

AtPMEI10	MN ILSQTQILHLS T AILLFITT SSSSLSPSSSSPSLSPSPSSSSPSSAPPSSLSPSSPPPLSLSPSSPPPPSSSSPLS	79
AtPMEI11	MAKQIFYTLFLFL L STAILTA.....	21
AtPMEI12	MKFLVSLVIFSLFLNGFATA.....	20
AtPMEI10	SLSPSLSPSPSSSSPSSAPPSSLSPSSPPPLSLSPSSPPPPSSSSPLSSLSPSSSSSTYSNQTNLDY I K T SC N I T LY K .	158
AtPMEI11SSAPRAAITSKRAINFI Q A S CKATTYP.	49
AtPMEI12QTL I Q D S C KKAF K D	35
AtPMEI10T I C N S L S P Y A S T I R S N P Q .K L A V I A L N L T L S A K S A S K F V K N I S ..H G G L T R L...E V V A V A D C V E E I G D S V	226
AtPMEI11T V C V N S L T G Y A N S I Q T S P R.R L A E T A L N V T V T Q A Q S T K V F V W R L G..R F T S L K K R ...E I Q A V K D C I E E I H D A V	117
AtPMEI12	P Q L S Y D F C V N S L T QD P Q S K A A T L E S L V L A S T K T A A K I T N L K G I V A Q D L K D Q R Y Q D I V E D L K L C L G F Y N D A N	108
AtPMEI10	T S L Q D S I R E L D S I N Y K D S A K ...F E M V M S D V E T W S A A L T N D D T C M D G F S ..L V K T A V K D L V R R H V V E V A R L T S N A L A L	300
AtPMEI11	D R L T M S I H E V K M C G ...S A K G R D Q F W H M S N A Q T W T S A A L T N A N T C S D G F A G R V M D G R V K N S V R A R I L N L G R G T S N A L A L	194
AtPMEI12	D D L T T A L A N I K S R DY Q G . A N I N L S A A L D V P G N C E D D F K ..E A K K T S P I T N E N S I L F K T I L I P ..L A F	170
AtPMEI10	I N Y A S T Q E N F S ..	312
AtPMEI11	I N A F A K K Y	202
AtPMEI12	T N M L	174

Figure S2. Alignment of amino acidic sequences of AtPMEI10, AtPMEI11 and AtPMEI12. The signal peptides are highlighted with a black rectangle. Highlight homology level: 100% black; >50% light blue

atgaacatcttatctcaaaccxaaatccttcatctctctatagctattcttctcttcatc
M N I L S Q T Q I L H L S I A I L L F I
acaacatcatcatcatcattatcaccatcatcatcaccatcattatcaccatcacca
T T S S S S L S P S S S S P S L S P S P
ccatcatcatcaccatcatcggcaccaccatcatcattatcaccatcatcaccaccacca
P S S S P S S A P P S S L S P S S P P P
ctatcattatcaccatcatcaccaccaccaccgcccaccatcatcatcacctctatcatca
L S L S P S S P P P P P S S S P L S S
ttatcaccatcattatcaccatcaccaccatcatcatcaccatcatcggcaccaccatca
L S P S L S P S P P S S S P S S A P P S
tcattatcaccatcatcaccaccaccactatcattatcaccatcatcaccaccaccaccg
S L S P S S P P P P L S L S P S S P P P P
ccaccatcatcatcacctctatcatcattatcaccatcatcattcatcaacataactca
P P S S S P L S S L S P S S S S S T Y S
aatcaaaccaacttagattacatcaaaacatcatgtaacattacactctacaaaaccatt
N Q T N L D Y I K T S C N I T L Y K T I
tgctacaactctctatctccttaagcctcaacaatccggtccaaccctcaaaaactcgc
C Y N S L S P Y A S T I R S N P Q K L A
gtcatcgccctcaatctcacctctcatccgccaatctgctccaattcgtcaaaaac
V I A L N L T L S S A K S A S K F V K N
atatctcacggagggtggtctatcttggaaagtagttgcggttgctgattgcggtgaa
I S H G G G L T R L E V V A V A D C V E
gagattgggagactcgggtgacttcgcttcaagattcgataaggggaattggattctatcaac
E I G D S V T S L Q D S I R E L D S I N
tataaagatagtgcaaagtttgagatgggtatgtcggatggtgaaacatggggttagtgct
Y K D S A K F E M V M S D V E T W V S A
gctctcacgaatgatgacacatgtatggatgggttcagtctagttaaaacggccgtgaaa
A L T N D D T C M D G F S L V K T A V K
gatttgggttcgacgacatggtggttgaggttagctcggcttactagcaacgcggttgctctc
D L V R R H V V E V A R L T S N A L A L
attaatatgtacgcctctacgcaagaaaacttttcttaa
I N M Y A S T Q E N F S -

Figure S3. Nucleotide and amino acid sequences of AtPMEI10. The signal peptide is in yellow. The Serine-Proline-rich repeat (SPRR) region (in grey) of the inhibitor starts and ends with SSSS domains (in red), includes SL(S)SPS(L)-SP(A)PPS(L) and -SPP repeated domains (in fuchsia) and includes interspersed PPPP motifs (in green) . The PMEI domain of the protein is indicated in cyano.

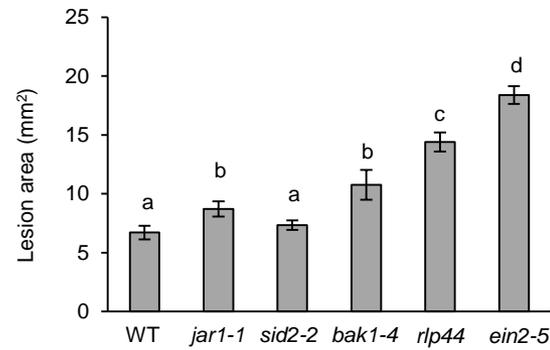


Figure S4. Disease symptoms produced by *Botrytis* infection in immune signaling mutants. *Arabidopsis* WT and the mutants indicated were inoculated with *Botrytis* and lesion area were determined at 48 hour post inoculation (hpi). The values are mean \pm SE. (n=36). The different letters indicate data sets significantly different according to ANOVA followed by Tukey's test ($P < 0.05$). The experiment was repeated three times with similar results.

AtPME10

GGTGGTCACTGTTTTATTAAATTTCAGATTTGATTTATTTGGCTATATTTGCCGACTGATCTTTTTCTGTATCCACTCTGAATTTATTT
TTCTTTTGGAAAGGATCTAGCTTCAATAAAAATGGGAATTATATAGCTGGACCAAAGTGTAGTTGGACGAATTAATTCATCATATACAACA
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AATAAATACAATAAATAAATAAATAAAGTAAAAATAGTTTTTACCTCAATTTGCTTTCTTAGATTACTAATAAACGTGTATGCCTAAA
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GTATGGAAGAAAAGTTCCATTGCTAAAATTTACGCAATTTAATGTATGAAAAATACTTATATGTGAAAAATACTTAACTTAATAAA
TTCAATTTAACAACTTCATATACAATAATTTATATTTTATATTTTTCATAAATTAATTTATAATCAATTAACGATTTCTTCCATACTTTAG
AAATATGGGAATCCATACTCTTCGGCCTTAAAGATATATATATTTGGTCGTTTATGAGTAAAATATGTTTTTTGAAGCATATGTGACAA
ATCCAGAATACGTTAAACATTTGAGCCGGATGTAATTTGTAAGCTTTCCCAAAAAACCTCAGCTTCAATCAAGTAAAAGTTATTAG
ATATGATTGAGTTATTAATAGACTCAACCAATTTCTTTTTCACAAGTTTTCTTTTACGTATAATTTATTTATATTTATATATACATGTT
CTATATATACACACTATATATACATACATGCACATGCCATTAGTTTCCACTAGGCCATTCTCTTTTAAAGCAATCGACGCCAA
AAAAGAAAACGACGATACAAA

AtPME11

ATTCATTAGTCGCCAAATGAACAATTTTTTTTACATGTTATTTTGTAAACATCACCTCTATTATTCTATGCTTAATTTTTAACTTAATCT
GTACATCTTTTGTCAATAATTAATGAGATACATACATTAGGGTTTATCAAAAAATATGAGATACACTAGTCGCCTAATTTGGTTACA
TAGCATATCTTTGGACGTGGATCAGACTAATTAGAAGTGTACCTATTGTTGCAATTTGGAACGATTTTCAGGGTCAAATCCACTATCCATC
TCTTTATGAATTTCAATTAATAGTCGCACAATCCAATTTCTATCTTTTGTAGTTGCCGGTGAATATAAAAATCTTTTGATATATGAC
GTAAAGGCAAAAGATACTTTGCGATGATAGTACAAAATGTATCCAAATACAAATATGATATATCTATACGTATATTTTTTTTTTTTCAAC
ACATAAAGCGAATAAAAAATGAACATTTTGTAAATTTG TGACTGATGGTGTATGCTAAAATGTTTCATTATATTGGAATGGAGATGTAAA
ATAAGTATACGTTGAAAATGCCATAATTCCTTGGAAATGAATCGATGATAAAGATCAACCTGTTTTTCATTATAACAACAATACAAAAGGT
TAAACCATGTTTACACCAAAAAGTCCATTTCTGATGATCATTACGAAAAATCCTAGATATTCGTTTGTATTTTTGTTCTTTTTCATTCCCA
TGTTACTTTTTGGGTCAAGAAGAAGAAATGAATATAATTTGCCATAGTACATTTCTTAATACCTAATAGCAGACAATTTCTGGTTATTTCCA
ACTAATAATCTAGACCTCAGTTGAAAAACAATAATCAACCAAAGCGTCAAAGTCAAAGCATATCAATTTATTTCTATTATATAAACC
TAAGAAAAACCAACGTACACCAAAGAGTACGTACTAGTCCACTATAAAAACTCAAACCAACTCTTTACCTCTCCACAAATATTATCT
AAACCAAAACATTTCAAAAGA

AtPME12

CAATCTTCCGTATTACAAAGACCGAAAAAATACAATAAAAGTAAGAATACATT TGTCAAAATAAATGCTAAAAGAGATAAGAAATAT
AACTTTTTTTTTTTTTTCTGATGTTCTTTTAT AACGT TAAGTTTTATTAACCTTAATTAATCTTTACACCAAATCCAGACCAGAAA
CAACAGCAAGAAAAAAACTTTGATTTGGTTGGAAGGTGGATCGAACGATAAATCCATAATCTAACATTTGAACAGAGTGTGCACAAT
GGAATGGAGAGTTGCTAAGAAAAATATTATGTCATGTTGATCAATATCATAAACAATTTATAAATAATCTAAACATAACGAACAAT
TGTTAAAAAATGTTATTGTCATGTTGGCCAATATCATAAACAATTAATAAATAATCTAAACATAAACGAACAATTTGTTAAAAAATAT
TATCTTTAATTTCTGTTATAAAAAATCAAAATGTTGATGAATGCATAATGATGATGACAAACGGCATAATGGGAGAAAAGAGTTCTCATTAT
TTCTGTTATGATGATTTTGGAAACACTCACAATCGT TGACTAAATTTGTGAATATATGGAAAACAATAAATCAATAATCTTTGATT
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AACGTGGGTATATGGAAAGCAAAATATTGTCATGTTGACCAATTTCCAAACAATAAATATGAATTTAGGAAAGAAATCATGAAAAGT GACT
ATCTTACCATTATTCATTAAGTGAATAATATCTCAATATGAATGATATCT ATTTCAAATGGGAACCTTTCCAAATTTTCAACAAT
TACCATAAAATTTATTTTTCTGTTTTACTATAAATTTGAACACAGGGTGACAAAAATTTTCATCAATCAATTTCTAACTTTCTCTGCTTC
TTCTCAAAGTGA AAAACAAAA

Figure S5. Analysis of cis-acting DNA elements with regulatory functions in plant immunity in the 5' flanking regions of *AtPME10*, *AtPME11*, *AtPME12*. About 1500 bp have been analysed for each gene. Green indicated Ethylene Responsive Element (ERE); Yellow indicated Brassinosteroid response element (BRRE); Fuchsia indicated Jasmonate responsive elements (JARE); Gray indicated responsive elements reported as involved in plant defence.

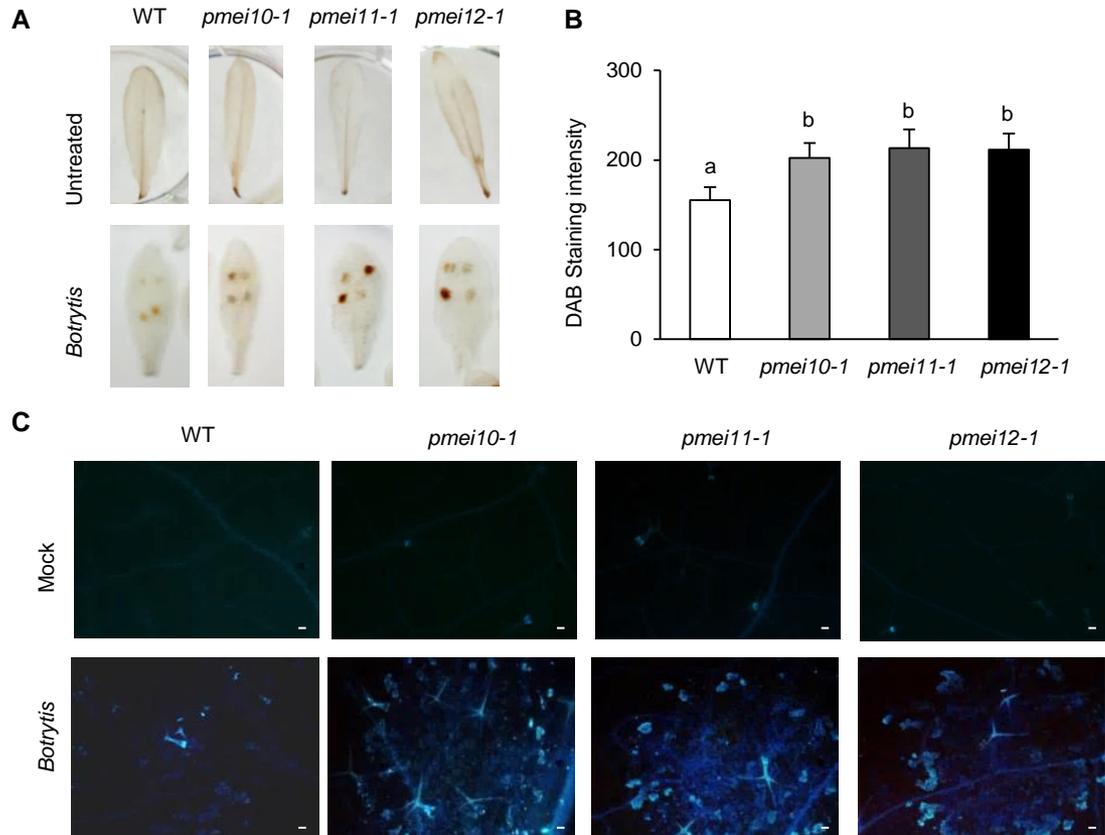


Figure S6. Arabidopsis *pmei10-1*, *pmei11-1* and *pmei12-1* mutants are not defective in H₂O₂ and callose accumulation against *Botrytis*. Representative pictures of untreated and *Botrytis* treated leaves of *pmei* mutants and WT plants (A) and accumulation of H₂O₂ (B). Untreated and *Botrytis*-treated leaves at 48 hour post infection were stained with 3,3'-diaminobenzidine and the intensity of the dark staining (mean gray value) quantified using IMAGEJ. The different letters indicate datasets significantly different according to analysis of variance (ANOVA) followed by Tukey's test ($P < 0.01$). C, Microphotographs indicating callose deposition at the level of lesion area produced by *Botrytis* at 48 hpi on *pmei* mutants and WT plants. The leaves were stained with aniline blue and callose deposition was visualized by epifluorescence microscopy. (scale bars = 100 μ m). The experiments were repeated three times with similar results.

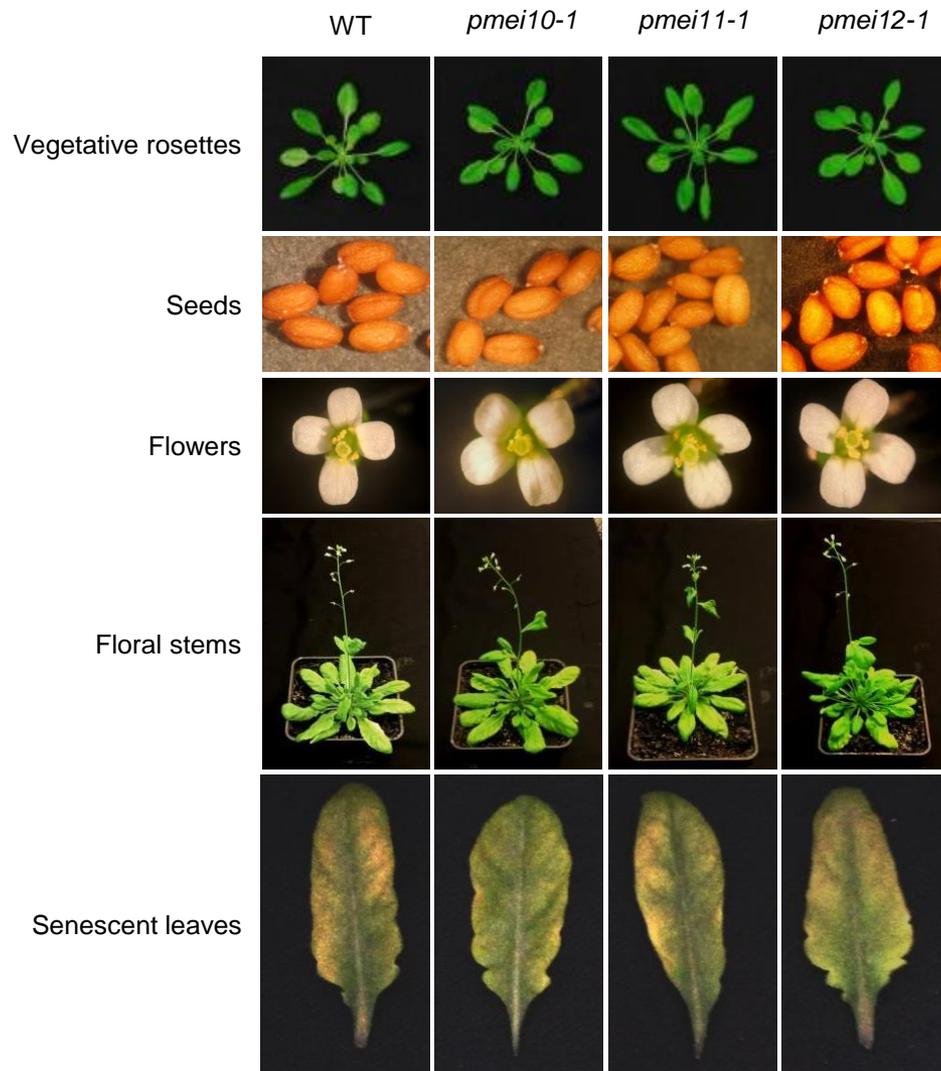


Figure S7. Representative pictures illustrating the morphology of different tissues of *pmei10-1*, *pmei11-1* and *pmei12-1* mutants and WT plants. Vegetative rosettes (25 days old plants), seeds, flowers (40-days old plants), floral stems (40-days old plants), senescent leaves (65 days old plants) of *pmei10-1*, *pmei11-1* and *pmei12-1* plants were compared with WT.

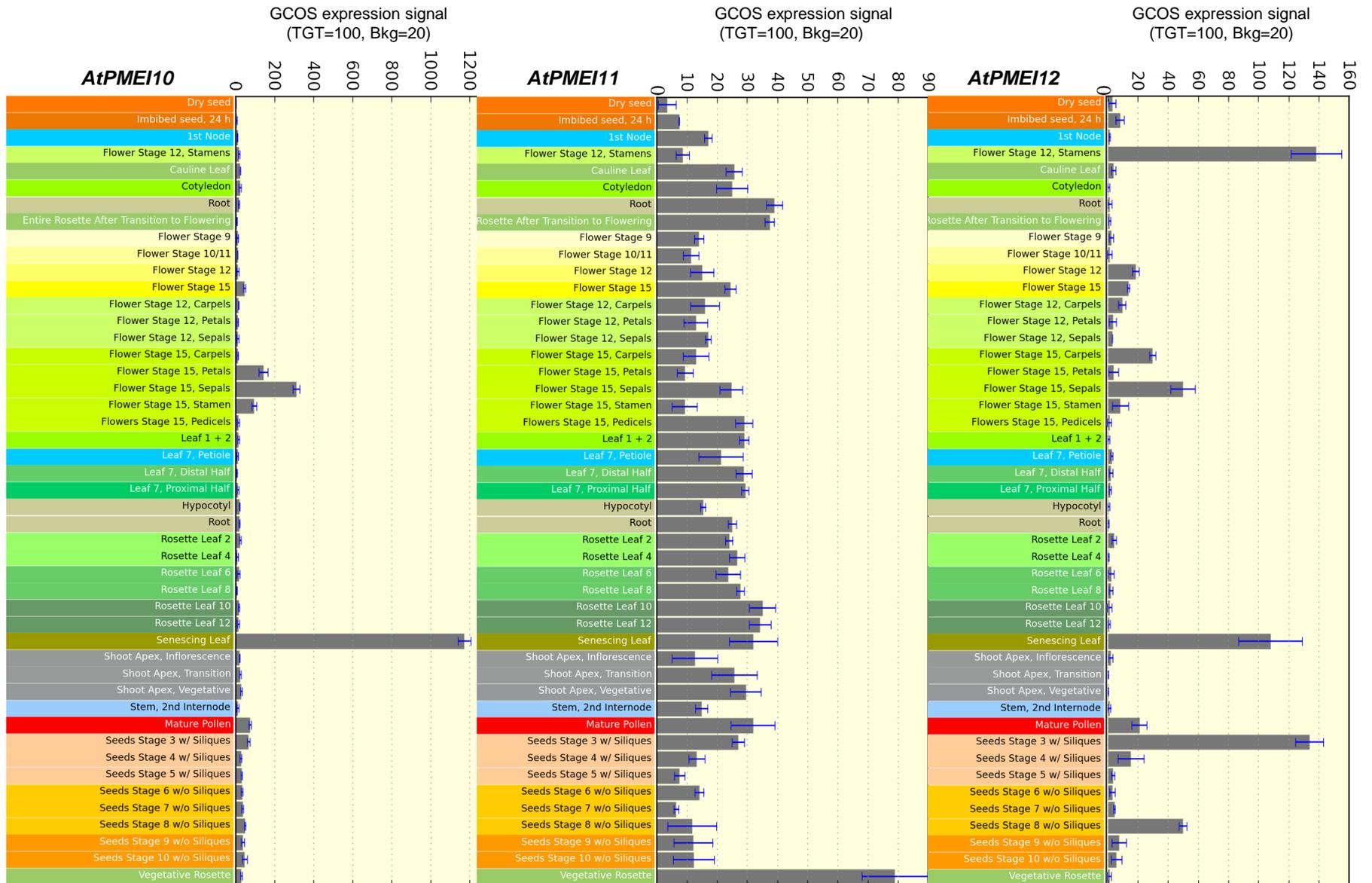


Figure S8. *AtPMEI10*, *AtPMEI11* and *AtPMEI12* gene expression in different tissues and developmental stages of Arabidopsis. ATH1 microarray data were gathered and processed using the eFP browser (Winter et al., 2007). Expression signals were generated by the eFP browser using the Gene Chip Operating Signal (GCOS) using target intensity (TGT) and background (bkg) values as indicated in figure.

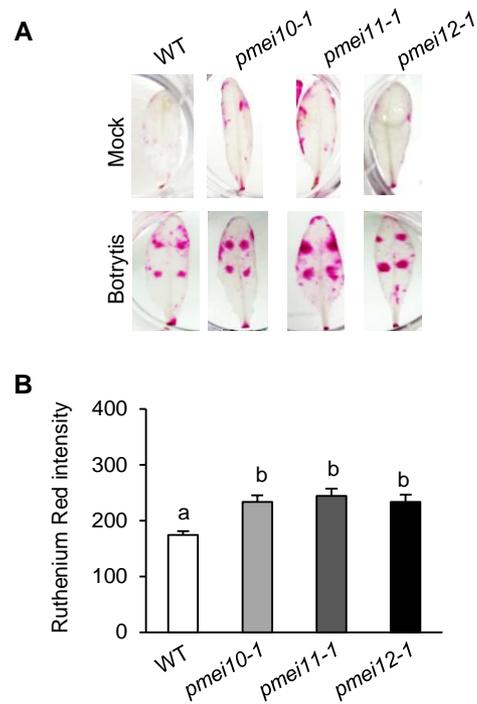


Figure S9. Histochemical evidence indicating the control of PME activity by AtPME10, AtPME11 and AtPME12 during *Botrytis* infection. Leaves of *Arabidopsis* WT and of *pmei* mutants were inoculated with *Botrytis* or mock and PME activity was evaluated at the level of lesion area (A) by staining leaves with ruthenium red and (B) by quantifying the mean gray value using IMAGEJ.

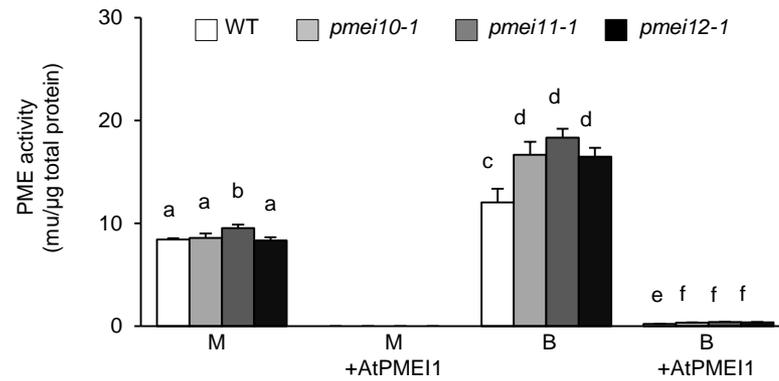


Figure S10 Effect of exogenous addition of recombinant AtPME1 on PME activity at 48 hour post inoculation. Value are mean \pm SD (n = 3). M = protein extracts from mock-inoculated leaves. B = protein extracts from *Botrytis* inoculated leaves. + AtPME1 = addition of AtPME1 to the relative protein extracts. mu = milliunits;

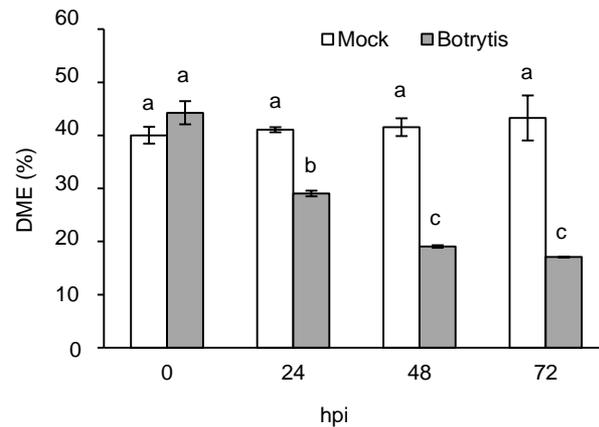


Figure S11. Dynamic modifications of DME in *Arabidopsis* leaves during *Botrytis* infection. The degree of pectin methylesterification (DME) was quantified in *Arabidopsis* leaves during *Botrytis* infection. The DME was quantified and expressed as methanol to uronic acid molecular ratio (%). hpi = hours post-inoculation. Results represent the mean \pm SD (n = 3). The different letters indicate datasets significantly different according to analysis of variance (ANOVA) followed by Tukey's test ($P < 0.05$). The experiments were repeated three times with similar results.

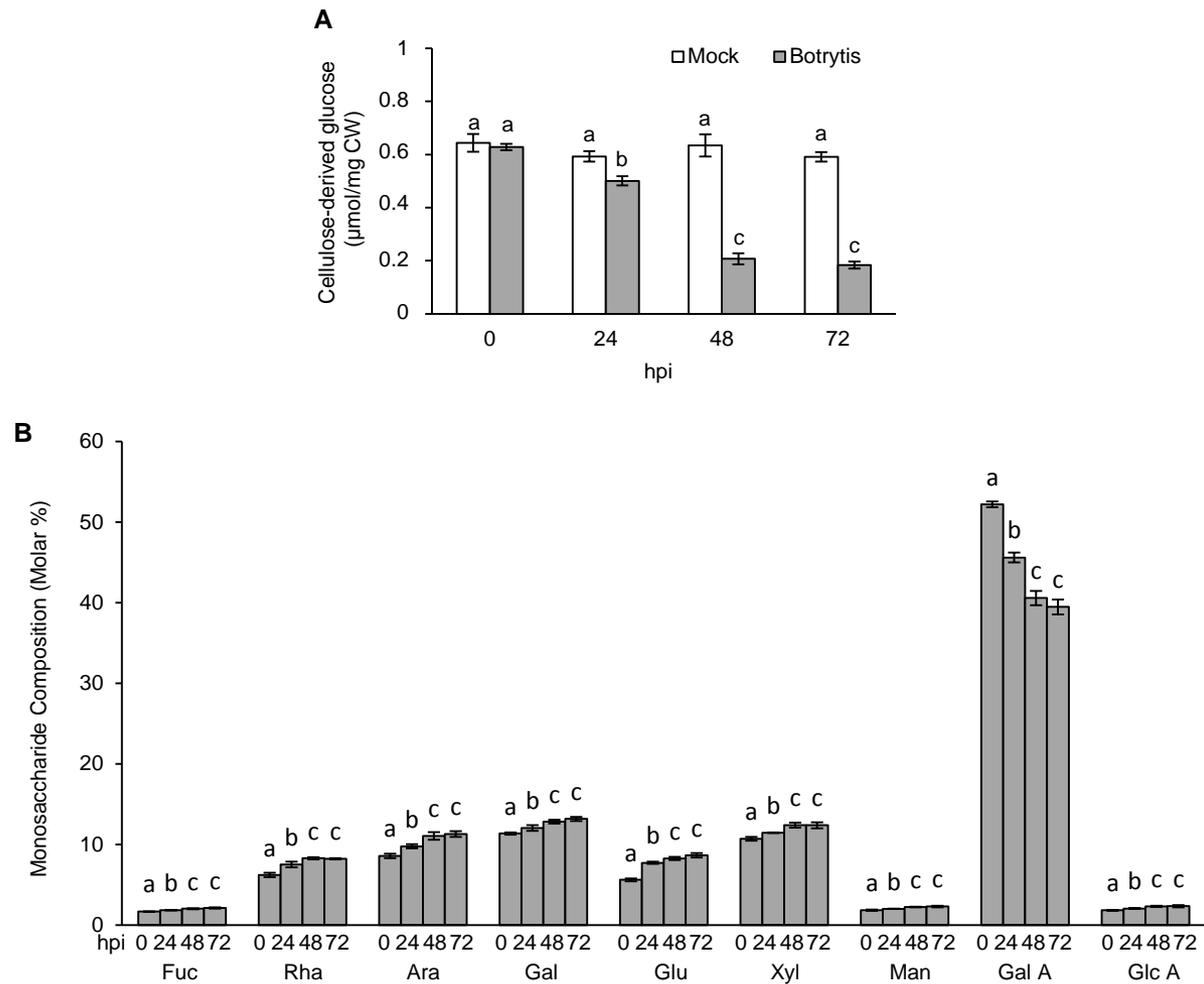


Figure S12. Dynamic modifications of CW in *Arabidopsis* leaves during *Botrytis* infection. The monosaccharide composition of cell wall was monitored in *Arabidopsis* WT leaves challenged with *Botrytis* and mock inoculated leaves. A, Cellulose-derived glucose. B, The molar percentages of fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glu), xylose (Xyl), mannose (Man), galacturonic acid (Gal A) and glucuronic acid (Glc A) released after 2M TFA hydrolysis were quantified by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) system. The results represent the mean \pm SD ($n = 3$). The different letters for each monosaccharide indicate datasets significantly different according to analysis of variance (ANOVA) followed by Tukey's test ($P < 0.05$). The experiments were repeated three times with similar results.

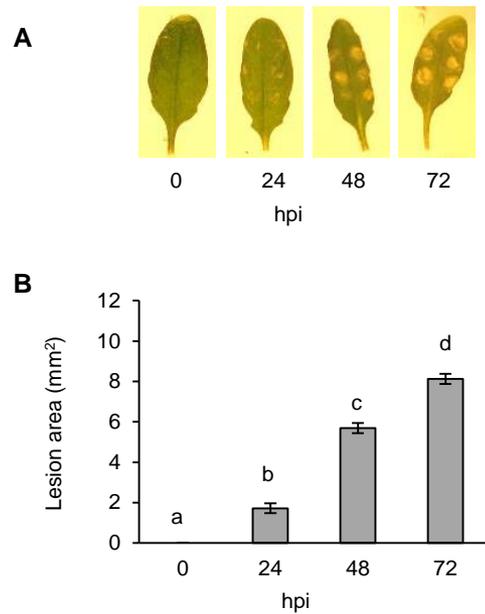


Figure S13. Evaluation of Arabidopsis susceptibility during *B. cinerea* infection. A, Representative pictures of *Arabidopsis* WT leaves challenged with *Botrytis* at different hours post inoculation (hpi). B, Measurements of lesion areas at different hpi. The values represent the mean \pm SD (n = 36). The different letters indicate datasets significantly different according to analysis of variance (ANOVA) followed by Tukey's test ($P < 0.05$). This experiment was repeated three times with similar results.

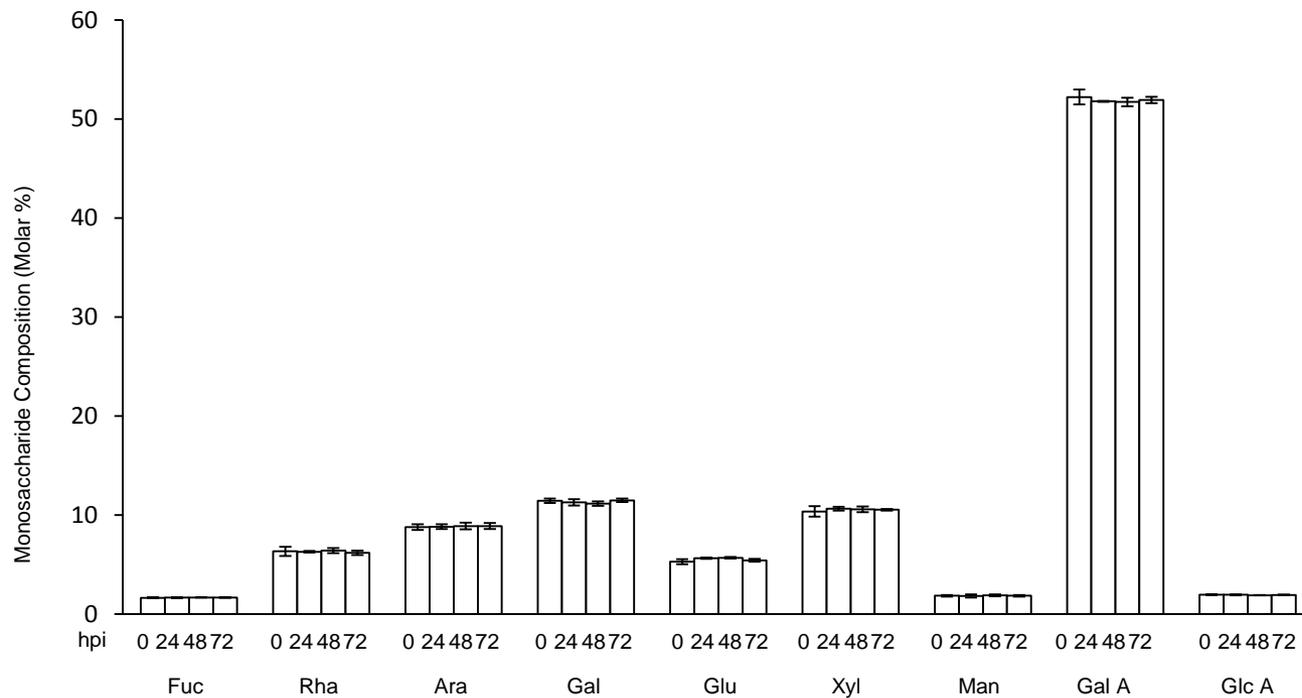


Figure S14. Monosaccharide composition at different times points of CW of mock inoculated leaves of *Arabidopsis WT* plants. The molar percentages of fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glu), xylose (Xyl), mannose (Man), galacturonic acid (Gal A) and glucuronic acid (Glc A) released after 2M TFA hydrolysis were quantified by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) system. The values are mean \pm SD ($n = 3$). Not significantly differences were observed for each monosaccharide according to analysis of variance (ANOVA) followed by Tukey's test ($P > 0.05$). hpi = hours post-inoculation. The experiment was repeated three times with similar results.

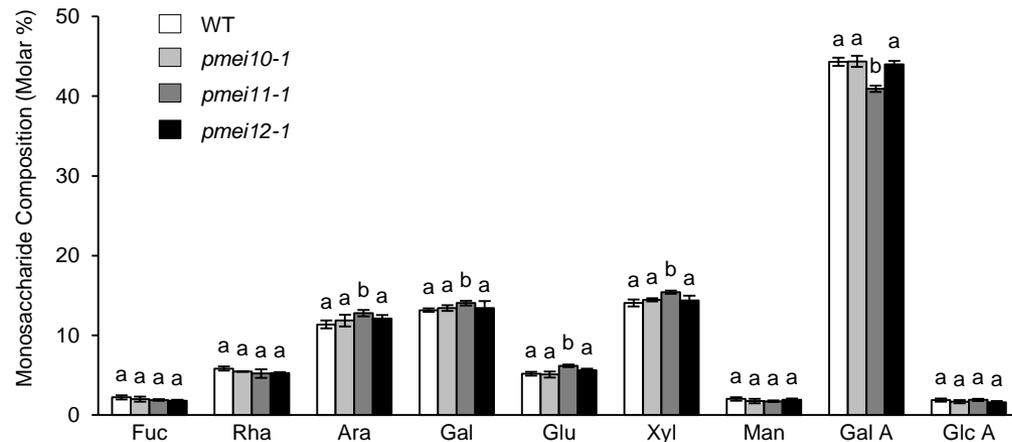
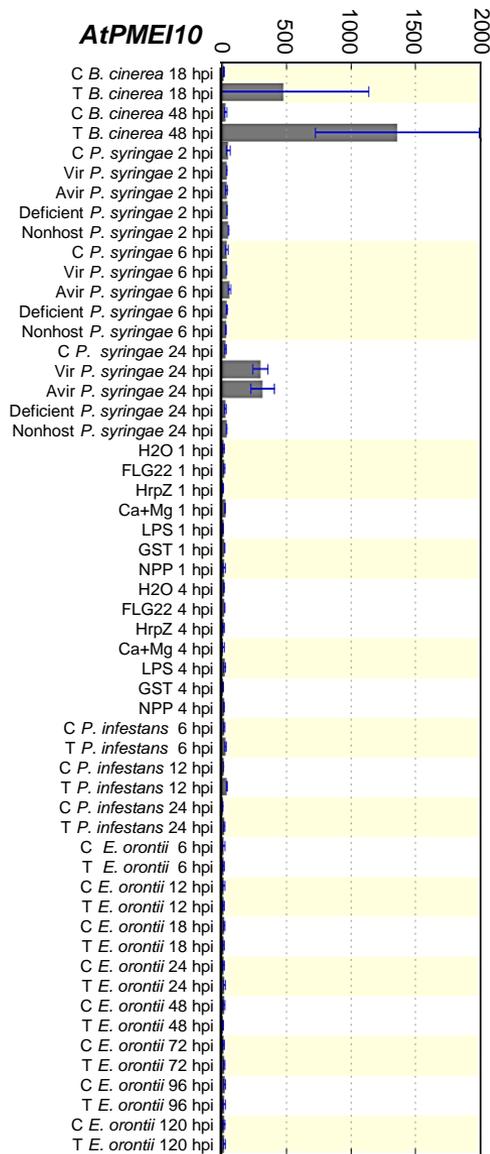
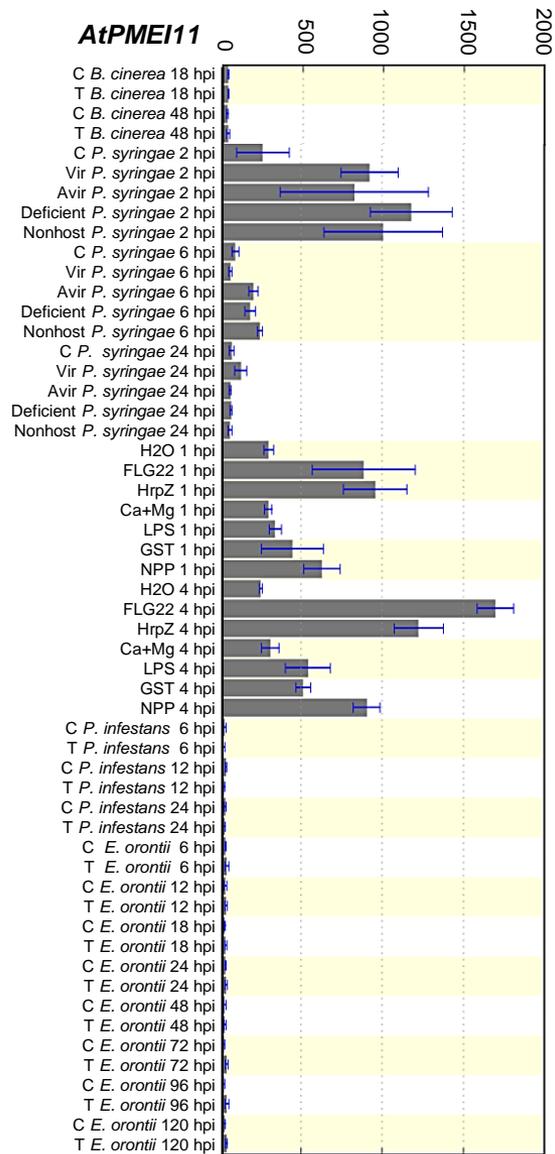


Figure S15. Monosaccharide composition of CW of mock inoculated leaves of *Arabidopsis* WT and *pmei* mutants. The monosaccharide composition of matricial cell wall polysaccharides was monitored in mock-inoculated leaves of six weeks-old *Arabidopsis* WT and *pmei* mutants. The molar percentages of fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glu), xylose (Xyl), mannose (Man), galacturonic acid (Gal A) and glucuronic acid (Glc A) released after 2M TFA hydrolysis were quantified by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) system. The results represent the mean \pm SD ($n = 3$). The different letters for each monosaccharide indicate datasets significantly different according to analysis of variance (ANOVA) followed by Tukey's test ($P < 0.05$). The experiments were repeated three times with similar results.

GCOS expression signal (TGT=100, Bkg=20)



GCOS expression signal (TGT=100, Bkg=20)



GCOS expression signal (TGT=100, Bkg=20)

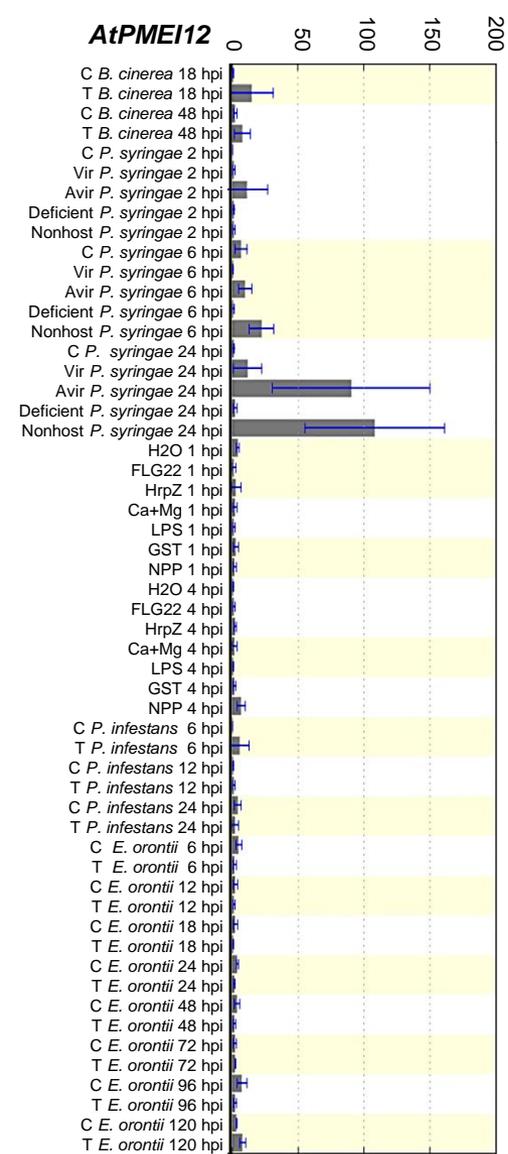


Figure S16. AtPMEI10, AtPMEI11 and AtPMEI12 gene expression during infection of Arabidopsis with different pathogens and follow treatment with several elicitors. ATH1 microarray data were gathered and processed using the eFP browser (Winter et al., 2007). Expression signals were generated by the eFP browser using the Gene Chip Operating Signal (GCOS) using target intensity (TGT) and background (bkg) values as indicated in figure. C; Control. T; Treatment. Vir; Virulent. Avir; Avirulent.

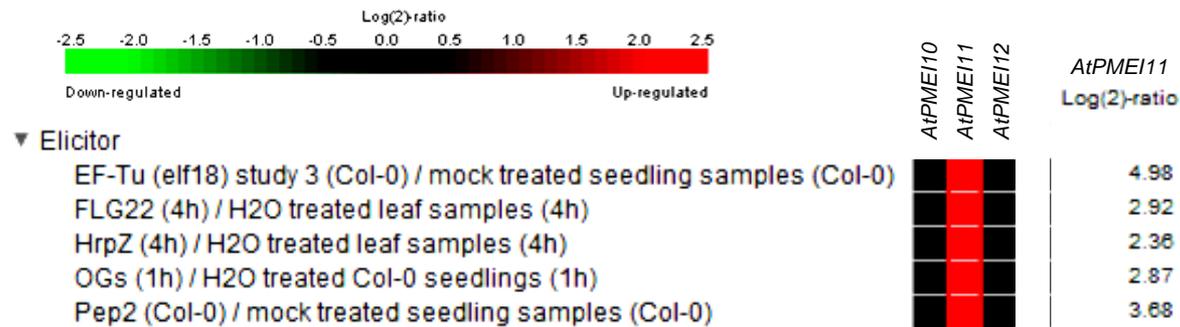


Figure S17. Expression pattern of *AtPMEI10*, *AtPMEI11* and *AtPMEI12* mRNA in *Arabidopsis* in response to different PAMPs, effectors and DAMPs. Elongation factor-thermo-unstable (EF-Tu - elf18), flagellin (flg22). Effector; HrpZ. DAMPs; oligogalacturonides (OGs) and Pep2. Data were analyzed using the Geneinvestigator Meta-Analyzer Tools (www.geneinvestigator.ethz.ch/at/). The specific expression level of *AtPMEI11* is indicated as Log(2)-ratio in treated plants relative to corresponding control.

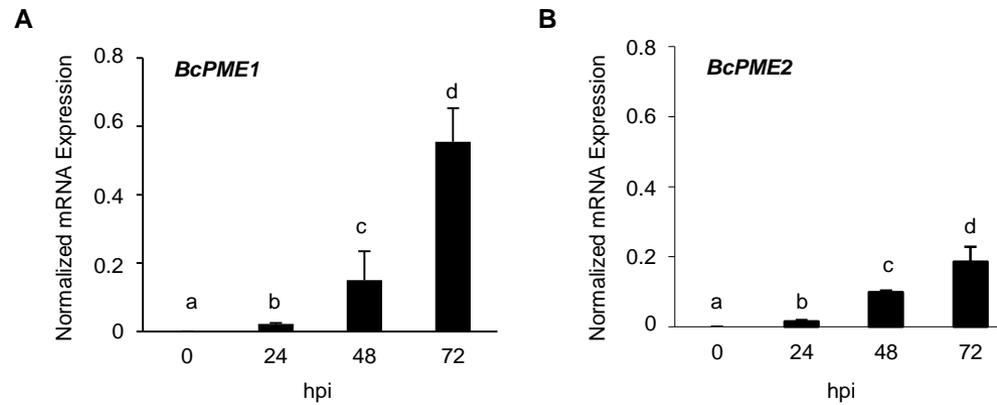


Figure S18. Level and kinetic of expression of *Botrytis* PMEs during infection. The expression of fungal *BcPME1* (A) and *BcPME2* (B) genes in *Arabidopsis* WT infected leaves was analysed by qPCR at 24, 48 and 72 hour post infection. In the figure the expression levels of each genes normalized to the *Botrytis Bc β-tubulin 2* expression is reported. The values represent the mean \pm SD (n = 6). The different letters indicate datasets significantly different according to analysis of variance (ANOVA) followed by Tukey's test ($P < 0.01$).