

Supplementary Information:

A bacterial acetyltransferase triggers immunity in *Arabidopsis thaliana* independent of hypersensitive response

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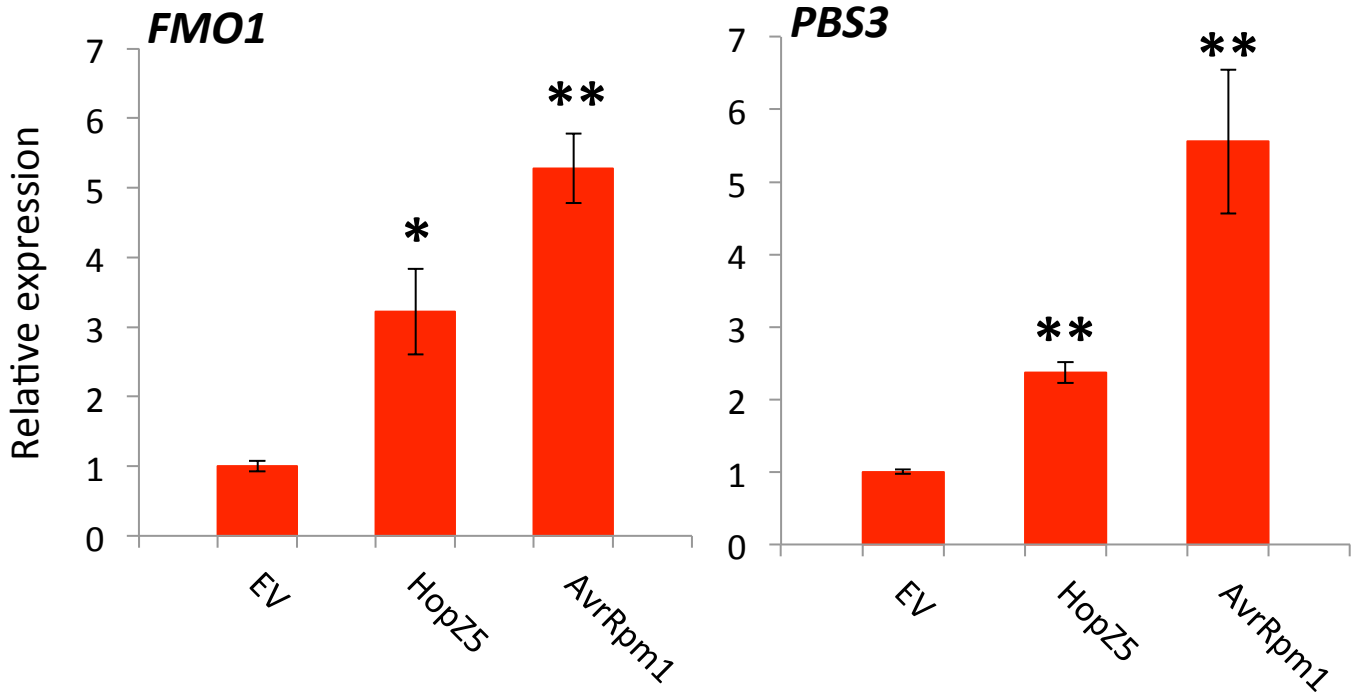
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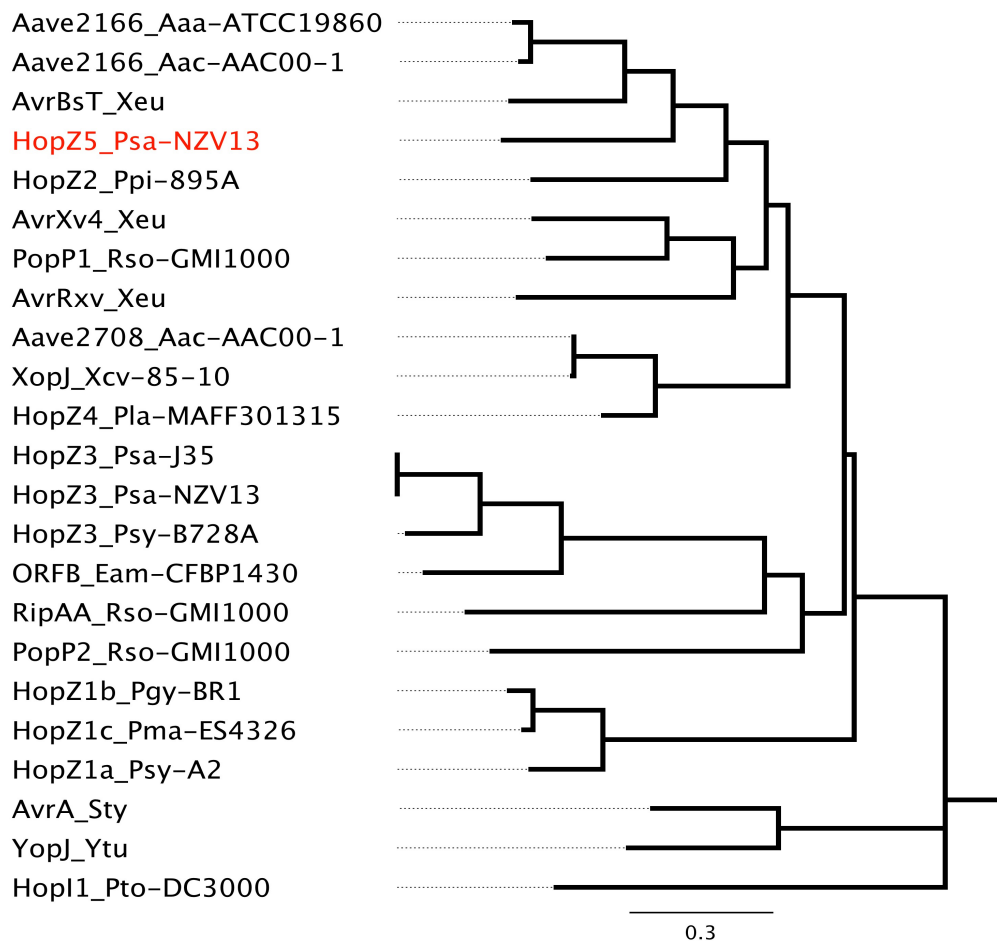
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Supplementary Figure S1



Supplementary Figure S1. *Pto* DC3000-delivered HopZ5 triggers ETI in *Arabidopsis thaliana* Col-0. Defence gene expression in *Arabidopsis* accession Col-0 in response to EV, HopZ5-HA or AvrRpm1. *Pto* DC3000 (2×10^7 CFU/mL) carrying EV, *hopZ5*-HA or *avrRpm1* was blunt syringe-infiltrated into leaves, samples taken at 8 hours post infiltration and defence gene expression determined from extracted RNA by quantitative polymerase chain reaction (qPCR). Expression for each defence gene is relative to internal *EF1 α* expression and defence gene expression for EV samples indicated. Error bars indicate standard error from three technical replicates. Asterisks indicate results of Student's t-test between selected sample and EV; * ($P < 0.05$), ** ($P < 0.01$). The experiment was conducted twice with similar results.

Supplementary Figure S2



Supplementary Figure S2. HopZ5 is a member of the YopJ family of putative acetyltransferases. HopZ5 (in red) from *Pseudomonas syringae* pv. *actinidiae* NZV-13 is a putative acetyltransferase from the YopJ family with *Xanthomonas* type-III secreted effector AvrBsT as its closest homolog. Jukes Cantor neighbour-joining tree using HopI1 from *Pto* DC3000 as outgroup and root, generated in Geneious software with various YopJ-family members: Aave2166_Aaa-ATCC19860 (*Acidovorax avenae* pv. *avenae* ATCC19860), Aave2166_Aac-AAC00-1 (*Acidovorax citrulli* AAC00-1), AvrBsT_Xeu (*Xanthomonas campestris* pv. *euvesicatoria* Bv5-4a), HopZ2_Ppi-895A (*P. syringae* pv. *pisii* 895A), AvrXv4_Xeu (*X. campestris* pv. *euvesicatoria* race T3), PopP1_Rso-GMI1000 (*Ralstonia solanacearum* GMI1000), AvrRxv_Xcv (*X. campestris* pv. *euvesicatoria* 85-10), Aave2708_Aac-AAC00-1 (*Acidovorax citrulli* AAC00-1), XopJ_Xcv-85-10 (*X. campestris* pv. *vesicatoria* 85-10), HopZ4_Pla-MAFF301315 (*P. syringae* pv. *lachrymans* MAFF301315), HopZ3_Psa-J35 (*P. syringae* pv. *actinidiae* J35), HopZ3_Psa-NZV13 (*P. syringae* pv. *actinidiae* NZV-13), HopZ3_Psy-B728A (*P. syringae* pv. *syringae* B728A), ORFB_Eam-CFBP1430 (*Erwinia amylovora* str. CFBP1430), RipAA_Rso-GMI1000 (*R. solanacearum* GMI1000), PopP2_Rso-GMI1000 (*R. solanacearum* GMI1000), HopZ1b_Pgy-BR1 (*P. syringae* pv. *glycinea* BR1), HopZ1c_Pma-ES4326 (*P. syringae* pv. *maculicola* ES4326), HopZ1a_Psy-A2 (*P. syringae* pv. *syringae* A2), AvrA_Sty (*Salmonella typhimurium* str. 14028s), YopJ_Ytu (*Yersinia pseudotuberculosis* str. IP32953). Node length is indicated.

Supplementary Figure S3

Myristoylation site

HopZ1a	1	MGNVCVG---	GSRMSHQVYSPDRADTPP	25
XopJ	1	MGLCVSKPSVAGSPEHYAAHVAEQATPS	28	
HopZ2	1	MGICVSKPSVRHDYNEGYRNYGADTAQ	28	
HopZ5	1	MGLCASKPRTTSGYNTYASYNSSSTPE	28	

Catalytic sites

PopP2	256	DDGSH	TRAADIRK	DASG-TSVIVVDPL-----RKEKDESAYVDYADNVNMEFGEHAKCAFIPVDIQKSFFD	CRILSLSLAL	330	
AvrA	119	SSGIH	ISVVDFRVM-DGKTSVILF	EPA-----ACSAFGPALA-LRTKAALEREQLPDCYFAMVELDIQRSSSE	CGIFSLALAK	194	
YopJ	105	EGGIH	FSVIDYKHI-NGKTSLILF	EPA-----NFNSMGPAMLAIRTKTAIERYQLPDCFHSMVEMDIQRSSSE	CGIFSFALAK	181	
HopZ1a	146	QTLKHHVMADVRLHQGGAPTIIITE	PAVIVG---ARYQQLQRHNLTL	EDLSESGVPLSQVAIIETQAQKTSDD	CVMSLNYAI	225	
XopJ	169	RDGEH	HVAADVRRRRP	NGEASVIVLEPA-----RLLTFVTGHTQLRRQALSQ	GENAKFAFIQVGAQKSAADCL	MFDLHFAL	244
PopP1	162	PN-L	HHAADVRRHHEDGRTTVIILE	EPA-----SAGNQENLPGYTELASALRYNLGSQCRMVVEIAEAQKSLSD	CVAFALDFAL	238	
HopZ2	156	PPSLH	HVAADVRRNHSNGQKTLIVLE	PITAYKDDVYPPAYLPGYPQLREEVNTRLRGN	AKMSVIETDAQRSWHDC	VIFSLNFAL	238
AvrBsT	150	PSSMH	HAAIDVRFK-DGKRTMLVIE	PALAYGMKDGEIKVMAGYETL	GKNVQNCLENGDMAVIQLGAQKSLFDC	VIFSLNMAL	231
HopZ5	146	PNSRH	HVAIDVRNQ-DDVRTMLIIE	SALAYGKSNNATGFLPGYLQMHNNVKNYAQDNGRMAVIQLGVQKSKYDC	IFSLNNAL	227	

Autoacetylation-required site

PopP2	373	GAPLVDARMMK	HGQAASSVSRYLGNHPEQSTVPVNKR-----NETL	GERTTRH	420	
AvrA	227	ADRYLPVSFYK	HQTQGAQRLNEYVEANPAAGSSIVNKK-----NET	LYERFDNN	274	
YopJ	214	LDPYLPVTFYK	HQTQGGKRLNEYLNTPQGVGTVVNKK-----NET	IVNRFDNN	261	
HopZ1a	279	GADVLPVDFYK	HGASLTQAKQLMKRPDGRMAGRVNSE-GHSEAENLVQR	NQAF	330	
XopJ	290	GEQMLPAIFYK	HHTHSSGVVEEVDRSQPGSAYTDVSTSGRQQHESLE	QRVQAF	342	
PopP1	289	GWGVLPPVYK	HAHSRETLKGVKQRQPGSLETDVSTGRNKDGAESLE	ERMEAF	341	
HopZ2	280	GKQVLPVAVYK	HAHSRGTVTAVENAQPHIANDNVSTN-RSSSRETL	NERAEAF	331	
AvrBsT	272	GDKFLPPIFYK	HSHSRGVVGEFISNQPEYAHKNVSTG-RTNP	SEDL	SERVENF	323
HopZ5	268	GTKILPAVFFK	HHTSRSTINDAIEEQPELADRNVSTN-RESAHQTL	SERVADF	319	

Supplementary Figure S3. HopZ5 shares predicted conserved residues with YopJ family members. Critically conserved residues in selected YopJ family members are shown: Putatively myristoylated glycine highlighted in purple, acetyltransferase catalytic core histidine, glutamate and cysteine residues highlighted in orange, and autoacetylation-required lysine residue highlighted in blue.

Supplementary Figure S4

HopZ5-C218A

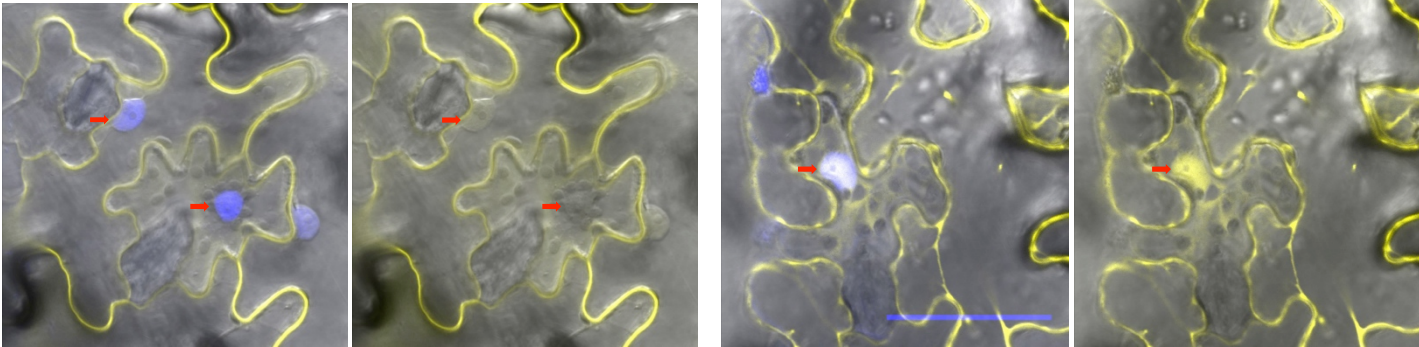
HopZ5-G2A

+ DAPI

- DAPI

+ DAPI

- DAPI



Supplementary Figure S4. The G2A variant of HopZ5 localizes to the plant cell cytoplasm and nucleus. *In planta* subcellular localization of HopZ5 variants was determined in *SGT1*-silenced *N. benthamiana*. Leaves were infiltrated with *Agrobacterium tumefaciens* AGL1 carrying YFP-tagged *hopZ5* (G2A) or *hopZ5* (C218A) under a constitutive CaMV 35S promoter. Leaf discs were taken at 2 days post infiltration, stained with DAPI, and visualized under confocal laser-scanning microscopy for localization of the DAPI signal and YFP tag. All panels are confocal images merged with corresponding bright-field images, with or without DAPI signal included as indicated. Red arrows indicate plant cell nuclei. The blue scale bar in G2A panel represents 50 μm.

Supplementary Figure S5

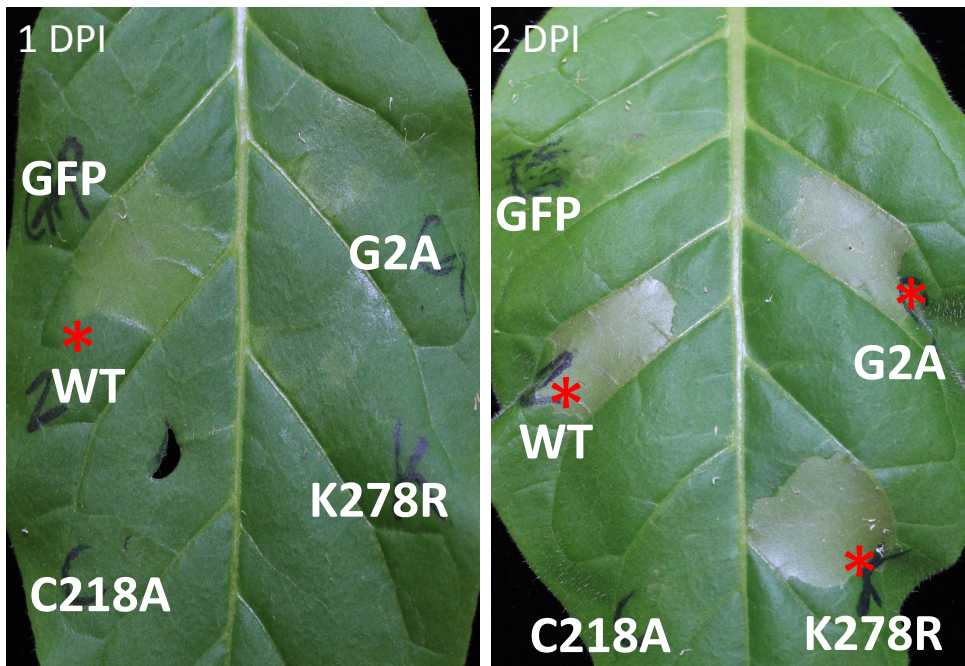
Pto DC3000



Supplementary Figure S5. HopZ5 variants are unaffected in expression and protein stability in *Pto* DC3000. *Pto* DC3000 carrying *hopZ5* (WT) or with mutated myristoylation (G2A), catalytic core (C218A), or autoacetylation-required (K278R) variants tagged with 6xHA were inoculated at $OD_{600}=0.15$ and grown for 7 hours on type-III inducing minimal medium (Huynh et al. 1989)*. Cells were harvested by centrifugation, boiled in 1xLeamli buffer and separated by SDS-PAGE for immunoblot using α -HA antibody.

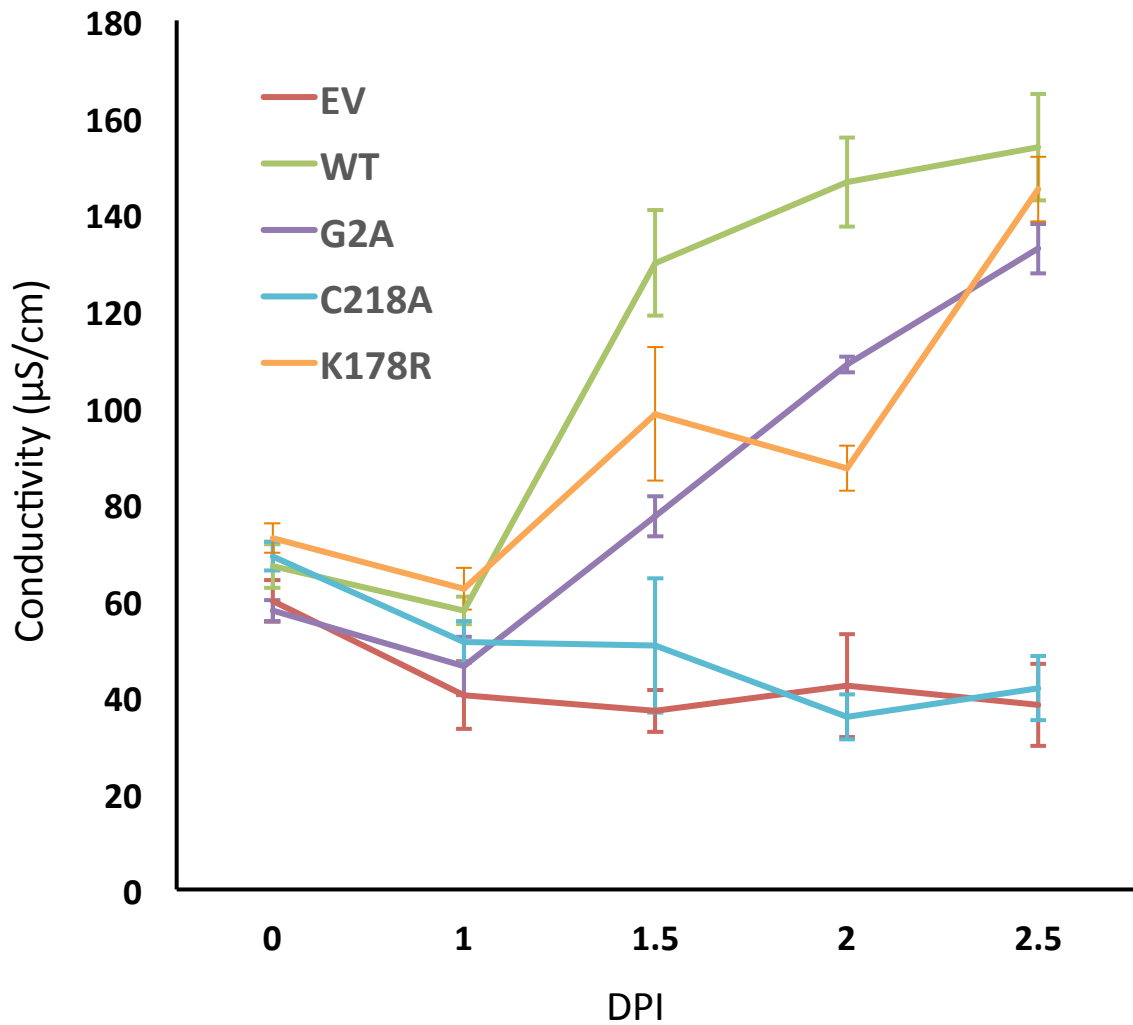
*Huynh, T., Dahlbeck, D. & Staskawicz, B., Bacterial blight of soybean: regulation of a pathogen gene determining host cultivar specificity, *Science* **145**, 1374-1377 (1989)

Supplementary Figure S6



Supplementary Figure S6. HopZ5 triggers a hypersensitive response in *Nicotiana tabacum* and requires G2, C218 and K278 residues. HopZ5 (WT) with mutated myristoylation (G2A), catalytic core (C218A), or autoacetylation (K278R) variants tagged with 3xFLAG were transiently expressed under the constitutive CaMV 35S promoter in *Nicotiana tabacum*. Photographs were taken at 1-2 days post infiltration (dpi). Red asterisks indicate development of hypersensitive response-like cell death symptoms. This experiment was repeated five times with identical results.

Supplementary Figure S7



Supplementary Figure S7. HopZ5 recognition in *Nicotiana benthamiana* requires acetyltransferase activity. Electrolyte leakage from *N. benthamiana* leaf discs triggered by *Agrobacterium*-mediated transient expression of HopZ5 or variants was determined at multiple time points (dpi). Infiltrations were carried out by blunt syringe at $\text{OD}_{600} = 0.4$ for all samples as described in Figure 5(b).

Supplementary Table 1. Summary of phenotypes for HopZ5 variants by assay

Assay	WT	G2A	K278R	C218A
HR (<i>Pseudomonas</i> delivery)	+++	+++	-	-
Electrolyte leakage (<i>Pseudomonas</i> delivery)	+++	++	-	-
Defence gene expression (<i>Pseudomonas</i> delivery)	+++	+ / -	-	-
<i>Pto</i> DC3000 growth restriction	+++	+++	+ / -	-
Defence gene expression (Col-0 stable expression)	+++	++	+ / -	-
<i>Pto</i> DC3000 growth restriction (Col-0 stable expression)	+++	-	-	-
HR (<i>Nicotiana</i> agroinfiltration)	+++	++	++	-
Electrolyte leakage (<i>Nicotiana</i> agroinfiltration)	+++	++	++	-

- +++ = strong phenotype with statistically significant difference compared to empty vector control
- ++ = intermediate phenotype with statistically significant difference compared to empty vector control
- +/- = weak phenotype; variable results and occasionally not statistically significant compared to empty vector control
- = not statistically significant as compared to empty vector control

Supplementary Table 2. Primers used for qPCR of marker genes

Gene	Forward	Reverse
<i>AtEF1α</i>	CAGGCTGATTGTGCTGTTCTTA	GTTGTATCCGACCTTCTTCAGG
<i>AtPBS3</i>	TTCGCTGGCTTGTATAGGATGA	CTGGAAATGTTGAGGTGTCAGC
<i>AtFMO1</i>	TGTGTTTGAAGATGGGACGACA	GTTTCGAGCTGCTTTGGACGTAT
<i>AtWRKY51</i>	AGGAAGTATGGCAAGAAATCTG	GAGTGTTGGTTCCAGTTATCAT
<i>hopZ5</i>	TGCATCAAAACCCCGCAC	GTGATATACCCTGACTGCC
<i>AtPR1</i>	ATACACTCTGGTGGGCCTTACG	TACACCTCACTTTGGCACATCC
<i>NbACTIN</i>	GATGAAGATACTCACAGAAAGA	GTGGTTTCATGAATGCCAGCA
<i>NbSGT1</i>	TAATGTGTCATCAGATGCC	ACTTCTTTCCAGTTTGTCGAC
<i>NbNDR1</i>	TAGTAAAGTGAAAGTGGATGGTTC	GCAATCAACTGAGTCCAACAT
<i>NbEDS1</i>	AACGAGGAAAAGATTGATGGTA	TCCTTTCTTCCCTCAAACATATC