# Wounding-Induced Pectin Methylesterases Enhance Banana (*Musa* spp. AAA) Susceptibility to *Fusarium oxysporum* f. sp. *cubense*

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**Supporting Information Table S1-S2, Figs S1–S8, Methods S1-S2 and References**

**Table S1** Primers for quantitative real-time PCR

| Gene | GeneBank number | Primer sequences (Forward/ Reverse) | Product size (bp) |
|---|---|---|---|
| *Ubiquitin* | HQ853254 | GGCACCACAAACAACACAGG<br>AGACGAGCAAGGCTTCCATT | 379 |
| *MaPME1* | FJ264505.1 | CTTTTACCGCAGGGTTGAACTAAG<br>GAAGAAACGTTTTATTCCACACATC | 125 |

**Table S2** Antibodies recognizing different HGs and their antigens

| Antibody | Antigen | Reference |
|---|---|---|
| LM7 | Non-blockwise de-esterification of HG | Mastroberti et al., 2008 |
| 2F4 | Un-esterified/Calcium ion cross-linked HG | Liners et al., 1989 |
| CCRC-M38 | Fully de-esterified HG | Pattathil *et al*., 2010 |
| JIM5 | Partially methyl-esterified HG epitope: unesterified and partially esterified residues (up to 40%) | Knox et al., 1990 |
| LM18 | Low methyl-esterified HG | Verhertbruggen et al., 2009 |
| LM19 | Low methyl-esterified HG | Verhertbruggen et al., 2009 |
| JIM7 | Partially methyl-esterified HG epitope: methyl-esterified residues (up to 80%) | Knox et al., 1990 |
| LM20 | Highly methyl-esterified HG | Pattathil *et al*., 2010 |
| CCRC-M34 | Partially methyl-esterified HG | Pattathil *et al*., 2010 |
| CCRC-M130 | A methyl-esterified epitope on HG | Pattathil *et al*., 2010 |

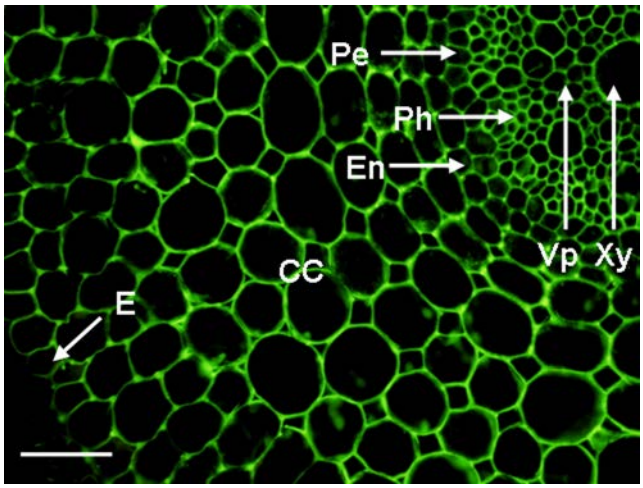



**Figure S1** A transection through banana (*Musa* spp. AAA) root in the elongation zone revealed by immunolabelling using LM18 antibody. Outermost layer of cells represents epidermis. The monolayer of cells encircling vascular cylinder is endodermis. Big parenchyma cells with intercellular spaces between epidermis and endodermis belong to the cortex. The vascular cylinder includes monolayer of pericycle cells and vascular tissue. The holes in the center of the vascular cylinder of the root are xylem elements (including metaxylem and protoxylem). Groups of small cells with dense content, lying between protoxylem elements represent phloem. Between these two vessel elements are vascular parenchyma cells. CC, cortical cells; E, epidermis; En endodermis; Pe, pericycle; Ph, phloem; Xy, xylem, Vp, vascular parenchyma. Bar represents 50 μm.

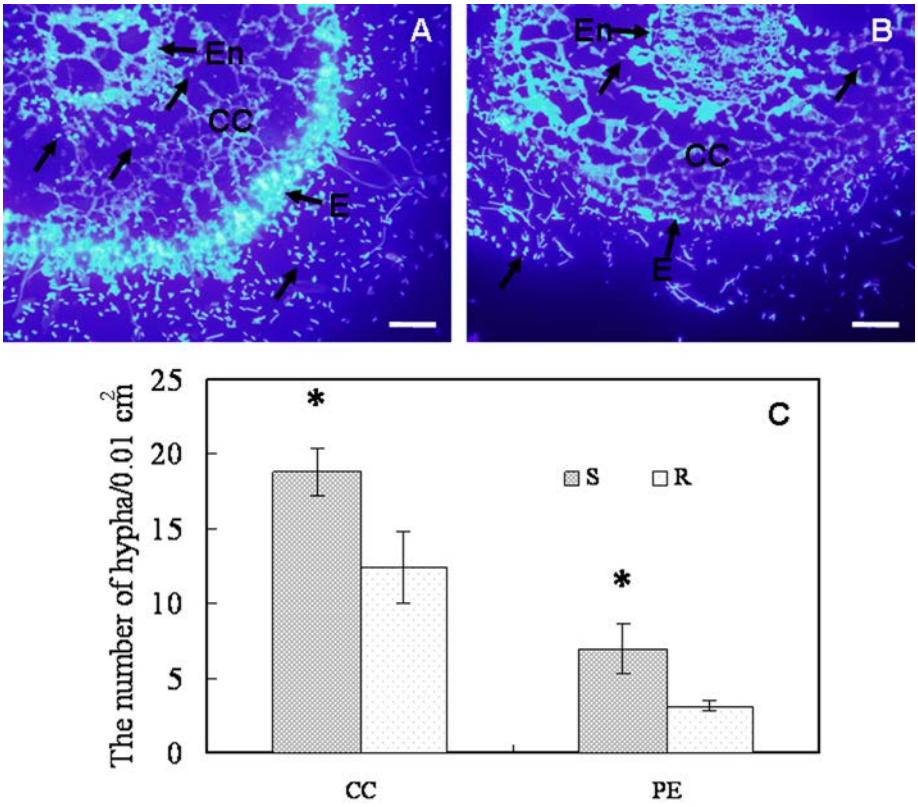



**Figure S2.** The tissue-specific pathogen diffusion in the roots of susceptible (A) and resistant (B) banana cultivar (*Musa* spp. AAA) and the corresponding quantification of the hypha (C) 48 h after infection with *Fusarium oxysporum* f. sp. *cubense*, showing much more hyphae (arrows) in the cortical cells, endodermis, epidermis and the periphery of the epidermis. In all cases cross-sections through roots are presented. CC, cortical cells; E, epidermis; En, endodermis; PE, the periphery of the epidermis; R, resistant cultivar 'Yueyoukang 1'; S, susceptible cultivar 'Brazil'. Bars represent 100 μm.

The quantitative data in the Fig. S2 (C) represents an average of three biological replicates (each was the average of hypha number counted from 10 banana cell area of 0.01 $cm^2$) ± standard deviation. A comparison of groups was conducted using a paired *t*-test of variance. Value marked with a star was considered significant at $P < 0.05$.

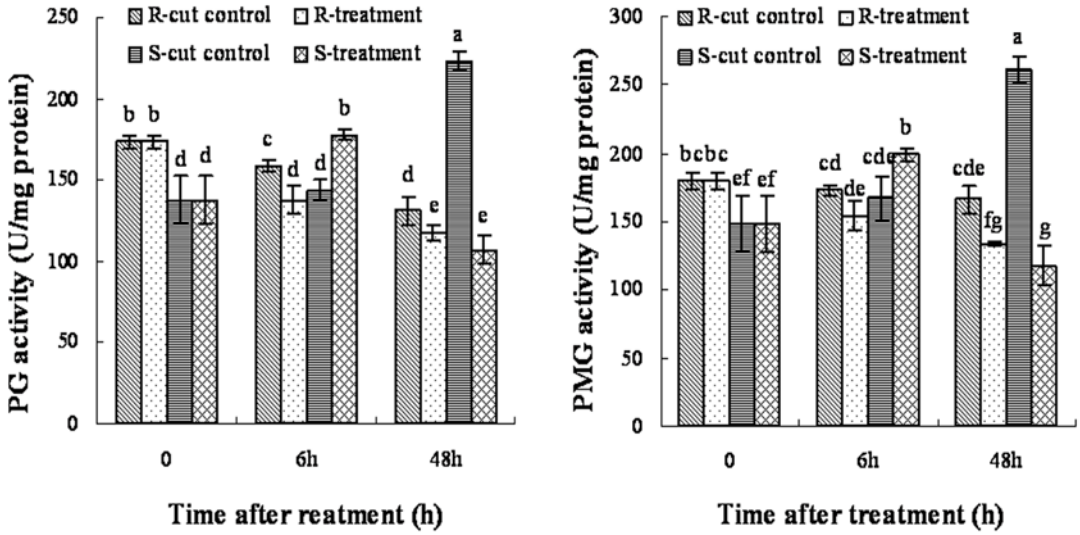



**Figure S3** The changes in PG and PMG activities in banana (*Musa* spp. AAA) after infection with *Fusarium oxysporum* f. sp. *cubense*

0, non-cut control; PG, polygalacturonase; PMG, polymethylgalacturonase; R, resistant cultivar ‘Yueyoukang 1’; S, susceptible cultivar ‘Brazil’. Data represent an average of three replicates ± SD. Values followed by the same letter are not significantly different using Duncan’s multiple range test at $p<0.05$ after angular transformation of the data.

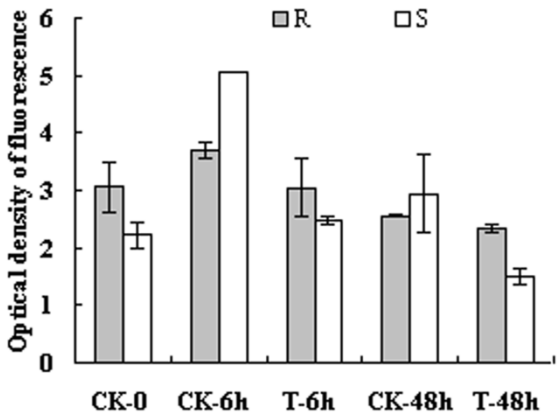
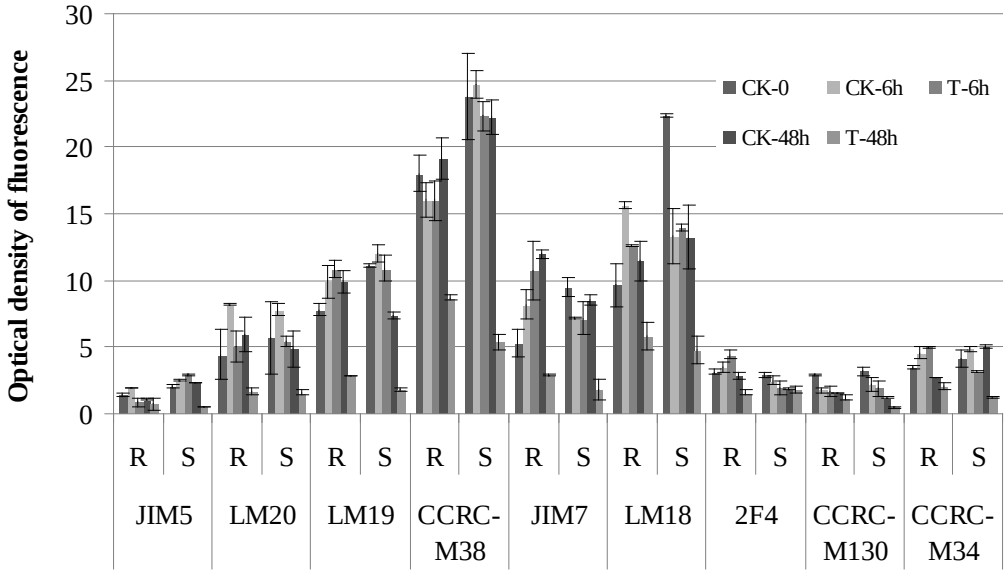



**Figure S4** The optical density of PME fluorescence in banana (*Musa* spp. AAA) roots after infection with *Fusarium oxysporum* f. sp. *cubense*

0, non-cut control; CK, the cut control; PME, pectin methylesterase; R, resistant cultivar ‘Yueyoukang 1’; S, susceptible culitvar ‘Brazil’ ; T, pathogen treatment. For quantification of fluorescence signal, the mean fluorescence intensity (n =3 sections of roots) was measured with Image J 1.44 software.

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172 **Figure S5** The optical density of fluorescence signal of pectins with different degree of
173 methylesterification.

174 0, non-cut control; CK, the cut control; R, resistant cultivar ‘Yueyoukang 1’ (*Musa* spp.
175 AAA); S, susceptible culitvar ‘Brazil’ (*Musa* spp. AAA); T, pathogen treatment. For
176 quantification of fluorescence signal, the mean fluorescence intensity (n =3 sections of
177 roots) was measured with Image J 1.44 software.

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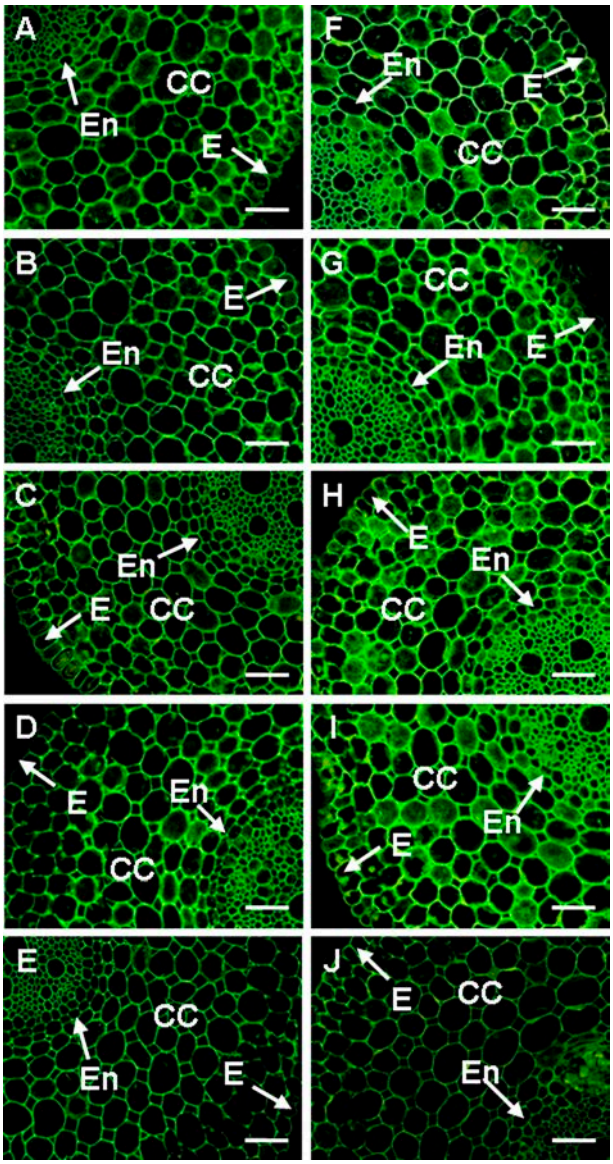


**Figure S6.** The changes in immunolocalization by CCRC-M38 antibody in banana (*Musa* spp. AAA) roots after infection with *Fusarium oxysporum* f. sp. *cubense*. In all cases cross-sections through roots are presented. Resistant cultivar: **A-E**; susceptible cultivar: **F-J**. The immunolabelling observed before the treatment (A, F), six hours after cut (B, G,), six hours after infection (C, H), 48 h after cut (D, I) and 48 after infection (E, J) are presented. CC, cortical cells; E, epidermis; En, endodermis. Bars represent 50 μm.

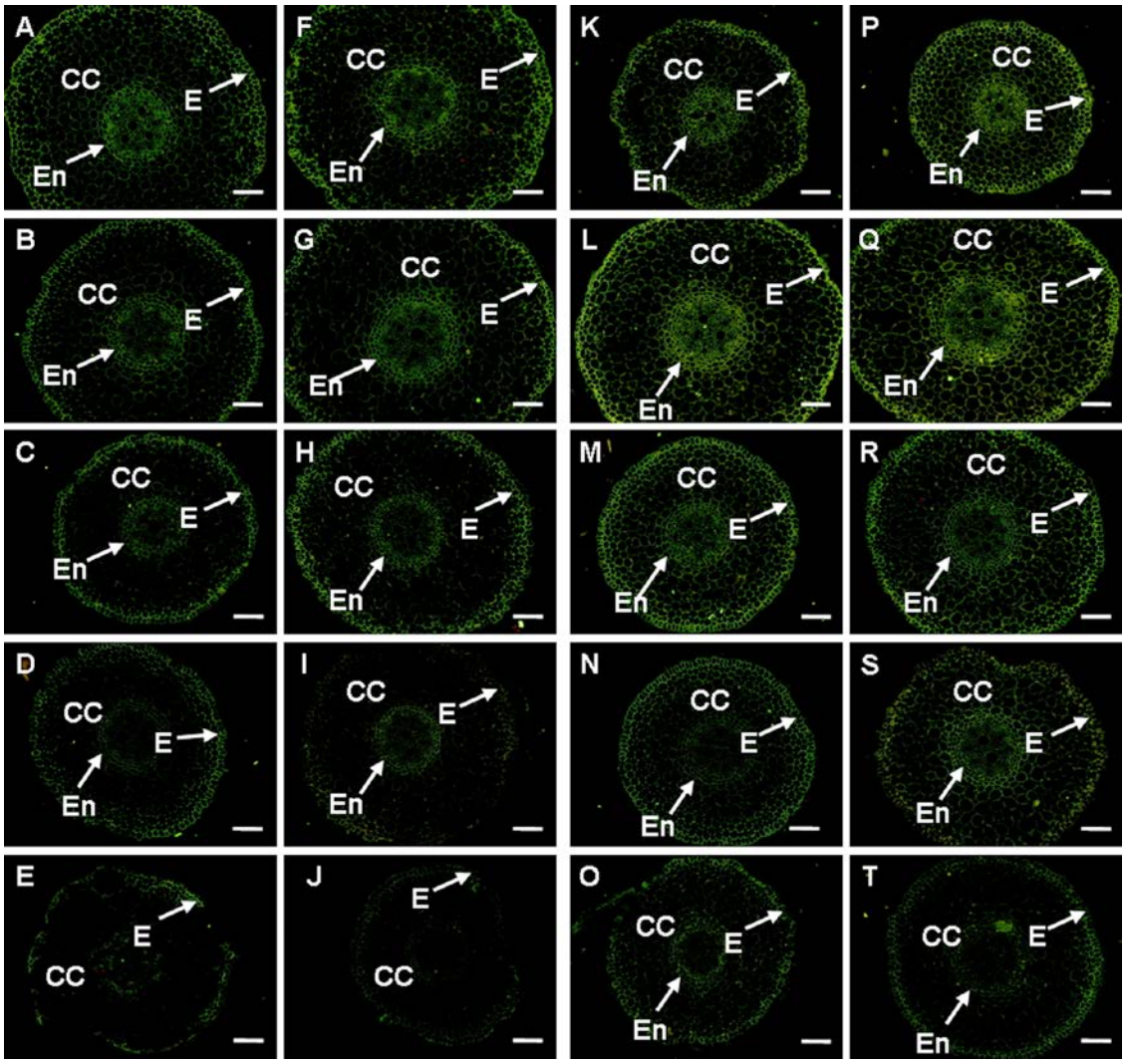


**Figure S7.** The changes in immunolocalization of CCRC-M130 (A-J) and CCRC-M34 (K-T) epitopes in banana (*Musa* spp. AAA) roots after infection with *Fusarium oxysporum* f. sp. *cubense*. In all cases cross-sections through roots are presented. Resistant cultivar: **A-E**, **K-O**; susceptible cultivar: **F-J**, **P-T**. The immunolabelling observed before the treatment (A, F, K, P), six hours after cut (B, G, L, Q), six hours after infection (C, H, M, R), 48 h after cut (D, I, N, S) and 48 after infection (E, J, O, T) are presented. CC, cortical cells; E, epidermis; En, endodermis. Bars represent 100 μm.

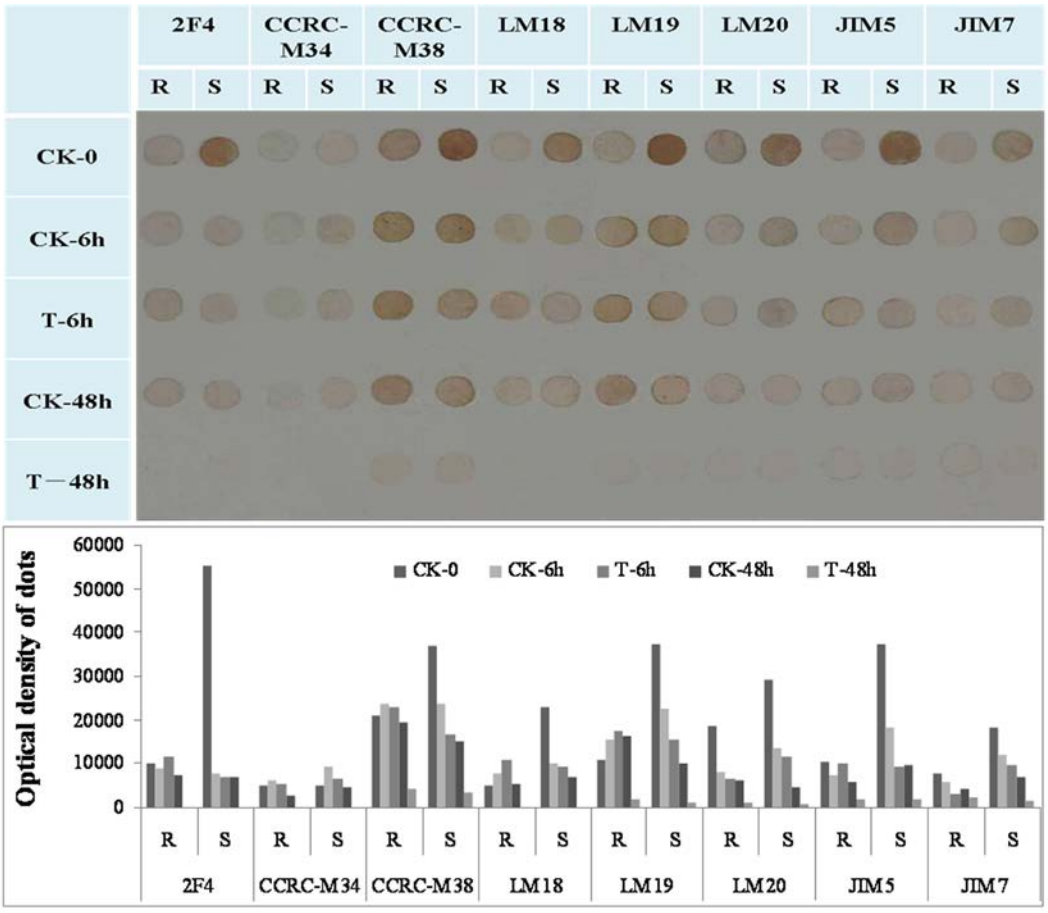


**Figure S8.** Immunodot analysis of changes in the abundance of HGs in banana cultivars (*Musa* spp. AAA) after infection with *Fusarium oxysporum* f. sp. *cubense* CK, the cut control; R, resistant cultivar 'Yueyoukang 1'; S, susceptible culitvar 'Brazil'; T, pathogen treatment. The quantification of dot signal was measured with Image J 1.44 software.

**Methods S1 Enzyme assay**

PG and PMG activities were assayed by measuring the release of reducing sugars by the modified Somogyi method (1952), using mono-D-galacturonic acid as standard. Aliquots of the supernatant (0.1 ml) were added to a 10 ml tube containing 0.3 ml of sodium acetate buffer (0.1 M, pH 5.0) and 0.2 ml of substrate (in sodium acetate buffer, pH 5.0; 0.25% polygalacturonic acid or 1% pectin for PG and PMG, respectively). The mixtures were incubated at 37°C for 30 min. The reaction was stopped by adding 1.5 ml of 3, 5-dinitrosalicylic acid (DNS) and placing the tubes in boiling water bath for 5 min. After cooled to room temperature and constant volume, the absorbance was read in a spectrophotometer at 540 nm against a suitable blank. The enzymatic activity was expressed in terms of Unit (U). One unit of enzyme activity was defined as the amount of enzyme that catalyzes the release of 1 μmol of galacturonic acid per hour under assay conditions. The protein content was determined by the Bradford (1976) method.

**Methods S2 Immunodot assay**

All antibodies used for immunodot assay and their corresponding antigens are presented in Table S2. Anti-mouse IgG (whole molecule)-peroxidase (Sigma A4416) was used as secondary antibody for 2F4 and CCRM antibodies while anti-rat IgG (whole molecule)-peroxidase (Sigma A5795) for all the other antibodies. Pectin was extracted from AIR with 0.5% (w/v) ammonium oxalate buffer at 100°C and the concentration was adjusted to 1mg/ml. Samples (5μl) were spotted onto nitrocellulose membrane by a micropipette. The membrane with dots was air dried at RT for 1h. Assays with antibodies JIM5, JIM7, LM7, LM18, LM19 and LM20, CCRC-M34 and CCRC-M38 were carried out as described by Willats *et al*. (2001). T/Ca/S buffer was used to replace PBS for the assay with 2F4 antibody. After the final wash, the membrane was developed by using 3, 3´-diaminobenzidine tetrahydrochloride kit (TCI Development Co., Ltd, Shanghai).

**References:**

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