</i>	1-Aminocyclopropane-1-carboxylate synthase	U73786	X82273					
EF423802	<i>BoDEF</i>	Plant defensin	AY063779	U59459					
EF123055	<i>BoGAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase	M64116	X04301		U93208	U17005		
EF123056	<i>BoLOX</i>	Lipoxygenase	NM104376, L23968	AY16214, AY162143		U37839, U37840	X96405, X96406	AY254349	X13302
EF423803	<i>BoMYC</i>	JA-responsive transcription factor	NM_102998		AY536428	AF011557	AJ63050, AJ630506		
DQ456999	<i>BoMYR</i>	Myrosinase	AY113880	X60214					
EF423804	<i>BoPAL</i>	Phenylalanine ammonia-lyase	AY303128	AY795078					
EF423805	<i>BoPIN</i>	Cysteine proteinase inhibitor	AB04405	DQ539645		X94946	U45450		
EF423806	<i>BoPR1</i>	Pathogenesis-related protein-1	AY117187	U70666		X68738		X05959	AF136636
EU921650	<i>BoVSP</i>	Vegetative storage protein	AY092991, AY114606						

Table S7. Specific primers used for quantitative PCR

Gene	Forward (from 5' to 3')	Reverse (from 5' to 3')
<i>BoLOX</i>	ACTTCCCGTCCCGTTCTTGG	GATTGTCGTGCCCGTGAATGC
<i>BoMYC</i>	GGCTGGACCTACGCTATATTCTGG	GCTCACGCAACACCTTCTTACG
<i>BoVSP</i>	GACTACCTCACTTCCACAG	CGGGTCTATCTTCTGTCC
<i>BoDEF</i>	GTTTGCTTCCATCATCACCTTCTC	CACTTGACCTCTCGCAACTTAG
<i>BoPAL</i>	TCGCTATGGCTTCTTACTGCTCTG	GAGGTCTTACGAGATGAGATGAGTCC
<i>BoPR1</i>	GTCAACGAGAAGGCTAACTATAACTACG	TTACACCTTGCTTGGCCACATCC
<i>BoACS</i>	ACTACGGTTGGCTGAAAGAC	GAGAAAACGTTTCAGCTTACC
<i>BoMYR</i>	GTTTCTTAGACCGCCAGATCATAAAG	CAGTGCTTACCTTCCACCAAATTC
<i>BoPIN</i>	TTCAAGAACATAACAACAAAGAGAACAAGG	GAGTTAGGTAGTACATCATTCCAGCAAC
<i>BoGAPDH</i>	GCTACGCAGAAGACAGTTGATGG	TGGGCACACGGAAGGACATAC