

Figure S1 Multiple sequence alignment of CmbHLH family proteins. The four-color modules at the top

of the figure are represented respectively: red represents the N-terminal basic region of bHLH, yellow and green represents two amphiphilic regions, blue represents variable length loop. Process the data into pictures by using Clustal W.

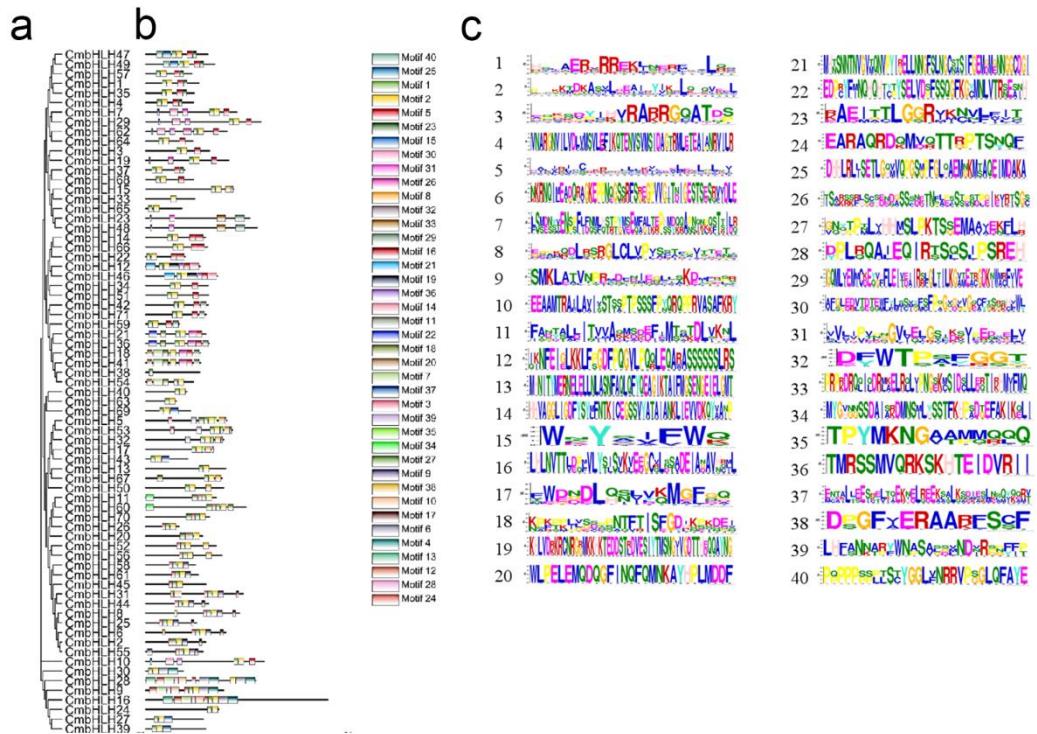


Figure S2 Motif composition of CmbHLH TF family. (a) Phylogenetic tree of CmbHLHs; (b) Conserved motifs of the CmbHLHs; (c) The sequence composition of each motif in CmbHLHs.

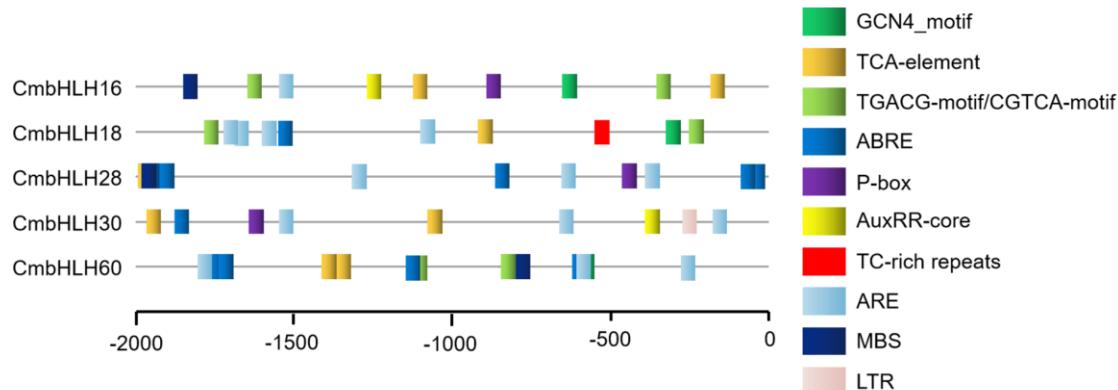


Figure S3 Visualization of *cis*-elements in the promoter sequences of *CmbHLH* genes. Different colors represent different *cis*-acting elements in the promoter sequences of *CmbHLH* genes.

Table S1 Promoter sequences of the candidate resistant genes to necrotrophic fungus.

Promoter sequences of the candidate genes
> <i>CmbHLH16</i>
TA GTCTGGTTAGTAAACTTGGCTATTAAATATGTTGAAAAGTTGATTAAATATACACAAG
TATATTGATCGCGCTATTATTTGTTCATCTTTTATATCACTACTGTATGATGGTTAAGTTG
CAGCATCAACTGGACATATATTTACCCACATCCTCAAGTGAATTCATGTTGTTGAGAT
CTTTCTTAAGTACTAAATGTATTCTTTGCCACATATAAAATAATGGAGTAGAACTAAAAG
AAAAAGAAAATTAAATTATATAAACCTCCAACCGTATCAAATTATTGTCGGGAGCGAA
GAATTAATAAAAGCGGGGCCAGTTAGCTGCAGCTAACAAATATAAATATTCTATGGGTAT
ATTCTTTTTTAACTTTCGTAAAAAGTGAACAAATTAATTGGATATCATAAAAGAAATATA

ACGATTATGAGACGGATGGAGTAATATTTATTATAAAAATTGATTTGTATCATTAAAATG
ATTAAGATCGCAATAAATGTCAAGTGTATGGTACGAGGTACATTTGTACTAGACAATCT
GAACATAATTGACAAAGAGGGACCAATGATGTGATAAAGAAGATTCAATGGTGGTGTCTATT
GTTGACCTTCCTGACTCATTATCTAAGACCATGATTGGGACTAAATTAAATTGCGTTTGCA
GCATATATGTATAACACATATTGCAAACATTTTTAAACATAGCCCTAAAAGTTGTGTT
CAAAGTATTCACACATGAAATAATTGTTGTATCTTACCGTTACTCTCTATAAATTACCTTAC
TCTCAGTAAATTACCTCTTCAATCGACTTATTATGACCAGATGAACATCTTACATTAA
ACTTAACCCTAAATGCTATATATCGATTTTTACATGTGAAACAGCATATAAACACCGCT
GTACTATTGAAAGGGTTTAACTATGTCTTTACAGAAATAACTTATGAAAAAGTCGTAC
AGAAAGCAATATATCCTACATAAAAGTCAGAACTGATTATCTTATGTGGATCCCATACTT
CAAATGGGCCTCTATAACATGTCTTTTATAACTTGTCTTATGATTAACCCATTGAAAAAA
AACTAGCCCTTTGTGAACAACGGCTATTATACATGGGGCTAATTAGTCGCTATCAATTATCA
AAGGAGGAATAACTTATAGAAAAGTATCAATATAATGGACCATCCATGGAAGAAGAAATCA
TCAAACAAATACCACGGTGAATTCAATCTATGACAAAATACTCCTCTGATGTCATCTTCTT
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GAATGAGTTGGAGCTTAAATCTGGCATCAAATTGACAGCTACAATTATCAAGTAATC
ACACTTCAT

>*CmbHLH18*

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TGCCTTTGTAAGGCTCGGTCAAGATTGCAACTTTATTTTATTAAAAGATTCTTAAA
TTCTAGCAACCTATAAGAACATAGCACCATTGTTACAAAATGCTTCAATTCTACACCCCC
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CCTCGAAGAACACAAATTCTGATTAT

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TCAATTATGTAAGATTGAAGCTAATGTGAATCCCTATTATCTTGCAGGAAGCAGGAATAAA
GGTATCCGATCTAGGCTGCATGGACACGGACACTGTCAAAGACGGCGATAAGCAAAATCTA
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CACGGCACATGCGTGTCCGACACGTGTCCGAGCGTGTCCGACACTGCCACGCC
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AGAGTTGCTAGTGCTTTAAGAGACATGGATCAAGTAATTAGGACCTATTAAACAACGAGTT
CCAAATCGACAAATCTCACAGAAGGTCAATTCTCCGAAATCTAAGTGAGGCTCGT
GCTCAAAGAGATCAA

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AAAGACTTATCAACACCATCCAGAAGAACATGCAGCCTGATCATTACATTATCAGACACG

CTTAGAGTACTTCAAGATACTGCTAAAGTATGCGAAAGGATCTGGCTGAAGTTCTCAAAGACGCTTAGGCCGGAGTAATTGAGATAAGGCAAAACGGAAAACCCTGGAAAGATAGAGACTCATCGAGAGTAGGGAAACCAACTTACATGAGGTGTCAGCACAGTAAAGCAACCATAGCCACACGATTGCACGTGTACCTTACTTCCAGTTGCTCTAGTTAGATATCATTGTTCTTGTAACTACTAACAGTGTATCTCCCTAGTTATAAAGGGAGGTTAGTAGTAGTGTAAAGCAATCGATTCCACCACTAGAAACTCCTCTTCTAAGTGGATCTTCTTCTCATAACTGAAGAGTAACATTGTCATTACCGGGAGTAGTATTCCGCAACTATCCCTCGTAAAGCCTCACCTTAACCTCTAAGTGAGACTAATACAGACGTAGTGTACAATGGTATTATTAGTGTACTCAGGTCAAGATTGTAAACATGCTTTATTCTAATGAACATCGTTATTTCAATTGCTCAAACACTCTTTCTACGTTACATGGTTTACACAAAAATATCGGACCAACAATCCAAGTTATTAGTCATCGGTTCTACCCAATAAACTCGAGAAAAGAGGGTAATTAAATCCAACACGATAATCCACAAACTACTTTAAA GTTGTCAATTATAAAATTCTTCAAGTTGATGTACTTAAAGGTTAGTAAACTTTGACTAGATAGATAACAAAGTCTAAAATCGTACGTGATTACACTTTAGATGTAGTTAAAACAAATTAAACCTA CTTAAAAACAAATATCCTCCGGATTATTGTTAAATCAAAGTCCCAGCTTAAATTAGAATCATCCTTCCAAATCACTAAATTACCATTTCAAATCAAGTACATAAATAATCTACTCAATCATCTAAATAAAGCTAAAATAGGCCTAATTAAATTACTAAACATGAAAAATAAGAAAAGTGAAGAGAGGGATTGCAAAGCACCAGTTGGTAAGGAATGATGATGACGAAACAAACTAAACTAAAACCAACCAAAGCTGTAAATAACCCAAATGCTCTTAAACACCCAATAATCTCGACTAACTTTGCATGAATGAAACTTTATGTAATTAAAGATGGTAAATGAATAAAGTTGCAACTTAA TATTGTAGAAAGCTTAAAACCTATAAAACTGATTAAAGAACTATATCTTAAATAATTGCTTAAAACCTTAAATTCAAGTCACAAACATCAAAGAAAATTACATCTCATCTCAAACACCCCTATTGTTGATAATACAAATATGTCACTTATGTGTGATAAAAGTTGTTG TAATCCAAACTATTAGACACTAGACACTGATATTGTCATGTAATATCTTCAATTGCAAAGAATCTGTGAAATTAGGAAAGAGTAGTTATTAAACGGTTGGATTAAACTGATGGTGGTATTAGGAAACACCAGCCGCTCAATTGAAACCATTATCTGAAACCTGCCTACTCCACGTAGGATCCTGACACTTACTAGCCAGCTAGGTTCTGTATGTAGTATTGTAGTTCCCTAAACATCCATGGCAATTAGGTATTAAATTCTTCACAAGAATCTCATGCTTCTATATAAAAGCTACACTTTCCCACTTCCCTTGTGCCATTTCACAAATAGATAACAAACAAACACACATTGTTGAAACATACGAATATTGTCAAATCCGTTAAATTGCGTACATATCTGTCACTAGAGGTACAAATTGTCAGATTCTGATA

Table S2 Number of bHLH TF in each subfamily of chrysanthemum and *Arabidopsis*.

Subgroup	Chrysanthemum	Arabidopsis	Rate (Chrysanthemum/Arabidopsis)
Ia	3	10	0.30
IIb	0	10	0.00
II	2	4	0.50
IIIa+c	3	5	0.60
IIIb	1	4	0.25
IIIe+d+f	5	12	0.42
IVa	8	4	2.00
IVb+c	4	6	0.67
IVd	4	1	4.00
Va	0	3	0.00
Vb	6	5	1.20
VIIa(1)	0	5	0.00
VIIa(2)	2	8	0.25
VIIa(3)+b	0	5	0.00
VIIIa	3	3	1.00
VIIIb	2	6	0.33
VIIIc	0	6	0.00
IX	5	5	1.00
X	4	5	0.80
XI	2	5	0.40
XII	10	17	0.59
XIII	2	3	0.67
XIV	0	5	0.00
XV	0	3	0.00
Orphans	5	16	0.31
Total	71	156	0.46

Table S3 Proportion of conserved amino acids at each point of CmbHLH protein.

Amino acid location	Conserved amino acids >50%	Ratio (%)
5	His, H	55
8	Ala, A	59
9	Glu, E	77
10	Arg, R	68
12	Arg, R	77
13	Arg, R	92
14	Glu, E	52
23	Leu, L	83
26	Leu, L	61
27	Val, V	51
28	Pro, P	76
35	Lys, K	56
37	Asp, D	65
38	Lys, K	59
39	Ala, A	79
40	Ser, S	52
42	Leu, L	82
45	Ala, A	56
46	Ile, I	63
48	Tyr, Y	70
49	Ile, I	54
50	Lys, K	52
52	Leu, L	92
56	Val, V	55
59	Leu, L	75

Table S4 Selection criteria for differential genes.

Gene ID (name)	Cm_A3 vs Cm_CK0		Cm_A5 vs Cm_CK0	
	log2FC	Padj	log2FC	Padj
c60363_g1(CmbHLH16)	3.9	8.29E-12	3.7	4.40E-16
c61229_g1(CmbHLH18)	2.4	0.00023616	1.6	0.0050347
c93607_g1(CmbHLH28)	3.5	4.53E-19	2.7	2.4474E-23
c93607_g2(CmbHLH30)	4.1	3.06E-14	3.7	1.1979E-20
c77068_g1(CmbHLH60)	3.8	2.27E-43	2.7	1.3631E-21

Note: Cm_A3 vs Cm_CK0 represents the ratio of the expression of this gene at day 3 of inoculation to that at day 0 of inoculation by *Alternaria* sp.; Cm_A5 vs Cm_CK0 represents the ratio of the expression of this gene at day 5 of inoculation to that at day 0 of inoculation by *Alternaria* sp. The screening criterion for differential genes is the default value of log2FC is greater than 1 and Padj less than or equal to 0.05.

Table S5 Relative activity of leaf cells of WT and transgenic chrysanthemum.

Lines	A _{600nm}	Relative cell activity/ %
WT	1.237±0.178 ^a	54.93 ^a
35S::CmbHLH18-5-1	0.525±0.067 ^b	80.86 ^b
35S::CmbHLH18-32-9	0.370±0.040 ^{bc}	86.53 ^{bc}
35S::CmbHLH18-14-4	0.276±0.008 ^c	89.95 ^c

Note: The different letters signified significant differences at $p < 0.05$ by one-way ANOVA.

Table S6 Primers used in this study.

Primer codes	Forword primer (5'-3')	Reverse primer (5'-3')	Purpose
qCmbHLH16	CTTCAAATGACACCAAC GAGGAT	GAGGCGGCGGAATACA	For RT-qPCR in Chrysanthemum.
qCmbHLH18	GGCTGCAATTCAAAGGA TGTTC	GTCATGCAGAAGTCGTCCG CC	
qCmbHLH28	TTCTGAGATGGCGGTAG	CGTGGCACATCCTCTTT	
qCmbHLH30	TGTAGTTGGCATCACCG	CAAATCCACCAAGTATCACA T	

qCmbHLH60	CTTCTGATGGCGAGTTT	GGTTTGTCCACCTATGTTAG	
CiUBI	AGCTGAGCAGACTCCCC	AGGCGAACATCATCAGTACCA	For RT-qPCR.
	ATG	AGT	
CmbHLH18	GGTGTCCAATCATCCC	GGATCGGACAAGCAACCGT	For isolation and
	GAA	A	transformation of
Super1300	GCCATTTCGCCCTTTCAG	TGATAATCATCGCAAGACC	<i>CmbHLH18</i> into
		G	vector.
CmbHLH18-V	CTAGTCTAGAATGGGAA	CGGGGTACCAAGAACATTGT	
	AGGTATCGAACACGATT	TGCAGATTCTTCGA	
CmbHLH18-At	TGAAGTGCAAATGTCAG	TACTTCCTACTCCCACCAA	For identification of
	GGAA		transgenic
			<i>Arabidopsis</i> .
AtActin	CTAAGCTCTCAAGATCAA	AACATTGCAAAGAGTTCA	For RT-qPCR in
	AGGC	AGG	<i>Arabidopsis</i> .
AtSOD	TGGAACTGCCACCTTCAC	GATGCCGCAAGCAACACG	
	AAT		
AtCAT	ACGCCGATTGCGAGATA	CAGACAGGAGCACCCGAG	
	CA	TTT	
AtPAL	AAGCCTGAGTTCACCGA	TACGAGCTCCGTCGAGGA	
	TCATC	T	
AtPOD	TGCGAGAGACGGTATTGT	TCTCCAAGTAGCTCCCTCT	
	CA		
AtCHT	CGGAAACTGGAAACCTA	CAGTTGAAGGGTTGAAAGG	
	CA		
AtGLU	GCAATGCAGAACATCGA	TCATCCCTGAACCTTCCTTG	
	GAA		
