ROLE OF CELL WALL ON TOMATO FRUIT SUSCEPTIBILITY TO CALCIUM DEFICIENCY DISORDER

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INTRODUCTION

Blossom-end rot (BER) is believed to be a Ca^{2+} deficiency disorder in tomato fruit (White & Broadley, 2003). However, no threshold of fruit Ca^{2+} concentration has been used to accurately predict BER (Saure, 2001). This suggests that BER may also be triggered by abnormal cellular Ca^{2+} partitioning and distribution that leads to a cellularly localized Ca^{2+} deficiency. Since the cell wall represents 60 to 75% of the total tissue Ca^{2+} content, it plays an important role in cellular Ca^{2+} partitioning and distribution (De Freitas et al., 2010) and possibly in fruit susceptibility to BER development. The objectives of this study were to evaluate the effect of Ca^{2+} binding to the cell wall on fruit susceptibility to BER.

MATERIALS AND METHODS

Wild type and *PME*-silenced tomato plants (*Solanum lycopersicum*) cultivar Rutgers were grown in 9.5 L pots containing 0.3 kg of perlite as substrate in a greenhouse environment. The *PME*-silenced plants (line 3781^) contain two copies of a *PME* type I antisense nucleotide sequence (GenBank: U70676.1) under the control of the cauliflower mosaic virus 35S promoter. Both wild type and *PME*-silenced plants were irrigated every day with a nutrient solution containing N (102 mg L^{-1}), P (26 mg L^{-1}), K (124 mg L^{-1}), Ca^{2+} (90 mg L^{-1}), Mg^{2+} (24 mg L^{-1}), S (16 mg L^{-1}), Fe (1.6 mg L^{-1}), Mn (0.27 mg L^{-1}), Cu (0.16 mg L^{-1}), Zn (0.12 mg L^{-1}), B (0.26 mg L^{-1}), and Mo (0.016 mg L^{-1}). After tagging and manually pollinating the flowers at full bloom, the plants were irrigated everyday with the same nutrient solution, but without Ca^{2+}. There were four replications with four plants each for wild type and *PME*-silenced plants. Fruit from the first and second clusters on each plant were harvested and analyzed at 15, 30, and 45 DAP. All tissue analyses were accomplished in fruit without visible BER symptoms using blossom end tissue. Fruit were analyzed for BER incidence, electrolyte leakage of pericarp tissue, *PME* expression, Ca^{2+} concentrations in pericarp tissue and soluble and insoluble pectins. Statistical differences between wild type and *PME*-silence tomato samples were calculated with one-tailed unpaired Student's t-test. P-values <0.05 were considered significant. Data are presented as means ± standard error (SE).

RESULTS AND DISCUSSION

Throughout fruit growth and development, *PME*-silenced fruit had a lower BER incidence and electrolyte leakage of pericarp tissue than wild type fruit tissue (Figure 1A, 1B). While more than 80 % of WT fruit exhibited BER, only about 30% of *PME*-silenced fruits were affected by this disorder by the time fruit reached full size (45 DAP). Higher electrolyte leakage is associated to higher tissue susceptibility to BER (Saure, 2001).

The expression of the six *PME* genes PMEU1, LOC544090, LOC544289, Les.9028, Les.10790, and Les.10560 in the wild type pericarp tissue increased 62, 491, 220, 77, 40, and 57 fold, respectively, from 15 DAP to 45 DAP (Figure 2). *PME*-silenced fruit also showed increased expression of all six *PME* genes during growth and development. However, expression of PMEU1, LOC544090, LOC544289, Les.9028, Les.10790, and Les.10560 were 48, 474, 214, 63, 18, and 42 fold lower in *PME*-silenced fruit, respectively, than in wild type fruit at 45 DAP (Figure 2).

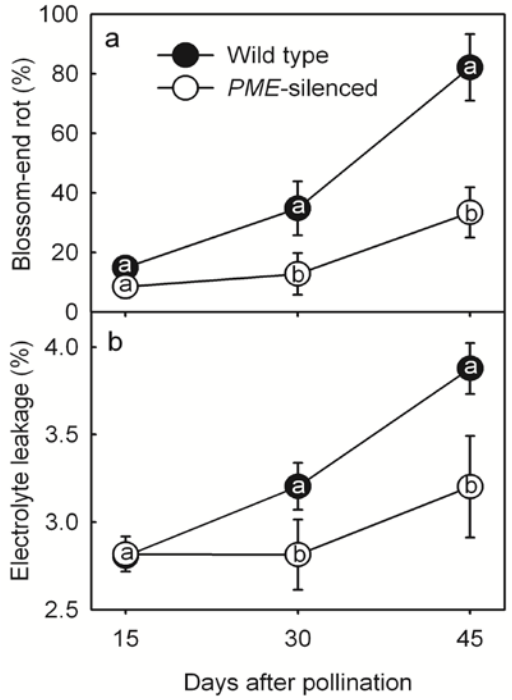

Figure 1. Blossom-end rot incidence (a) and electrolyte leakage of pericarp tissue (b) of wild type and *PME*-silenced tomato fruit cultivar Rutgers. Different letters on each day represent statistical difference between wild type and *PME*-silenced samples (P-value < 0.05). Data are means ± SE.

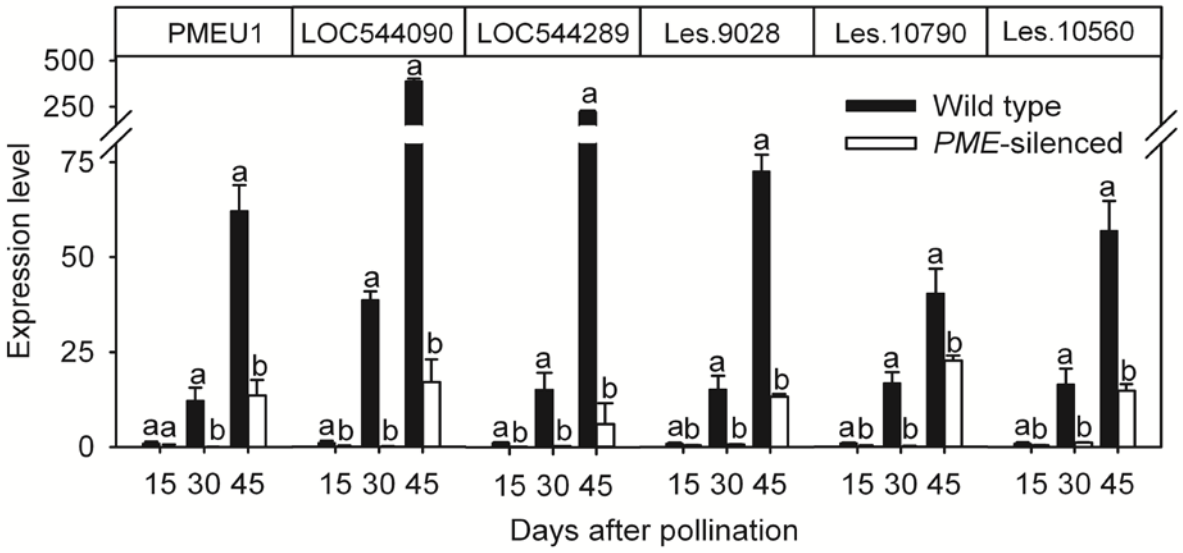

Figure 2. Changes in *PME* expression during wild type and *PME*-silenced fruit growth and development. Different letters on each day represent statistical difference between wild type and *PME*-silenced samples (P-value < 0.05). Data are means ± SE.

The total concentrations of Ca^{2+} in the pericarp tissues of wild type and *PME*-silenced fruit decreased from 15 to 45 DAP, but the values for each fruit type were similar at each developmental time point (Figure 3A). The *PME*-silenced fruit pericarp showed a slightly lower Ca^{2+} concentration in the water soluble pectin fraction than wild type fruit at 45 DAP (Figure 3B). The Ca^{2+} concentrations in the water insoluble pectin were similar in wild type and *PME*-silenced fruit pericarp at 15 DAP and increased steadily from 30 to 45 DAP in wild type fruit while the Ca^{2+} concentration remained unchanged in the *PME*-silenced fruit (Figure 3C). These results show that higher cell wall Ca^{2+} binding capacity due to higher *PME* expression increase fruit susceptibility to BER. Accordingly, plants that have more binding sites for Ca^{2+} in the cell wall are known to require higher levels of Ca^{2+} for normal growth and development. For instance, dicotyledeonous plants require more Ca^{2+} in their tissues than monocotyledonous plants, a phenomenon attributed to the larger cation exchange capacity of their cell walls (Kirkby & Pilbeam, 1984). Therefore, susceptibility of tomato genotypes to BER development may be determined by the capacity of their cell walls to bind Ca^{2+} during rapid cell expansion and vacuolation under conditions in which fruit Ca^{2+} uptake is restricted.

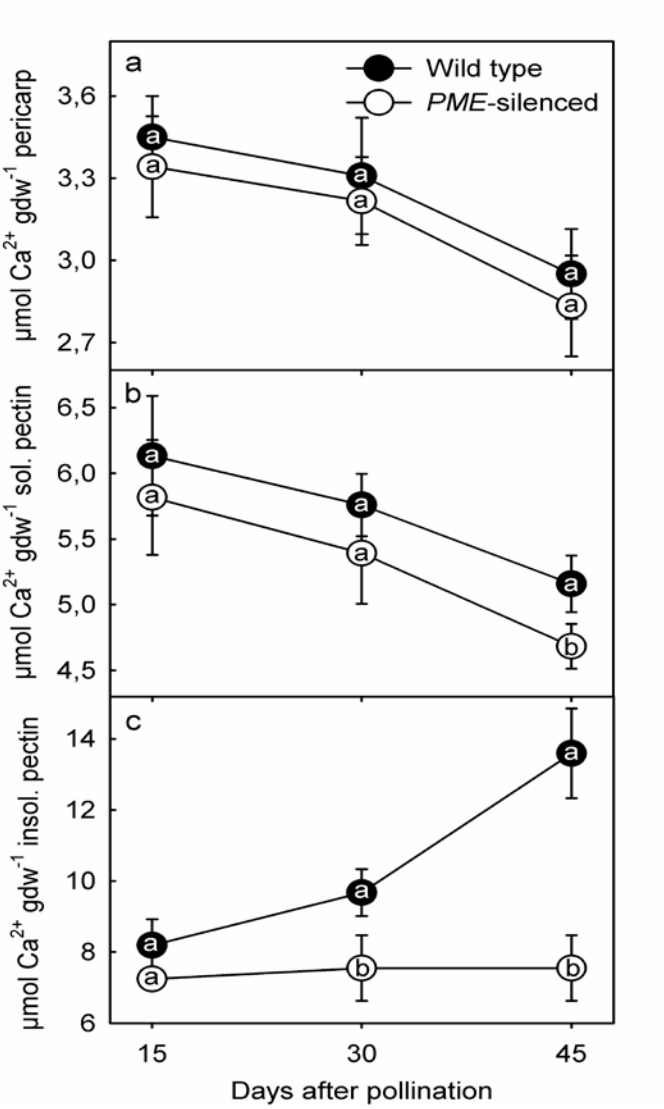

Figure 3. Calcium concentration in pericarp tissue (a), as well as in water soluble pectin (b) and water insoluble pectin (c) fractions extracted from pericarp tissue of wild type and *PME*-silenced tomato fruit cultivar Rutgers. gdw = grams of dry weight. sol. = soluble, insol. = insoluble. Different letters on each day represent statistical difference between wild type and *PME*-silenced samples (P-value < 0.05). Data are means ± SE.

CONCLUSIONS

Suppressing expression of *PMEs* in tomato fruit reduces the amount of Ca^{2+} bound to the cell wall.

Decreasing Ca^{2+} binding to the cell wall decreases fruit tissue susceptibility to BER.

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