

Supplemental Figure 1. Bayesian phylogenetic tree based on amino acid alignment of full-length products of Arabidopsis *PBS1* (*AtPBS1*), all characterized Arabidopsis *PBS1*-like (*AtPBL*) genes, and barley *PBS1*-like (*HvPBL*) genes homologous to Arabidopsis *PBS1*. *AtPBS1* and *AtPBL* sequences were obtained from The Arabidopsis Information Resource (TAIR10) website (arabidopsis.org). Homology searches were performed using BLASTp to identify barley amino acid sequences homologous to Arabidopsis *PBS1* and *PBS1*-like proteins. Thirty-two barley protein sequences were identified as homologous to the 29 Arabidopsis sequences used in the analysis. Bayesian phylogenetic trees were generated for the collected sequences using the program MrBayes under a mixed amino acid model. Scale bars indicate amino acid substitutions per site and nodes are labeled with Bayesian posterior probabilities as a percentage. The gray box highlights the clade presented in Figure 2.

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AtPBS1      1  MGCFSredCF-DSSDDEKLNblueNPVDESgreyNG-----QKKQSQgreyTVSNgreyNIgreyS
HvPBS1-1   1  MGCFFCF-DSSSDGELLYPKQGGGGGGGGNGTGGRTAAAASSSGVGAREERPMVPPRVE
HvPBS1-2   1  MGFLSCLFRCPredEEEEVVVKEHDDNE-D-----SSGIDHGVASESEgrey

AtPBS1      39  GLPSGGgreyEKLSSKgreyTNGGSKRELLLPgreyRDGLGQ-IAAHTFAFRELAAATMNFHPDgreyFLGEGGF
HvPBS1-1   60  KLPAGAEKARAKGNAgreyGKELS-DLRDANGNVLgreySAQTFTFRQLTAATRNFRgreyECCFgreyTGEGGF
HvPBS1-2   41  SVPLRAESTHIEG-----IQgreyRNGTN--NEATIFTLRELVDATKNFSQDSQLGRGGF

AtPBS1      98  GRVYKGRLDSTGOVVAVKQLDRNGLOGNREFLVEVLMLSLgreyLHHPNLVNLIGYCADGDQRL
HvPBS1-1   119  GRVYKGRLDG-GOVVAIKQLNRDGNgreyOGNKEFLVEVLMLSLgreyLHHPNLVNLVGYCADGEQRL
HvPBS1-2   90  GCgreyVYKAYLND-GOVVAVKQLDgreyNGLOGNREFLVEVLMLgreyNLHHPNLVNLIGYCVgreyDGDQRL

AtPBS1      158  LVYEFMPLGSLEDHLHDLPPDKEALDWNMRMKIAAGAAKGLEFLHDKANPPVIYRDFKSS
HvPBS1-1   178  LVYEFMPLGSLEDHLHDLPPDKEPLDWNTRMKIAAGAAKGLEYLHDKAgreyPPVIYRDFKSS
HvPBS1-2   149  LVYEFMPLGSLEDHLHDLPPNKEPLDWTTRMKIAAGAAAGLEYLHDKANPPVIYRDKgreyKPS

AtPBS1      218  NILLDEGFHPKLSDFGLAKLGPgreyTGDKSHVSTRVMGTgreyYGYCAPEYAMTGQLTgreyTKSDVYSFG
HvPBS1-1   238  NILLGDDFHPKLSDFGLAKLGPVGDKSHVSTRVMGTgreyYGYCAPEYAMTGQLTgreyTKSDVYSFG
HvPBS1-2   209  NILLAEGVHAKLSDFGLAKLGPVGDKgreyTHVgreyTRVMGTgreyYGYCAPEYAAgreyTGQLTgreyNKSDgreyYgreySFG

AtPBS1      278  VVFLLELITGRKAIDSEMPHGEONLVgreyAWARPLFNDRRKFgreyIKLADPRLKGRFFgreyTRALYQALA
HvPBS1-1   298  VVFLLELITGRKAIDSTRPHGEONLVgreySWARPLFNDRRKLgreyPKMADPGLQGRVgreyPMRGLYQALA
HvPBS1-2   269  VVFLLELITGRALDgreySNRPREQDLVgreySWARPLFKDgreyQRKFPKgreyMADPLLgreyKGRFFgreyKRGLYQALA

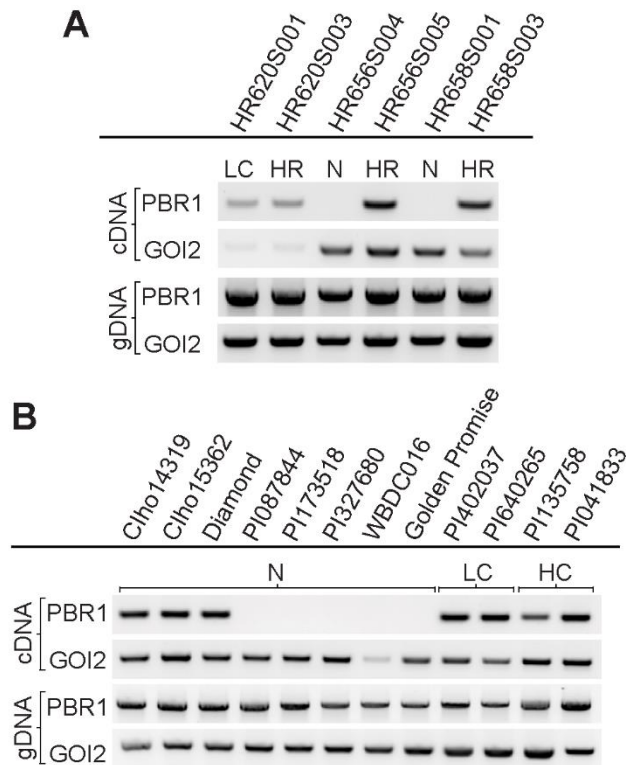
AtPBS1      338  VASMCIOEQAAgreyTRPLIADVVTALSgreyYLANQAgreyYDPSKDDSRRN-----RDER-----
HvPBS1-1   358  VASMCIOSEAAgreySRPLIADVVTALSgreyYLAgreySIYDPNAIHASKKAGGDQgreyRSRVSD-----
HvPBS1-2   329  IAAMCIOEKSRNgreyRPLIREVAAALSgreyYSSQTYNGNDAAGRRCLDGPSTPKVSEEQVNQDDA

AtPBS1      383  -----GARLITRN--DDGGGSGSKFDLEGSEKEDSPRETARILNRDINRERAVAEAKMWG
HvPBS1-1   410  -----SGRALLKN--DEAGSSGSK-----SDRDDSPREPPPGIL--NDRERMVAEAKMWG
HvPBS1-2   389  LPSQLGAQTSgreyMHDRMgreyNDFIPEGKEHCRSGSNRgreyGVGRVVPNG-----VDRDRALADANVWA

AtPBS1      436  ESLREKRROS--EQGTSES-NSTG
HvPBS1-1   456  ANLREKTRAANAgreyQGSgreyLDSPTETG
HvPBS1-2   445  EAWRRHEKAS--KVRVTD--EILG

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Supplemental Figure 2. Full-length amino acid sequence alignment between Arabidopsis PBS1 and the barley PBS1 homologs. Conserved residues and conservative substitutions are highlighted with black and grey backgrounds, respectively. Predicted myristoylation and palmitoylation sites are indicated with red and blue boxes, respectively. The activation segment is indicated with a green box and the AvrPphB cleavage site with a black arrow.



Supplemental Figure 3. Expression of *Pbr1* and *Goi2* in additional representative barley lines. PCR amplification from cDNA showing expression and from gDNA showing primer compatibility for **A**) two recombinant inbred lines each from each of the three NAM subpopulations used for GWAS (HR620, HR656, and HR658), exhibiting the parental phenotypes **B**) Additional lines bringing the total lines tested (excluding RILS) to 12 responding and 12 non-responding when considered with Figure 5. cDNA was generated from RNA extracted from 10-day old plants, the same age used for AvrPphB response assays. The AvrPphB response for each line is indicated: N, no response; LC, low chlorosis; C, chlorosis; HC, high chlorosis.

Information on Additional Supplemental Tables and File:

Supplemental Table 1. Responses of 150 barley lines when infiltrated with *Pseudomonas syringae* DC3000(D36E) expressing AvrPphB. A catalytically inactive AvrPphB(C98S) mutant was used as a negative control and never elicited a response. Lines were scored as no response (N), low chlorosis (LC), chlorosis (C), high chlorosis (HC), and hypersensitive reaction (HR).

Supplemental Table 2. Responses of 193 RILs from the UMN Spring Barley Nested Association Mapping (NAM) population when infiltrated with *Pseudomonas syringae* DC3000(D36E) expressing AvrPphB. The inactive protease AvrPphB(C98S) never elicited a response. Response phenotypes were used in genome wide association analysis. Hypersensitive reaction = 1; no response or low chlorosis = 0.

Supplemental Table 3. Responses of wheat varieties to *P. syringae* DC3000(D36E) expressing AvrPphB. The second leaves of 14-day old wheat seedlings were inoculated with *P. syringae* DC3000(D36E) carrying AvrPphB ($OD_{600} = 0.5$) by infiltration with a needleless syringe. Wheat responses were scored as no response, low chlorosis, or chlorosis three days post-inoculation. Wheat varieties were obtained from the U.S. Department of Agriculture Wheat Germplasm Collection or generously provided by Scot Hulbert (Washington State University).

Supplemental File 1. Nexus file with alignment of *Pbr1* alleles from 12 barley lines with diverse responses. The file was generated by Clustal Omega nucleotide alignment. The tree derived from the alignment is shown in Figure 5C.