Supplementary material

Enhanced *Nicotiana benthamiana* immune responses caused by heterologous plant genes from *Pinellia ternata*

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Fig. S1. Agarose gel of cDNA inserts. M, 100bp marker.



Fig. S2. Hypersensitive response induced by *ptHR* genes on *L. esculentum*, *G. hirustum*, *N. benthamiana* and *P. ternata* leaves.

L. esculentum and *G. hirustum* and *N. benthamiana* leaves were infiltrated with EHA105 Agrobacterium strain, empty vector and *ptHR* genes. Pictures were taken at 48 h of post infiltration. **a-d** *L. esculentum* leaves treated with **a** Agrobacterium EHA105 strain, **b** pTRV empty vector, **c** pTRV₁ as control and **d** *ptHR941*. **e-h** *G. hirustum* leaves treated with **e** Agrobacterium EHA105 strain, **f** pTRV empty vector, **g** pTRV₁ as control and **h** *ptHR941*. **i-l** *N. benthamiana* leaves treated with **i** buffer, **j** Agrobacterium EHA105 strain, **k** pCAMBIA3301 and **l** *ptHR941*. **m-o** *L*. *esculentum* leaves treated with **m** Agrobacterium EHA105 strain, **n** pCAMBIA3301 empty vector and **o** *ptHR941*. *P. ternata* leaves were infiltrated with empty vector and *ptHR* genes. **p-q** *P. ternata* leaves treated with **p** pCAMBIA3301 empty vector and **q** *ptHR941*. Each experiment was repeated three times and each time same results were observed. Representative pictures are shown here.



Fig. S3. Relative expression levels of pathogenesis-related genes in *N. benthamiana*.

N. benthamiana leaves were infiltrated with *ptHR375* and an empty vector as control for RT-qPCR analysis. Leaves infiltrated with empty vector were used as control for relative quantification of gene expression. *EF-1a* was used as indigenous control. **a-g** Relative expression levels of, **a** *PR-1a* vs control, **b** *PR-5* vs control, **c** *PDF1.2* vs control, **d** *NPR1* vs control, **e** *PAL* vs control, **f** *RBOHB* vs control and **g** *ERF1* vs control. Significance was determined by t-test: **P*<0.05, ***P*<0.01. Results are the mean values from three independent experiments. Vertical bars indicate SD.



Fig. S4. Relative expression levels of *ptHR941* and *ptHR375* genes in transformed *N. benthamiana*.

Leaves were sampled from transformed *N. benthamiana* (T₃) to extract total RNA for RT-qPCR analysis. Transformed (T₂) *N. benthamiana* was used as control for relative quantification of gene expression. *EF-1a* was used as internal control. Figure showing the relative expression of *ptHR941* and *ptHR375* genes in transformed *N. benthamiana* (T₃) compared with control. Results are the mean values from three independent experiments. Vertical bars indicate SD. Significance was determined by t-test: **P*<0.05, ***P*<0.01.



Fig. S5. Mass spectra for the induced bioactive compounds detected in *ptHR375* transformed *N*. *benthamiana*. **a** Oxytetracycline, **b** Cuelure, **c** Allantoin, **d** Diethylstilbestrol and **e** 1,2-Benzisothiazol-3(2H)-one.

Table S1	Primers	used t	for	qRT	-PCR.
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Gene	Forward Primer (5'-3')	Reverse Primer (5'—3')
EF-1a	TGTGATGTTTTTGTTCGGTCTTTAA	TCAAAAGAAAATGCAGACAGACTCA
PR-1a	CCTCGTACATTCTCATGGTCAAT	CCATTGTTACACTGAACCCTAGC
<i>PDF1.2</i>	GGAAATGGCAAACTCCATGCG	ATCCTTCGGTCAGACAAACG
NPR1	ACATCAGCGGAAGCAGTAG	GTCGGCGAAGTAGTCAAAC
PAL	GTTATGCTCTTAGAACGTCGCCC	CCGTGTAATGCCTTGTTTCTTGA
RBOHB	TTTTCTCTGAGGTTTGCCAGCCACCA	GCCTTCATGTTGTTGACAATGTCTTT
ERF1	GGCGAATTTTCCGGGAGACT	GGCTCCGATTTTACTTCGCC
ptHR941	TCAGGTTATGCCGTGCTCG	AAGGAAGCGTCTACAGGGAG
ptHR375	CAGGATCTCCACGCCGAAGC	AGCAGCTTCTACTTGAATCGAT

Gene	Length (bp)	Homology		
ntHD771	137	Echinostoma caproni genome assembly E_caproni_Egypt,	80	
<i>p</i> (<i>H</i> K 274 157		scaffold ECPE_scaffold0025465		
ptHR284	410	Spirodela polyrhiza strain 9509 chromosome 1 sequence	77	
ptHR298	395	Spirodela polyrhiza strain 9509 chromosome 2 sequence	79	
ntHD310	80	Zantedeschia aethiopica rubisco activase (rca4) mRNA, partial		
pillK310	80	cds	74	
<i>ptHR317</i>	408	Spirodela polyrhiza strain 9509 chromosome 14 sequence	88	
		PREDICTED: Asparagus officinalis ATP-dependent Clp protease		
ptHR359	346	proteolytic subunit-related protein 1, chloroplastic	78	
		(LOC109835634), mRNA		
ntHR375	254	PREDICTED: Daucus carota subsp. sativus metallothionein-like		
<i>pinks73</i> 234		protein type 3 (LOC108220108), mRNA		
ntHR388	763	PREDICTED: Phoenix dactylifera UV-B-induced protein At3g17800, chloroplastic-like (LOC103713309), mRNA		
pillisoo	105			
ptHR449	173	Spirodela polyrhiza strain 9509 chromosome 15 sequence		
<i>ptHR478</i> 498 Oryza s		Oryza sativa Indica Group cultivar Shuhui498 chromosome 3	3 79	
		sequence		
ptHR615	711	Spirodela polyrhiza strain 9509 chromosome 7 sequence	88	
ptHR620 476 PREDICTED: Musa acuminata subsp. mala		PREDICTED: Musa acuminata subsp. malaccensis 60S ribosomal	86	
		protein L21-1 (LOC103980608), mRNA		
ptHR805	547	PREDICTED: Musa acuminata subsp. malaccensis		
<i>p</i>	0.17	uncharacterized LOC103976420 (LOC103976420), mRNA		
<i>ptHR812</i> 414		PREDICTED: Phoenix dactylifera glutamate dehydrogenase 2-	82	
		like (LOC103710782), mRNA		
		PREDICTED: Eucalyptus grandis 5-		
ptHR813	94	methyltetrahydropteroyltriglutamatehomocysteine		
		methyltransferase (LOC104443139), mRNA		

 Table S2 NCBI blast results showing homology with known sequences.

ptHR830	346	PREDICTED: Asparagus officinalis ATP-dependent Clp protease proteolytic subunit-related protein 1, chloroplastic (LOC109835634), mRNA		
ptHR844	465	Phaseolus vulgaris clone BE5D669 mitochondrial import inner membrane translocase subunit tim17 mRNA, complete cds; nuclear gene for mitochondrial product		
ptHR849	232	Mouse DNA sequence from clone RP23-169H17 on chromosome 2, complete sequence		
ptHR897	897 602 PREDICTED: Elaeis guineensis RGG repeats nuclear RNA binding protein A (LOC105048011), mRNA		70	
ptHR941	190	PREDICTED: Gossypium raimondii sulfite reductase [ferredoxin], chloroplastic-like (LOC105794174), mRNA		
ptHR1028	278	278 PREDICTED: Elaeis guineensis 125 kDa kinesin-related protein- like (LOC105044532), mRNA		
ptHR1041	533	Phyllostachys edulis cDNA clone: bbasst002k07, full insert sequence		
<i>ptHR1067</i>	126	Spirodela polyrhiza strain 9509 chromosome 12 sequence		
ptHR1070	971	Spirodela polyrhiza strain 9509 chromosome 2 sequence	75	
ptHR1092186PREDICTED: Zea mays uncharacterized LOC (LOC103636543), ncRNA		PREDICTED: Zea mays uncharacterized LOC103636543 (LOC103636543), ncRNA	77	

 $\label{eq:source} \textbf{Table S3} \text{ NCBI blast results showing homology with transcription factors.}$

Gene	Length (bp)	Homology	
ptHR69	177	PREDICTED: Ananas comosus nuclear transcription factor Y subunit B-3-like (LOC109711511), mRNA	100
ptHR293	749	PREDICTED: <i>Setaria italica</i> probable WRKY transcription factor 33 (LOC101782717), mRNA	
ptHR759	363	PREDICTED: <i>Musa acuminata</i> subsp. malaccensis NAC domain- containing protein 21/22-like (LOC103995621), mRNA	89
ptHR1015	123	PREDICTED: <i>Nelumbo nucifera</i> probable sucrose-phosphate synthase 3 (LOC104602206), transcript variant X2, mRNA	92

<i>ptHR1028</i> 278	278	PREDICTED: Asparagus officinalis kinesin-like protein KIN-5A	
	270	(LOC109848469), transcript variant X1, mRNA	

Gene	Length (bp)	Gene	Length (bp)
ptHR40	47	ptHR772	607
ptHR47	166	<i>ptHR</i> 788	609
ptHR99	207	ptHR829	129
ptHR2-20	310	ptHR831	60
ptHR268	203	ptHR835	47
ptHR292	196	ptHR841	530
ptHR519	166	ptHR917	91
ptHR601	89	ptHR943	47
ptHR602	47	ptHR1124	91
ptHR612	60		

Table S4 NCBI blast results showing no homology with known sequences.

Table S5 List of induced bioactive compounds present in *ptHR375* transformed *N. benthamiana*

Compound	Retention time	m/z	Molecular weight
Compound	(min)	$[M+H]^+$	(g/mol)
Oxytetracycline	5.31	460.83	460.43
Cuelure	1.20	103.95	206.24
Allantoin	6.04	86.98	158.11
Diethylstilbestrol	0.91	466.87	268.36
1,2-Benzisothiazol- 3(2H)-one	6.61	188.95	151.18