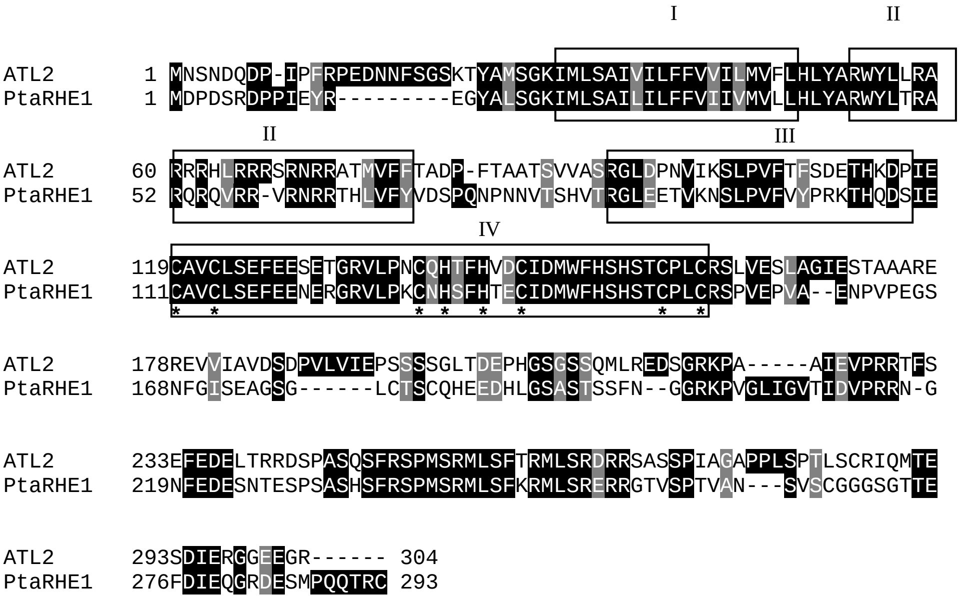
Ectopic expression of *PtaRHE1*, encoding a poplar RING-H2 protein with E3 ligase activity, alters plant development and induces defence related responses. *J Mukoko Bopopi, OM Vandeputte, K Himanen, A Mol, Q Vaessen, M El Jaziri, and M Baucher*

SUPPLEMENTARY DATA



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ATL2       1 MNSNDQDP-IPFRPEDNNFSGSKTYAMSGKIMLSAIVILFFVVILMVFLHLYARWYLLRA
PtaRHE1    1 MDPDSRDPPIEYR---------EGYALSGKIMLSAILILFFVIIVMVLLHLYARWYLTRA

ATL2      60 RRRHLRRRSRNRRATMVFFTADP-FTAATSVVASRGLDPNVIKSLPVFTFSDETHKDPIE
PtaRHE1   52 RQRQVRR-VRNRRTHLVFYVDSPQNPNNVTSHVTRGLEETVKNSLPVFVYPRKTHQDSIE

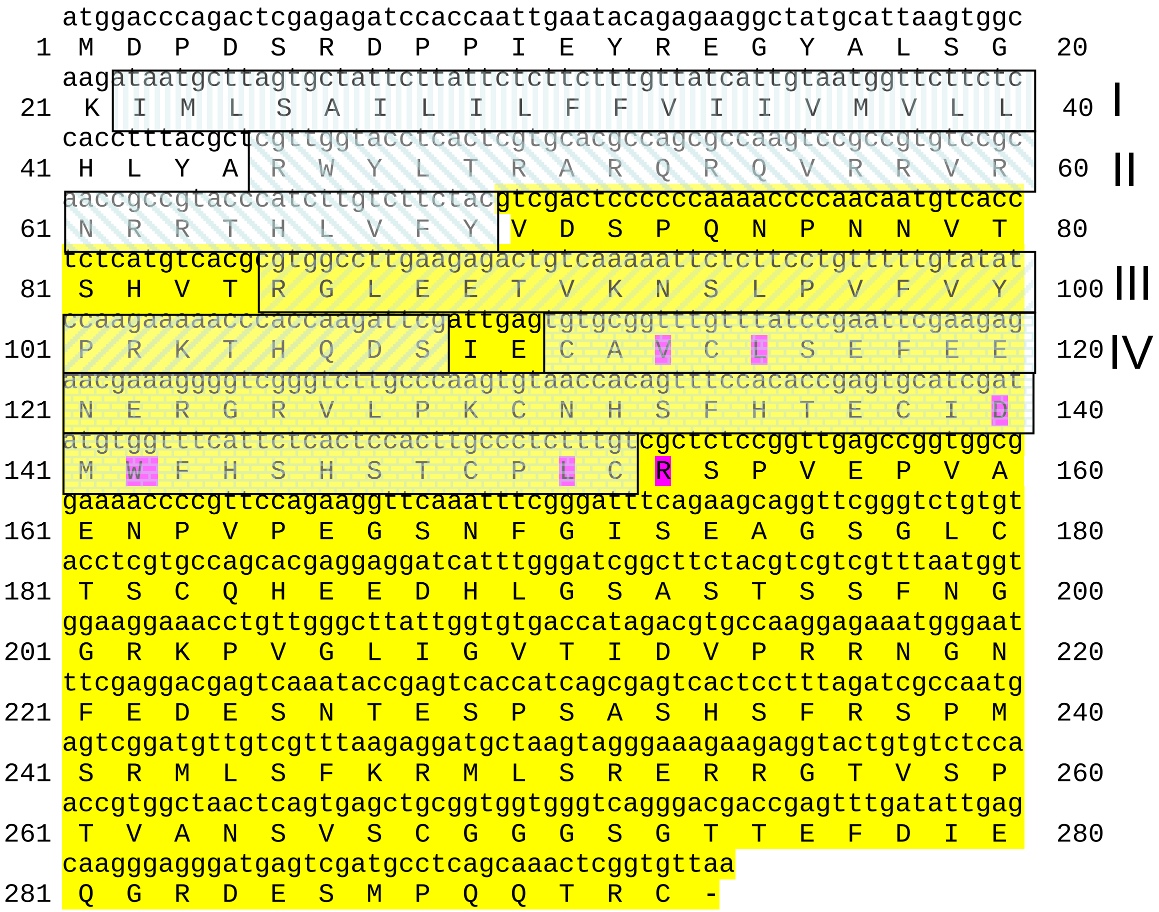
ATL2      119CAVCLSEFEESETGRVLPNCQHTFHVDCIDMWFHSHSTCPLCRSLVESLAGIESTAAARE
PtaRHE1   111CAVCLSEFEENERGRVLPKCNHSFHTECIDMWFHSHSTCPLCRSPVEPVA--ENPVPEGS
             *  *               * *  *  *              *  *

ATL2      178REVVIAVDSDPVLVIEPSSSSGLTDEPHGSGSSQMLREDSGRKPA-----AIEVPRRTFS
PtaRHE1   168NFGISEAGSG------LCTSCQHEEDHLGSASTSSFN--GGRKPVGLIGVTIDVPRRN-G

ATL2      233EFEDELTRRDSPASQSFRSPMSRMLSFTRMLSRDRRSASSPIAGAPPLSPTLSCRIQMTE
PtaRHE1   219NFEDESNTESPSASHSFRSPMSRMLSFKRMLSRERRGTVSPTVAN---SVSCGGGSGTTE

ATL2      293SDIERGGEEGR------ 304
PtaRHE1   276FDIEQGRDESMPQQTRC 293
```

Figure S1. Alignment of the PtaRHE1 amino acid sequence with the *Arabidopsis* ATL2 (At3g16720) amino acid sequence. I. Hydrophobic region (TM domain). II. Basic region. III. Conserved region. IV. RING-H2 zinc finger domain. Black and grey boxes indicate strictly conserved amino acids and closely related amino acids, respectively. Asterisks indicate the position of cysteine and histidine residues of the RING-H2 domain.



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    atggacccagactcgagagatccaccaattgaatacagagaaggctatgcattaagtggc
  1  M  D  P  D  S  R  D  P  P  I  E  Y  R  E  G  Y  A  L  S  G   20
    aagataatgcttagtgctattcttattctcttctttgttatcattgtaatggttcttctc
 21  K  I  M  L  S  A  I  L  I  L  F  F  V  I  I  V  M  V  L  L   40  I
    caccttttacgctcgttggtacctcactcgtgcacgccagcgccaagtccgccgtgtccgc
 41  H  L  Y  A  R  W  Y  L  T  R  A  R  Q  R  Q  V  R  R  V  R   60  II
    aaccgccgtacccatcttgtcttctacgtcgactcccccaaaaccccaacaatgtcacc
 61  N  R  R  T  H  L  V  F  Y  V  D  S  P  Q  N  P  N  N  V  T   80
    tctcatgtcacgcgtggccttgaagagactgtcaaaaattctcttcctgtttttgtatat
 81  S  H  V  T  R  G  L  E  E  T  V  K  N  S  L  P  V  F  V  Y  100  III
    ccaagaaaaacccaccaagattcgattgagtgtgcggtttgtttatccgaattcgaagag
101  P  R  K  T  H  Q  D  S  I  E  C  A  V  C  L  S  E  F  E  E  120  IV
    aacgaaaggggtcgggtcttgcccaagtgtaaccacagtttccacaccgagtgcatcgat
121  N  E  R  G  R  V  L  P  K  C  N  H  S  F  H  T  E  C  I  D  140
    atgtggtttcattctcactccacttgccctctttgtcgctctccggttgagccggtggcg
141  M  W  F  H  S  H  S  T  C  P  L  C  R  S  P  V  E  P  V  A  160
    gaaaaccccgttccagaaggttcaaatttcgggatttcagaagcaggttcgggtctgtgt
161  E  N  P  V  P  E  G  S  N  F  G  I  S  E  A  G  S  G  L  C  180
    acctcgtgccagcacgaggaggatcatttgggatcggcttctacgtcgtcgtttaatggt
181  T  S  C  Q  H  E  E  D  H  L  G  S  A  S  T  S  S  F  N  G  200
    ggaaggaaacctgttgggcttattggtgtgaccatagacgtgccaaggagaaatgggaat
201  G  R  K  P  V  G  L  I  G  V  T  I  D  V  P  R  R  N  G  N  220
    ttcgaggacgagtcaaataccgagtcaccatcagcgagtcactcctttagatcgccaatg
221  F  E  D  E  S  N  T  E  S  P  S  A  S  H  S  F  R  S  P  M  240
    agtcggatgttgtcgtttaagaggatgctaagtagggaaagaagaggtactgtgtctcca
241  S  R  M  L  S  F  K  R  M  L  S  R  E  R  R  G  T  V  S  P  260
    accgtggctaactcagtgagctgcggtggtgggtcagggacgaccgagtttgatattgag
261  T  V  A  N  S  V  S  C  G  G  G  S  G  T  T  E  F  D  I  E  280
    caagggagggatgagtcgatgcctcagcaaactcggtgttaa
281  Q  G  R  D  E  S  M  P  Q  Q  T  R  C  -
```

Figure S2. Sequences of PtaRHE1 full length and PtaRHE1-Ct (where the TM domain and the basic region were deleted). The PtaRHE1-Ct part is marked in yellow. I. Hydrophobic region. II. Basic region. III. Conserved region. IV. RING-H2 zinc finger domain (Salinas-Mondragón *et al.*, 1999). Residues marked in magenta were shown to be crucial for EL5-OsUBC5b binding (Katoh et al., 2005).

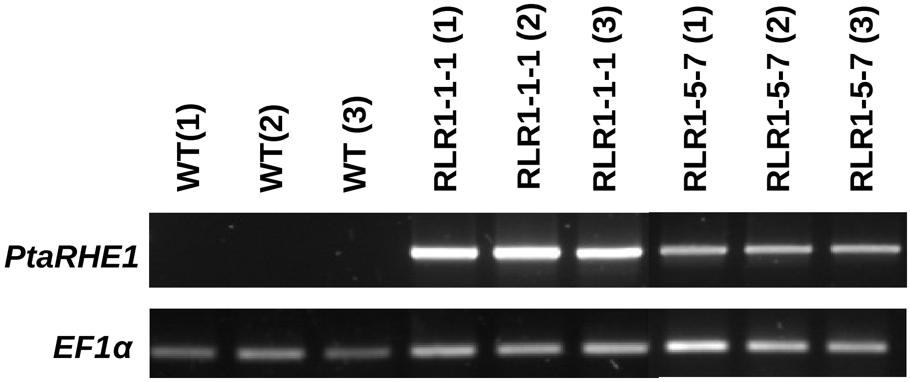
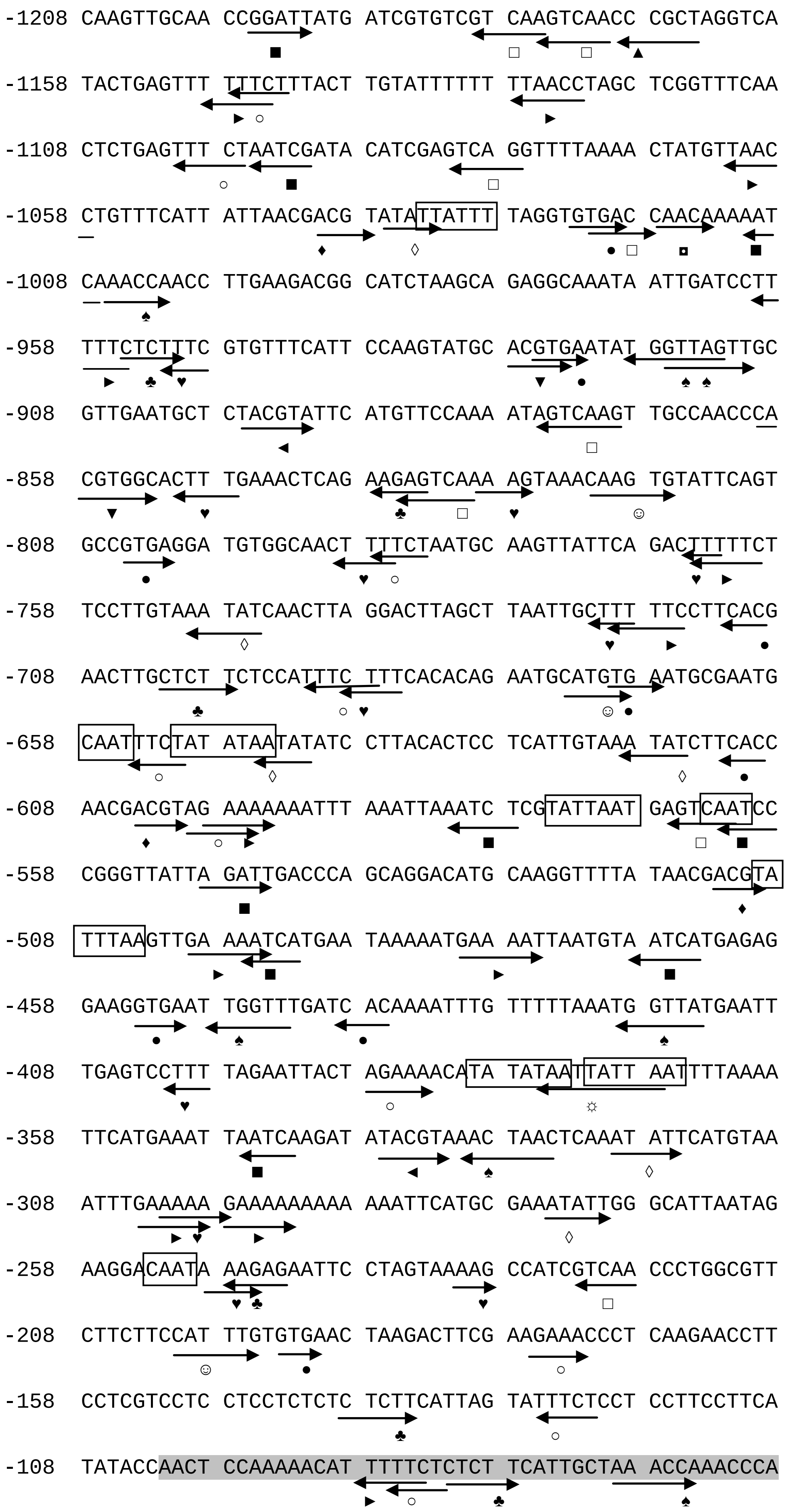


Figure S3. RT-PCR expression analysis of *PtaRHE1* in 3 individual plants of WT, RLR1-1-1, and RLR1-5-7. *EF1*-α was used as loading control. These lines were further used for RT-qPCR analysis (see Table 1).



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-1208 CAAGTTGCAA CCGGATTATG ATCGTGTCGT CAAGTCAACC CGCTAGGTCA
      ■ □ □ ▲
-1158 TACTGAGTTT TTTCTTTACT TGTATTTTTT TTAACCTAGC TCGGTTTCAA
      ► ○ ►
-1108 CTCTGAGTTT CTAATCGATA CATCGAGTCA GGTTTTAAAA CTATGTTAAC
      ○ ■ □ ►
-1058 CTGTTTCATT ATTAACGACG TATATTATTT TAGGTGTGAC CAACAAAAAT
      ♦ ◊ ● □ ◘ ■
-1008 CAAACCAACC TTGAAGACGG CATCTAAGCA GAGGCAAATA ATTGATCCTT
      ♠
-958  TTTCTCTTTC GTGTTTCATT CCAAGTATGC ACGTGAATAT GGTTAGTTGC
      ► ♣ ♥ ▼ ● ♠ ♠
-908  GTTGAATGCT CTACGTATTC ATGTTCCAAA ATAGTCAAGT TGCCAACCCA
      ◄ □
-858  CGTGGCACTT TGAAACTCAG AAGAGTCAAA AGTAAACAAG TGTATTCAGT
      ▼ ♥ ♣ □ ♥ ☺
-808  GCCGTGAGGA TGTGGCAACT TTTCTAATGC AAGTTATTCA GACTTTTTCT
      ● ♥ ○ ♥ ►
-758  TCCTTGTAAA TATCAACTTA GGACTTAGCT TAATTGCTTT TTCCTTCACG
      ◊ ♥ ► ●
-708  AACTTGCTCT TCTCCATTTC TTTCACACAG AATGCATGTG AATGCGAATG
      ♣ ○ ♥ ☺ ●
-658  CAATTTCTAT ATAATATATC CTTACACTCC TCATTGTAAA TATCTTCACC
      ○ ◊ ◊ ●
-608  AACGACGTAG AAAAAAATTT AAATTAAATC TCGTATTAAT GAGTCAATCC
      ♦ ○ ► ■ □ ■
-558  CGGGTTATTA GATTGACCCA GCAGGACATG CAAGGTTTTA TAACGACGTA
      ■ ♦
-508  TTTAAGTTGA AAATCATGAA TAAAAATGAA AATTAATGTA ATCATGAGAG
      ► ■ ► ■
-458  GAAGGTGAAT TGGTTTGATC ACAAAATTTG TTTTTAAATG GTTATGAATT
      ● ♠ ● ♠
-408  TGAGTCCTTT TAGAATTACT AGAAAACATA TATAATTATT AATTTTAAAA
      ♥ ○ ☼
-358  TTCATGAAAT TAATCAAGAT ATACGTAAAC TAACTCAAAT ATTCATGTAA
      ■ ◄ ♠ ◊
-308  ATTTGAAAAA GAAAAAAAAA AAATTCATGC GAAATATTGG GCATTAATAG
      ► ♥ ► ◊
-258  AAGGACAATA AAGAGAATTC CTAGTAAAAG CCATCGTCAA CCCTGGCGTT
      ♥ ♣ ♥ □
-208  CTTCTTCCAT TTGTGTGAAC TAAGACTTCG AAGAAACCCT CAAGAACCTT
      ☺ ● ○
-158  CCTCGTCCTC CTCCTCTCTC TCTTCATTAG TATTTCTCCT CCTTCCTTCA
      ♣ ○
-108  TATACCAACT CCAAAAACAT TTTTCTCTCT TCATTGCTAA ACCAAACCCA
      ► ○ ♣ ♠
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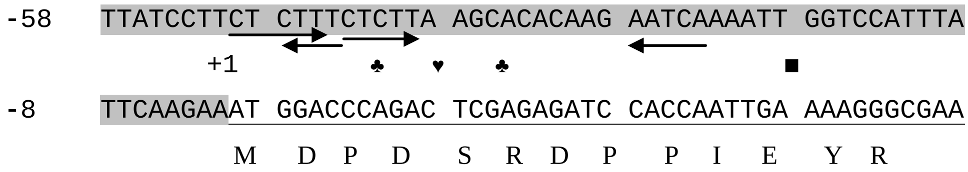

Figure S4. Nucleotide sequence of the 5'-flanking promoter region and putative *cis*-acting elements of the *PtaRHE1* promoter. The 5'UTR region is marked in grey and the coding sequence is underlined. The translational start site is denoted as +1. Putative CAAT- and TATA- boxes are boxed. Putative *cis*-acting elements are represented by a symbol and underlined by an arrow. □, W-box are binding sites for WRKY transcription factors (Eulgem *et al*., 2000); WRKYs are involved in elicitor-responsive transcription of defense genes in tobacco (Yamamoto *et al*., 2004); ▲, BS1 (binding site 1) found in *Eucalyptus gunnii cinnamoyl-CoA reductase* gene promoter and is required for vascular expression (Lacombe *et al*., 2000); ►, GT-1 binding motif found in the promoter of many light-regulated genes; Binding of GT-1-like factors to the PR-1a promoter influences the level of SA-inducible gene expression; Plays a role in pathogen- and salt-induced SCaM-4 gene expression (Buchel *et al*., 1999; Park *et al*., 2004); ▼, ABRE; ABA-responsive elements found in the promoter of ABA and/or stress-regulated genes (Nakashima *et al*., 2006); ♦, ACGTERD1; ACGT sequence required for induction of expression of *erd1* in *Arabidopsis thaliana* by dehydration stress and dark-induced senescence (Simpson *et al*., 2003); ◄, ACGTABOX, "A-box" ACGT elements which are binding sites for bZIP proteins (Foster *et al*., 1994); ■, ARR1AT "ARR1-binding element" found in *A. thaliana* (Sakai *et al*., 2000); ●, GTGANTG10; "GTGA motif" found in the promoter of the tobacco late pollen gene g10 (Rogers *et al*., 2001); ☼, HDZIP2ATATHB2, binding site of the *A. thaliana* homeobox gene *ATHB-2* found in its own promoter (Ohgishi *et al*., 2001); ♠, MYB1(AT) sites are MYB recognition sites found in the promoters of the dehydration-responsive gene rd22 and many other genes in *A. thaliana* (Abe *et al*., 2003); ☺, MYC recognition sequence (from -466 to -461) necessary for expression of *erd1* in dehydrated Arabidopsis (Simpson *et al*., 2003) and binding site of ATMYC2 which function as transcriptional activator in ABA signaling (Abe *et al*., 2003); ○, POLLEN1LELAT52; one of two co-dependent regulatory elements responsible for pollen specific activation of tomato *lat52* gene (Bate and Twell, 1998); ◘, RAV1AAT; binding consensus sequence of *A. thaliana* transcription factor, RAV1 (Kagaya *et al*., 1999) ; ◊, ROOTMOTIFTAPOX1; motif found in both promoters of rolD (Elmayan and Tepfer, 1995); ♣ NODCON2GM, one of two putative nodulin consensus sequences (Stougaard *et al*., 1990); ♥, Core site required for binding of Dof proteins (DNA binding with one finger), regulating the expression of plant genes in response to light, developmental stage and hormone treatment such as gibberellin (Washio 2001).

Figure S4, references

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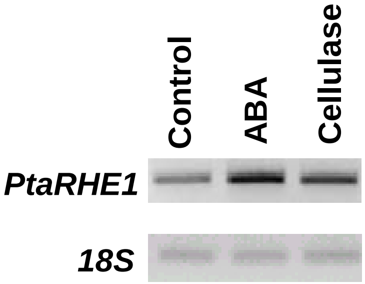

Figure S5. RT-PCR expression analysis of *PtaRHE1* in poplar leaves treated during 8 h with Cel (100 µg/ml) and ABA (150 µM). *18S* was used as a loading control.

Table S1. Primer sequences and amplicon size for RT-PCR and RT-qPCR. The gene classification was based on literature data.

Gene	Accession	Forward primer (5′->3′)	Reverse primer (5′->3′)	Size (bp)	References
PtaRHE1	AY780430	ATGGACCCAGACTCGAGAGA	TTAACACCGAGTTTGCTGAGG	882	This study
1. Defence/elicitor inducible genes					
PR1A	X06361	ATGCGCAAAATTATGCTTCC	CCTAGCACATCCAACACGAA	242	Cornelissen *et al.*, 1986
PR1B	X17680	GCAGACTGCAACCTCGTACA	CCTAGCACATCCAACACGAA	213	Cornelissen *et al.*, 1986
TIZZ	AB024510	TGCCCAGTCAAGAAGAAGGT	AAGGAAGATGCCATTTGTGC	224	Yoda *et al.*, 2002
PR5 (osmotin)	X95308	ATCGAGGTCCGAAACAACTG	AGGACTCCACCACAGTCACC	203	Kumar and Spencer, 1992
SAR8.2A	M97194	GCCTTTCTTTGGCTATTTTGG	AGCTTTGCCAATGATTTTGC	200	Alexander *et al.*, 1992
PR3 (CHN50)	X51599	CAGCAAATTTGGTTGGTGTG	CTTGGCATGCATTATCGTTG	170	Fukuda *et al.*, 1991
HSR201	X95343	TGGGGAAAAGCAGTTTATGG	TCTCAAAGGGCAGGTCTGAT	233	Czernic *et al.*, 1996
HMGR2	AF004232	GGCAACCACTGAAGGATGTT	TGGTTGAAAACAGCAGCAAG	207	Genschik *et al.*, 1992
WRKY12	DQ460475	ACCGAGCTTGAGATCTTGGA	AAGGGGCTTTCATGATTGTG	200	van Verk *et al.*, 2008
EDS1	AF480489	AGGCCGAAGCGTTATAGGTT	AAAACATCATCGCCCAGAAG	203	Zhang *et al.*, 2004
PR4	AF154635	TTCCATGTGGCGAGTCATTA	CAACATGCAATGCCAAGTCT	208	Ward *et al.*, 1991
WIPK	AB098729 (NB)	TCATGTGAACCCATTGGCTA	GATTCAGCGACAAAGCTTCC	227	Yang *et al.*, 2001
LSD1	EB448923 (EST NT)	CAGCTCGAGGTCCTAACCAG	GTTCTGAACGACCACGGTTT	245	Dietrich *et al.*, 1997
SAMDEC	AF033100	CCCATCCTAAAGTTGGCTGA	ATAGGTCCTGCAGAGGCAGA	233	Yoda *et al.*, 2003
BECLIN1	BP534310 (EST NT)	TAGCCGCTTTAAGGAGTTGG	CCCACTCAACCGGAATTTTA	245	Liu *et al.*, 2005
DND1	DV162472 (EST NT)	TGAGCTTCTTTCCTGGTGCT	AACGACGCCAAGCTAACTGT	239	Clough *et al.*, 2000
ABAKIN	EB684220 (EST NT)	GAACACCAGCAGTGGGATCT	CAGTCCAGTGCACAAAGCAT	216	Kim *et al.*, 2006
NPR1	AF480488	TAGCGTATTGCGATGCAAAG	TAGTGAGCCTCTTGGCGATT	213	Liu *et al.*, 2002
HSP90	AB264546	GAAGGCACAAGCACTTAGGG	TGCCAAAGGTGTTAGGTTCC	215	Takabatake *et al.*, 2007
SIPK	AB098730 (NB)	CCGTCGACAATCATTCACTG	GCGACTCCCTGTAAATCAGC	245	Yang *et al.*, 2001
S26-PR6	U44760	ACATGATTTTGCCTTGCACA	TGTTTGCCAAGTCACTCCTG	245	Kim *et al.*, 2003

NTMEK2	AB264547	ATTGCTGACTTTGGGGTGTC	TCCCAACAGAAAACGGAAAC	205	Yang *et al.*, 2001
HSR203J	AB091430	TATCCGGCTGGCTTACAGTT	ACGGAATTGTCGTTTCGTTC	199	Pontier *et al.*, 1994
SperSy	AF321139	CACATTGGTGATGGAGTTGC	GCAATTGGCAACGATTTCTT	222	Yoda *et al.*, 2003
WIZZ	AB028022	AGGCAGGTGATTTGTTGGAG	TTGTTGTTGTTGGGATTGGA	208	Yoda *et al.*, 2002
NOA1	AB303300 (NB)	GTGTTGGTGACTGGGTTGTG	GGCTGCAACTGGGTCATTAT	227	Kato *et al.*, 2008
Polyox	AB200262	GTGTTCTCCAAAGCGACCTC	CGGGTAAGCATTCTCCATGT	215	Yoda *et al.*, 2003
PR1C	X17681	CTCCGGTGCCAGGTAAAATA	TCCTGTTTCCCAGGTTACCA	202	Cornelissen *et al.*, 1986
HSR515	X95342	GACAGTGGAGTGGGCAATTT	CTCGACACTCCGTAGCCTTC	200	Czernic *et al.*, 1996
2. Cell death induced by proteasome malfunction related genes					
20S prot.	DV162364 (EST NT)	AATGGCCAGCATAGGAACAG	GGAATGGCGATGAACAGAGT	223	Kim *et al.*, 2006
UBIPROT6	EB446639 (EST NT)	ACGGTTTTGCGGAAGAAATA	GCCACTTTCAGCACAATGAA	204	Kim *et al.*, 2006
UBIPROT12	EB426207 (EST NT)	GTATCCAGCCGCTTTCAGAG	GGGCCTATGTGAAGAGCAAG	225	Kim *et al.*, 2006
BS2	AM847972 (ESTNT)	CTGGAAAGCCACAGATGACA	GAACATCCACGGGTTTGATT	206	Kim *et al.*, 2006
NAM-like	EB441345 (EST NT)	TTTGCAAAAACTTGGTGCTG	CCCAAGGCTCGAGTTTACAG	237	Kim *et al.*, 2006
CLpP PROTEASE	U32397	CCTGGGGTGGCTATTTATGA	CAGGACAAATTCGCCTGTTT	201	Kim *et al.*, 2003
3. Both defence and proteasome malfunction cell death induced genes					
PR2	M60460	CAACCCGCCCAAAGATAGTA	CCAAAAGGGCATCAAAAAGA	218	Ward *et al.*, 1991; Kim *et al.*, 2003
HIN1	AB091429	CAGCTTGCGTCCAGTATTCA	GGCATCTGGTTTCCTCAAAA	245	Gopalan *et al.*, 1996; Kim *et al.*, 2003
NtCP-23	AB032168	TCGATCGCACAACAAGAAAG	GTGCTGAATGTCCAGCAAGA	240	Ueda *et al.*, 2000; Kim *et al.*, 2003
SGT1	EB449104 (EST NT)	TCATCGACGACCACTTTGAG	AGCTGCTTTTGCAGTTTGGT	239	Peart *et al.*, 2002; Kim *et al.*, 2006
4. Apoptosis related genes					
BI-1	AF390556	GGCTTTTGGTTGCTTCTCAG	CCAAGGTGTGCCTTCTCAAT	231	Bolduc and Brisson, 2002
DAD1	DW001598 (EST NT)	AGAGACGCCCAAGCTCTTTT	TCGAAGACAAACAGCAAGGA	213	Gallois *et al.*, 1997
5. Leaf senescence specific gene					
CP1	AY881011	CGAGAAAGAAGTGCGTTTCC	TGGCGGTATGTCTGTCACAT	229	Beyene *et al.*, 2006

6. Oxidative stress related genes					
APX	U15933	GGACCTGATGTTCCCTTTCA	GGGGATTGGTAGTCCAAGGT	223	Mittler *et al.*, 1999
SOD	X14482	GTGAGCAGACGGACCTTAGC	GGCGTCATGTAGCTGTTCAA	204	Bowler *et al.*, 1989
NBRBOHA	AB079498 (NB)	TCAAGAACTCAAGCGGGTCT	CCATTTTCACGATGTTGACG	242	Yoshioka *et al.*, 2003
EF1ALPHA	D63396	TGCTACCACCCCCAAGTACTC	TAAAGCTGGCAGCACCCTTAG	512	This study

NB, *N. benthamiana*; NT, *N. tabacum*

Table S1: References

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