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e-Xtra*

Pectin Methylesterase Is Induced in *Arabidopsis* upon Infection and Is Necessary for a Successful Colonization by Necrotrophic Pathogens

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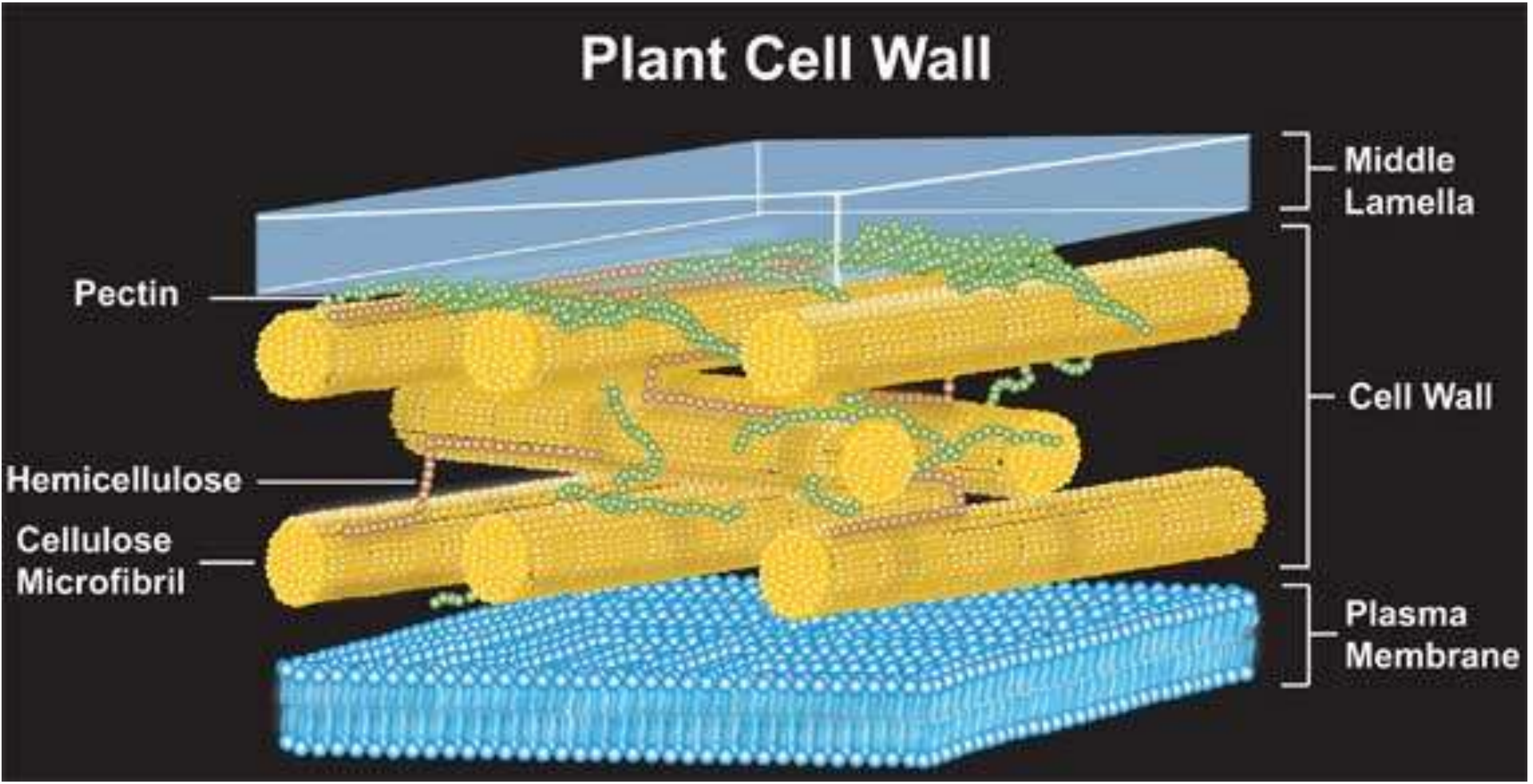
INTRODUÇÃO



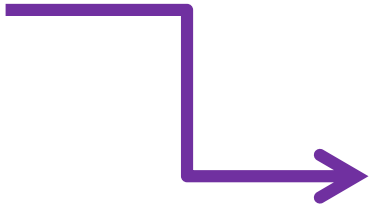
Fonte: fwcdscience.com

INTRODUÇÃO

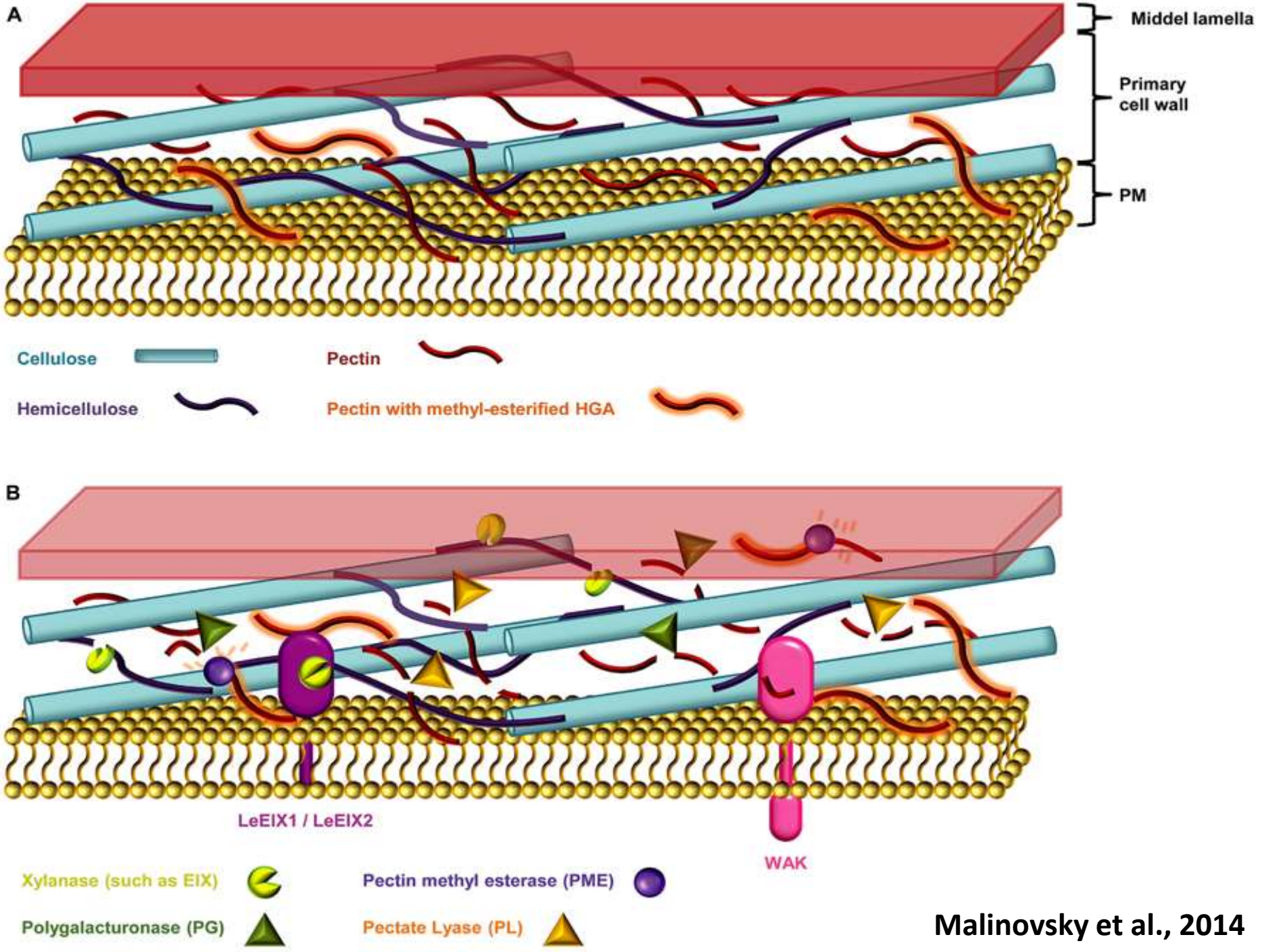
Homogalacturonana (HGA)

Pectina metilesterase (PME)

De-esterificação de HGA



Porosidade e alteração de pH



Malinovsky et al., 2014

INTRODUÇÃO

- *Botrytis cinerea*
- *Pectobacterium carotovorum (Erwinia carotovora)*
- Fatores de virulência: Pectinases e EndoPG
- Necessidade de PME

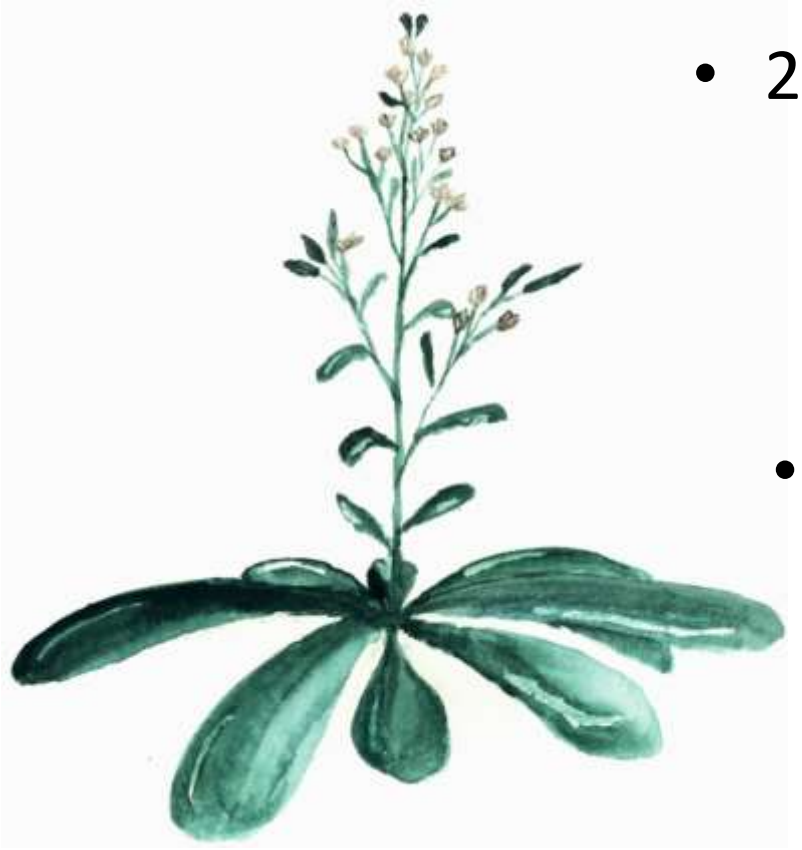
Objetivos

Investigar a indução de AtPME3 em *Arabdopsis* infectada por dois patógenos necrotróficos e a necessidade da expressão deste gene para a colonização.

Material e métodos

- Wyld-type
- Overexpressing AtPMEI-1 ou AtPMEI-2
- *pme3* knock-out mutant

Material e métodos



- 12 folhas

- 2 x 5 µL de suspensão de esporos (5×10^5 conídios/mL)
  - Diâmetro da lesão por 3 dias

- 5 µL suspensão de bactérias (5×10^7 UFC/mL)
  - Área das lesões 16 hpi

Material e métodos

- Grau de esterificação (DM)
- Composição de monossacarídeos
- Análise por immunodot (PAM1 e JIM5)
- Expressão gênica por RT-PCR



RESULTADOS

Expression of AtPME3 induced upon infection

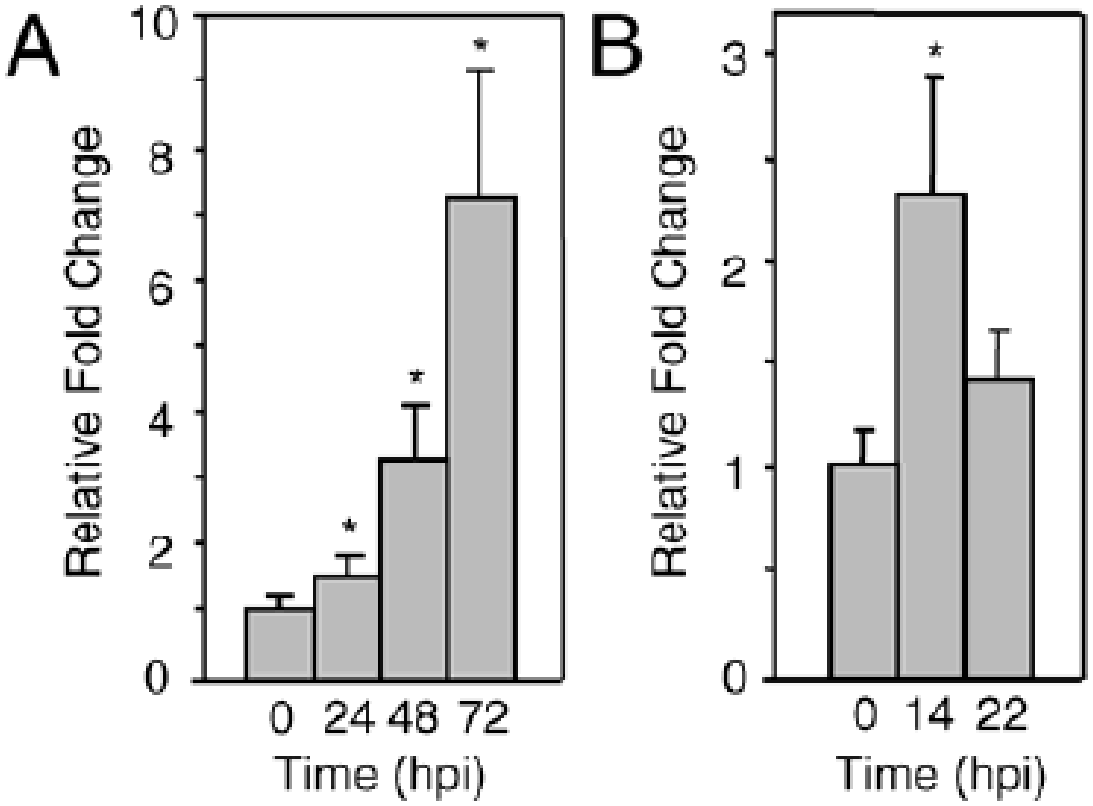
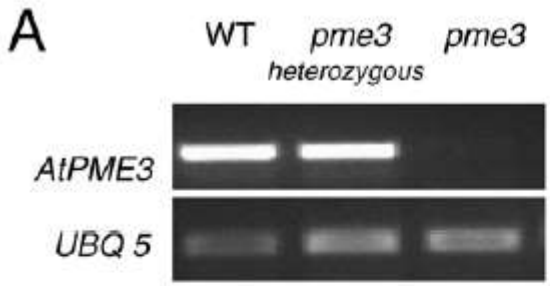
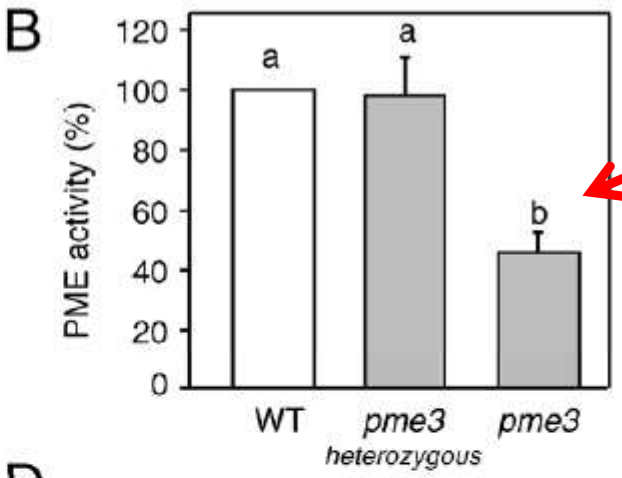


Fig 1. Expressão de AtPME3 após inoculação de **(A)** *B. cinerea* e **(B)** *P. carotovorum* em wyld-type (WT) de *A. thaliana*

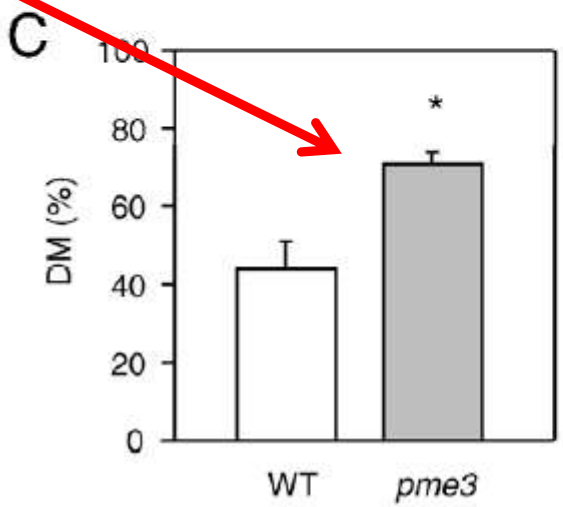
AtPME3 expression influences pectin methylesterification of the cell walls

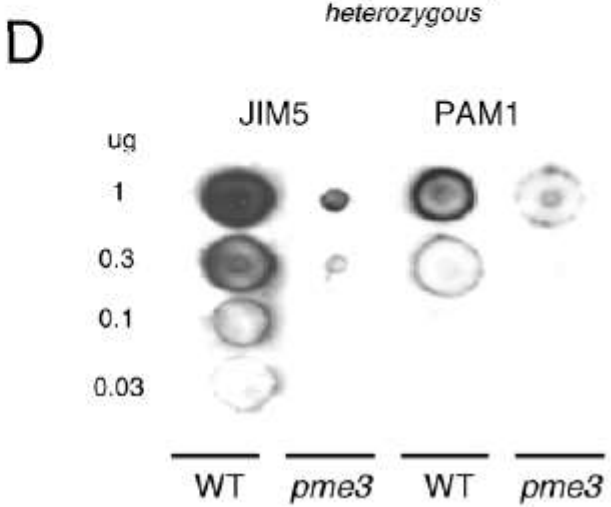




- 50%

+ 40%





Composição dos monossacarídeos dos três fenótipos de *A. thaliana*

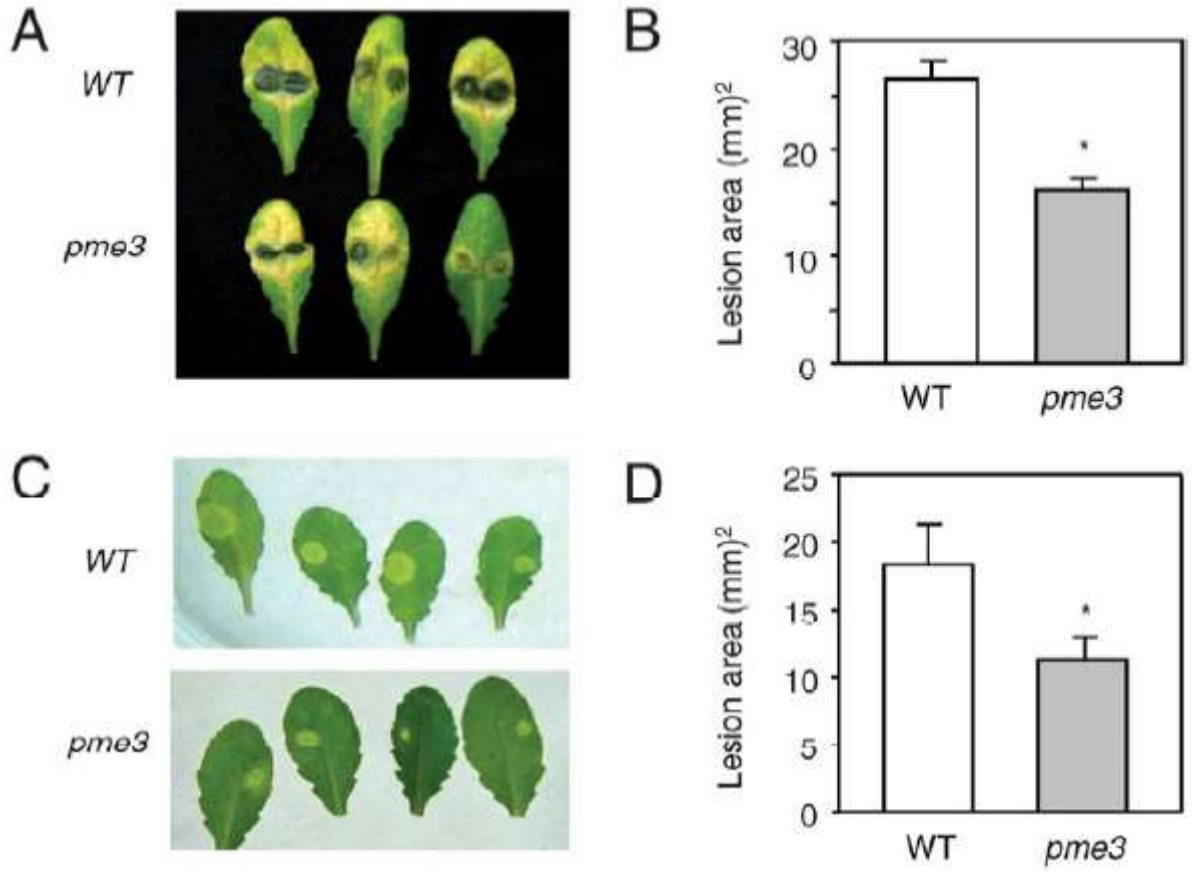
Table 1. Percentage of neutral sugars and uronic acid composition in cell walls extracted from rosette leaves of *pme3* and *Arabidopsis thaliana* pectin methylesterase inhibitor (AtPMEI) plants compared with the wild type (WT)[a]

	Transformant mol%/WT mol% × 100		
Monosaccharide	***pme3***	**AtPMEI-1**	**AtPMEI-2**
Rha	74 ± 26	94 ± 38	99 ± 20
Fuc	126 ± 18	96 ± 15	104 ± 11
Ara	112 ± 19	109 ± 33	103 ± 34
Xyl	115 ± 13	92 ± 19	107 ± 11
Man	103 ± 19	81 ± 16	100 ± 22
Gal	110 ± 16	97 ± 7	105 ± 13
Glc	102 ± 36	105 ± 25	95 ± 18
UA	86 ± 24	106 ± 29	95 ± 12

[a] Molecular percentage of *pme3* and AtPMEI cell wall monosaccharides was normalized with respect to WT. Data represent percent cell wall glycosyl residue composition ± standard deviation (WT $n = 8$, *pme3* $n = 11$, AtPMEI $n = 6$). Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acids. Differences in monosaccharides of *pme3* and AtPMEI plants compared with WT were not statistically different according to analysis of variance followed by Tukey's test.

Alta DM da pectina é o que determina a redução suscetibilidade

Susceptibility of *pme3* to *B.cinerea* and *P.carotovorum*



B. cinerea

P. carotovorum

Redução no desenvolvimento dos patógenos em plantas *pme3*

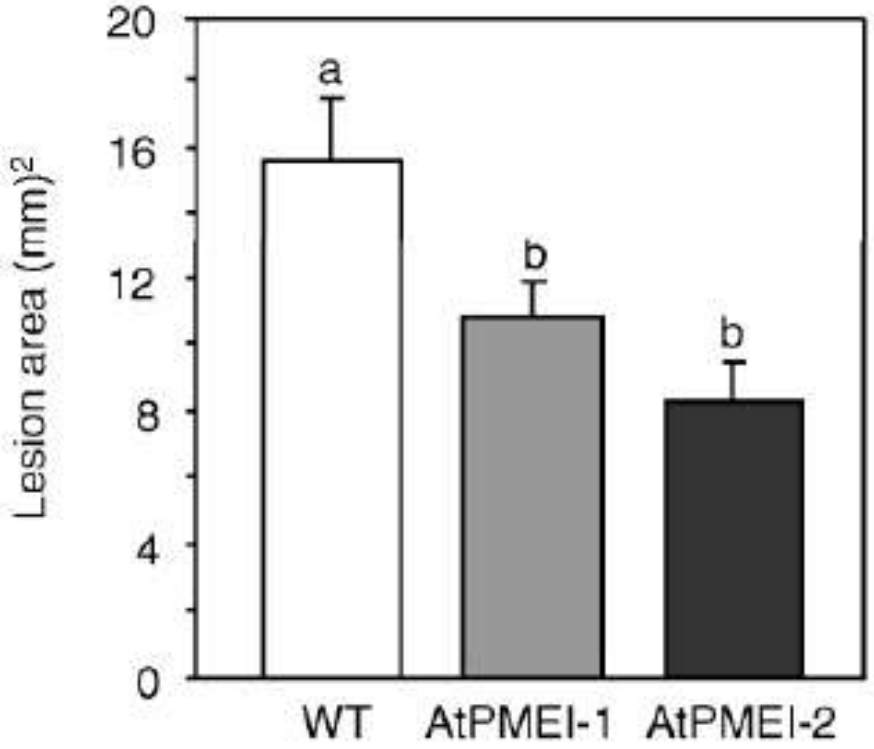


Fig. 4. *Pectobacterium carotovorum* symptoms were reduced in *Arabidopsis* plants overexpressing *AtPMEI-1* or *AtPMEI-2* (AtPMEI-1 or AtPMEI-2 plants). Bar represents the average lesion area ± standard error of 12 expanding lesions determined 16 h postinoculation. The experiment was repeated three times with similar results. Different letters indicate data sets significantly different according to analysis of variance (ANOVA) followed by Tukey's test ($P < 0.01$). Bacterial growth in planta was quantified by determining the number of bacteria isolated from leaves by plate count. Average of logarithm of CFU per leaf ± standard deviation are the following: wild-type (WT), 6.98 ± 0.08; AtPMEI-1, 6.62 ± 0.07; AtPMEI-2, 6.48 ± 0.05. Reduction of bacterial growth on AtPMEI-1 and AtPMEI-2 leaves with respect to WT leaves is significantly different according to ANOVA followed by Tukey's test ($P < 0.01$).

Menor suscetibilidade aos patógenos em plantas com superexpressão de inibidores de AtPME3

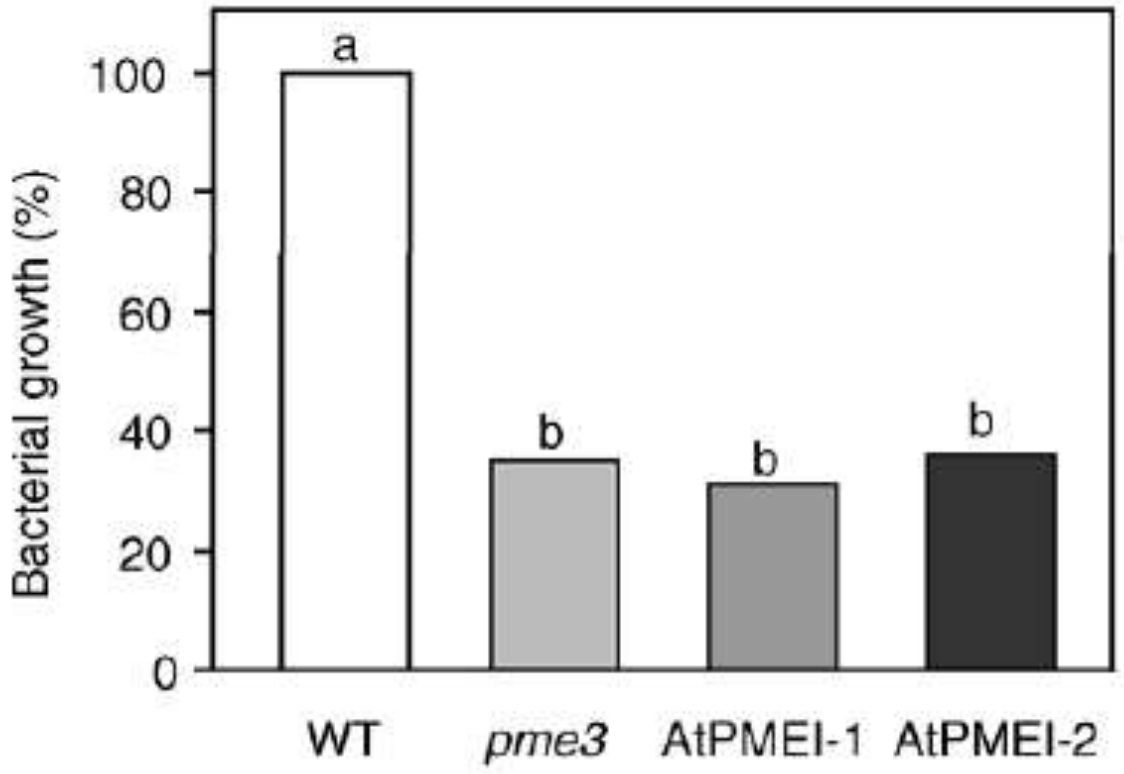


Fig. 6. Reduction of bacterial growth on cell walls isolated from transformed AtPMEI-1, AtPMEI-2, and *pme3* plants. Bars represent relative percentage of growth and different letters indicate data sets significantly different ($P < 0.01$) according to analysis of variance followed by least significant difference range test. Number of bacterial colonies was determined by 1,000-fold dilution from three independent experiments, with $n = 3$.

Redução do crescimento da bactéria em parede celular de plantas transformadas

Reduced suscepbility of *pme3* correlates with an impaired ability of pathogens to grow on methylesterified pectin

- Redução da suscetibilidade é consequência de indução de respostas de defesa?

- Sem acúmulo de H_2O_2 e metabólitos secundários

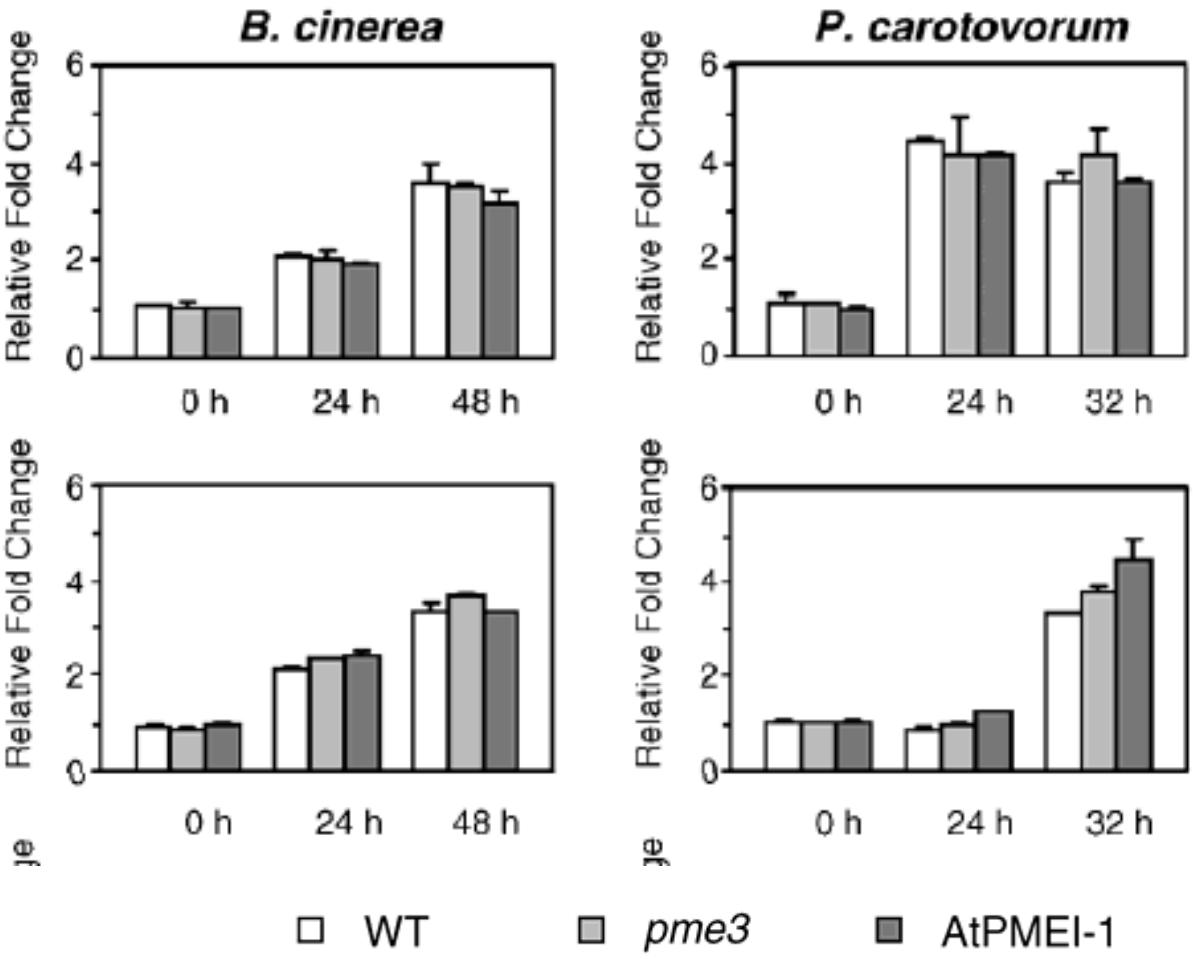
Enhanced activation of –SA, -JA, -ET pathways in *WT, pme3* and AtPMEI-1

PDF1.2

Codifica indicador de ativação da via JA/ET

PR1

Marcador de respostas dependentes de SA



PAD3

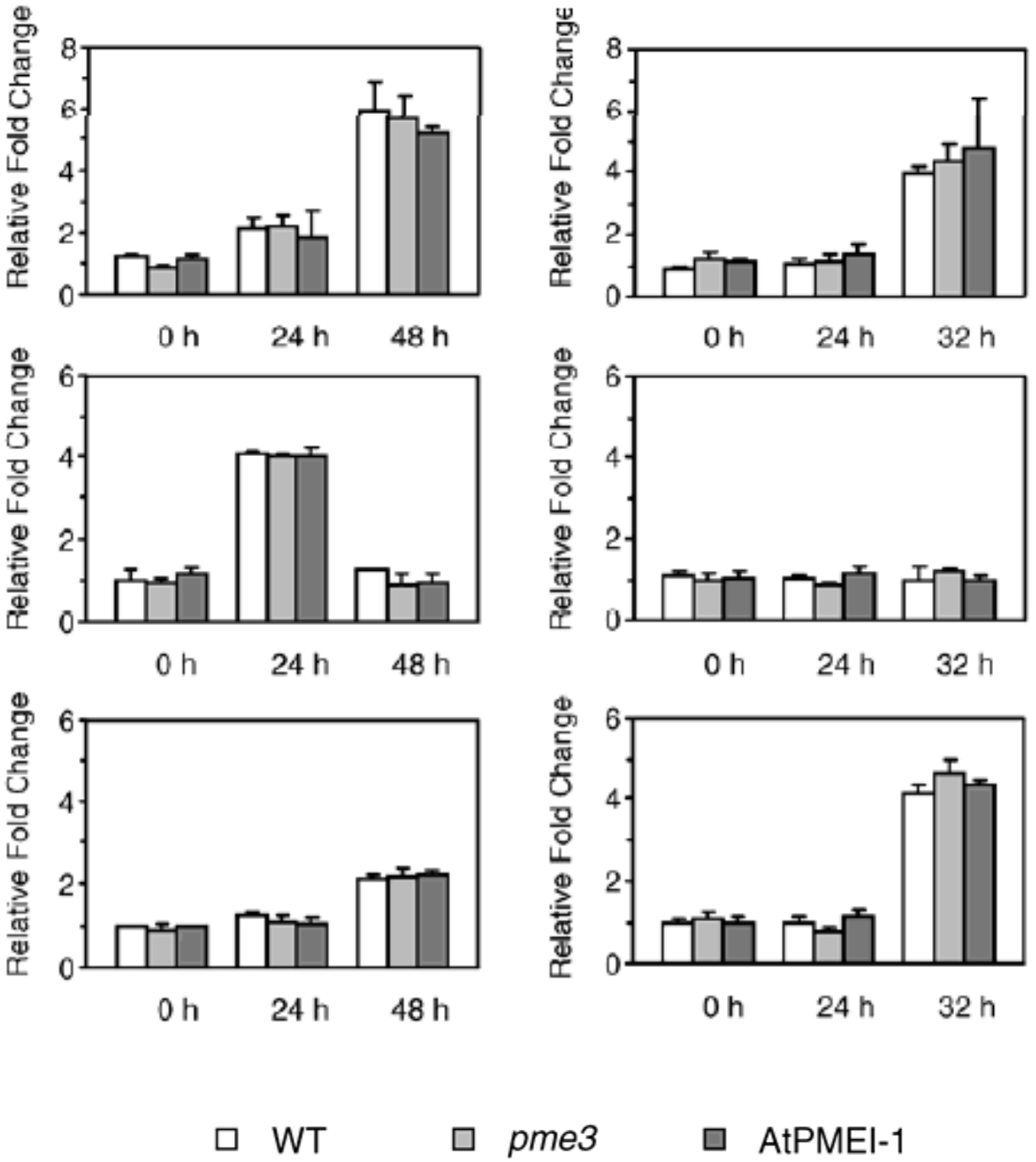
Codifica citocromo (P450) requerido para síntese de camalexina

JR1

Envolvido na transdução de sinal JA-dependente, em resposta a injúria

WR3

Transportador envolvido na transdução de sinal JA-dependente, em resposta a injúria



CONCLUSÕES

- AtPME3 é um fator de suscetibilidade requerido para rápida colonização do hospedeiro pelos patógenos *B. cinerea* e *P. carovotorum*

- Isoformas de PME podem contribuir na seleção de cultivares resistentes

Obrigada