Partial purification of tomato fruit peroxidase and its effect on the mechanical properties of tomato fruit skin

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Abstract

Peroxidase (EC 1.11.1.7)-mediated stiffening of cell walls within the fruit skin of tomato is hypothesized to regulate fruit growth. However, to date, there is no experimental evidence demonstrating that peroxidase affects the mechanical properties of skin tissue. Here, the mechanical properties of skin strips excised from a range of fruits at different ages were determined using an ‘Instron’ universal material testing instrument. The stiffness of tomato fruit skin strips increases 3-fold with increasing fruit age. Application of partially-purified peroxidase from the cell walls of mature tomato fruit skin significantly increased the stiffness of fruit skin irrespective of the age of fruit. Furthermore, the application of hydrogen peroxide significantly increased the stiffness of skin strips excised from fruit of an age when endogenous peroxidase isozymes associated with the termination of growth are first detected. The results support the hypothesis that the tomato fruit skin plays an integral role in the regulation of tomato fruit growth, and that changes in its mechanical properties may be mediated by peroxidase. As far as is known, this is the first demonstration that peroxidases alter the mechanical properties of the plant cell wall.

Key words: Cell wall, fruit growth, Lycopersicon esculentum, peroxidase, stiffening, tomato.

Introduction

Peroxidase-mediated stiffening of cell walls within the exocarp (skin) of tomato was hypothesized to be the mechanism by which turgor-driven expansion within the fruit mesocarp (fruit ‘flesh’), and hence fruit growth is controlled (Thompson et al., 1998; Andrews et al., 2002). Tomato fruits follow a sigmoidal pattern of growth. An initial short lag phase is followed by a phase of rapid fruit expansion, after which the rate of growth declines approaching maturation (Monselise et al., 1978). An increase in peroxidase activity has been observed with increasing fruit age, coinciding with the appearance of at least three peroxidase isozymes following the phase of rapid fruit expansion (Andrews et al., 2000). The use of near non-isogenic lines of five non-ripening mutants (nor, rin, Nr, Cnr, and Gr) indicated these isozymes were not involved in ripening. Isoelectric focusing showed that these peroxidase isozymes were highly anionic (pI~3) whilst subcellular localization confirmed that they were present in the cell wall of the exocarp in the latter stages of tomato fruit growth (Andrews et al., 2002). However, no conclusive evidence has established a relationship between the mechanical properties of the exocarp of tomato fruit and the presence of these isozymes.

Although peroxidase is thought to mediate changes in the mechanical properties of plant cell walls (Fry, 1986; Thompson et al., 1998; Brownleader et al., 1999; Hatfield et al., 1999; Andrews et al., 2000, 2002; Thompson, 2001), the evidence primarily reflects changes following the application of hydrogen peroxide as a peroxidase hydrogen acceptor (Schopfer, 1996), or peroxidase-mediated cross-linking extensin assays (Schnabelrauch et al., 1996). Little or no evidence is available on changes in the mechanical properties of tissue following direct application of peroxidase. Several hypotheses are currently postulated to explain how peroxidase may stiffen cell walls. The hypotheses include peroxidase catalysed cross-linking of cell wall components resulting in the formation of diferuloyl bridges between pectin residues, and isodityrosine bridges between hydroxyproline-rich extensin molecules (Fry, 1986; Brownleader et al., 1999; Hatfield et al., 1999). The deposition of lignin in the primary cell walls of epidermis from maize seedlings has also been suggested as

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a mechanism by which peroxidase might regulate growth (Müsel et al., 1997; Schopfer et al., 2001). The presence of ‘lignin-like’ phenolics within the epidermis of the tomato fruit exocarp has been demonstrated (Hunt and Baker, 1980; Andrews et al., 2002). However, neither the role of these phenolics in the cell wall nor their relationship to peroxidase isozymes within the fruit exocarp is known.

To assess the hypothesized role of the tomato fruit exocarp in the regulation of tomato fruit growth, the mechanical properties of exocarp strips excised from a range of fruit ages were examined. The effect of peroxidase on the mechanical properties of exocarp strips was measured following the application of ‘wall-bound’ peroxidase extracted from mature fruit exocarp tissue. In order to assess whether changes in the tensile properties of these exocarp strips were due to the enzymic activity of peroxidase, further measurements were made following the application of both hydrogen peroxide and denatured ‘wall-bound’ peroxidase. The results are discussed in relation to the regulation of tomato fruit growth.

Materials and methods

Plant material

Plants of Lycopersicon esculentum Mill. cv. Espero were grown as a conventional long-season glasshouse crop following normal commercial practice (Adams et al., 2001).

Bulk extraction of ‘wall-bound’ peroxidase

The exocarp of 80 mature green tomato fruit (~55 dpa) was excised into a beaker on ice using a scalpel blade. The excocarp sample was immediately frozen in liquid nitrogen before freeze-drying (Edwards Modulyo 4K) for 72 h. Once dry, the sample was powered in a domestic coffee grinder, prior to homogenization in 200 ml ice-cold 10 mM sodium acetate/citric acid buffer at pH 6.0. The resulting exocarp suspension was then filtered under vacuum through a Whatman 541 filter disc. The filtrate represented the soluble (symplastic) fraction and was discarded (Andrews et al., 2000). The residue was resuspended in 200 ml ice-cold 10 mM sodium acetate/citric acid buffer at pH 6.0, prior to vacuum filtration (Whatman 541 filter paper). The filtrate was again discarded, and the residue was put through the same procedure a further five times. Following this washing procedure to remove the soluble peroxidase fraction, the residue was resuspended in 200 ml ice-cold 100 mM sodium acetate/citric acid buffer at pH 6.0 containing 1.0 M sodium chloride. After 2 h of constant agitation, the suspension was again filtered (Whatman 541 filter paper) under vacuum. The resulting filtrate represented the salt-extractable ‘wall-bound’ fraction (Andrews et al., 2002) and was desalted by dialysis at 3 °C for 48 h against regular changes of double-distilled water.

Partial purification of ‘wall-bound’ peroxidase

The dialysed, ‘wall-bound’ fraction was mixed with 30 g of bentonite (Sigma Chemicals, UK) which had been previously hydrated for 12 h at 3 °C in 200 ml of 100 mM MES buffer pH 6.0. After 2 h with constant agitation the ‘wall-bound’/bentonite slurry was centrifuged for 30 min at 8000 g. The resulting supernatant was carefully decanted and immediately dialysed at 3 °C for 48 h against regular changes double-distilled water. Little or no protein precipitation was observed following dialysis, and particular care was taken to ensure maximum recovery of extracts from the dialysis tubing.

The dialysed extract was frozen at −30 °C, prior to freeze-drying (Edwards Modulyo 4K) for 72 h. Once dry the residue was resuspended in 75 ml of distilled water and spun for 30 min at 10 000 g, prior to final dialysis at 3 °C for 24 h against regular changes of double-distilled water. Following dialysis the extract was divided into 5 ml aliquots and freeze-dried (Edwards Modulyo 4K) for 72 h.

Peroxidase and protein assays

Peroxidase activity was determined in a microtitre plate using a microplate reader (Bio-Rad, model 3550UV). The chromogen 3,3',5,5'-tetramethylbenzidine (TMB) was used as an Ames test negative peroxidase substrate (Bos et al., 1981). TMB was dissolved at 20 mg ml⁻¹ in dimethyl sulfoxide (DMSO) and stored in aliquots at −20 °C. A 5 μl aliquot of sample was added to each microtitre well followed by 200 μl of 100 mM sodium acetate/citric acid buffer, pH 6.0, containing 0.1 mg ml⁻¹ TMB and 0.5 μl ml⁻¹ of 6% (w/v) hydrogen peroxide. Peroxidase activity was measured by following the initial increase in A₄₅₀ at 35 °C (Bos et al., 1981; Andrews et al., 2000). A unit of activity was defined as the amount causing an increase of 1 absorbance unit min⁻¹. This was equivalent to 4.16 nmol min⁻¹ using the absorption coefficient (35 800 m⁻¹ cm⁻¹) of the one oxidation state blue complex of TMB (Cattaneo and Luong, 1994).

Protein concentration was determined using bicinchoninic acid (BCA) as the detection reagent for Cu⁺, formed following the reduction of Cu²⁺ by protein in an alkaline environment (BCA³¹⁩ protein assay, Pierce, Rockford, USA.). Measurements were made using a microwell plate protocol A₅₉₀ (as per the manufacturer’s instructions) on a microplate reader (Bio-Rad, model 3550UV). The more commonly used Bradford reaction is not suitable for anionic peroxidases, as the dye Coomassie blue R-250 has a low sensitivity to the acidic amino acids present in anionic proteins (Scopes, 1982).

SDS–PAGE

SDS–PAGE was performed using a Bio-Rad Mini Protein II apparatus (as per the manufacturer’s instructions) with a 12% acrylamide resolving gel and a 4% stacking gel. Prior to loading, duplicate samples from dialysed salt-extractable and post-bentonite treated ‘wall-bound’ fractions were treated with either: cold 1% (w/v) SDS, 10% glycerol (v/v) and 0.002% bromophenol blue or boiled in 1% (w/v) SDS, 1% (v/v) mercaptoethanol, 10% glycerol (v/v), and 0.002% bromophenol blue (Andrews et al., 2000). Once run, the gels were stained for peroxidase activity by immersion in a solution containing 25 ml sodium acetate/citric acid buffer pH 6.0, 15 mg 4-chloro-1-naphthol dissolved in 10 ml methanol and 0.3 ml 6% hydrogen peroxide. Protein detection was visualized using a silver staining protocol for proteins (Plusone, Pharmacia Biotech).

Sample preparation for tensile measurements

Using single-edged, single-bevel blades (Durham-Duplex, type ‘D’) clamped to give a 2 mm gap in a spatial holder, the exocarp of a developmental range of tomato fruits was scored around the equatorial region of the fruits. Eight fruit exocarp strips (40×2 mm) per fruit were excised from a total of five fruits for each of the following approximate age groups 20, 30, 40, 50, and 60 dpa using a scalpel blade. For smaller fruits (~10 dpa) only four strips per fruit could be excised, and for this age group the number of replicate fruits was increased to ten. The average thickness of each strip was approximately 0.5 mm. Each individual exocarp strip was mounted using double-sided adhesive tape onto individual acetate templates (64×18 mm), across a cut-out measuring 20×6 mm (Hole et al., 2000).
 Mounted exocarp strips were frozen at −20 °C for 12 h prior to thawing at 20 °C for 30 min. Preliminary studies indicated that peroxidase penetration into the exocarp strips was limited without this freeze–thaw procedure (data not shown). Freeze–thaw is commonly used in rheological measurements of plant tissue as it eliminates the effects of turgor on tissue extensibility (Thompson, 2001). To prevent desiccation, thawed mounted exocarp strips were placed on moist capillary matting in a plastic container with a close-fitting lid. Prior to fitting the lid, 5 μl of each of the four following solutions were applied to two exocarp strips per fruit (one strip per fruit for ~10 dpa). The four solutions were the four factorial combinations of two levels of partially-purified peroxidase (present or absent), and two levels of hydrogen peroxide (present or absent): (i) 10 mM MES buffer pH 6.0 containing 5 mM CaCl2; (ii) lyophilized partially-purified peroxidase (5 ml original volume) dissolved in 55 μl 10 mM MES buffer pH 6.0 containing 5 mM CaCl2; (iii) 10 mM MES buffer pH 6.0 containing 5 mM CaCl2 and 0.06% H2O2; (iv) lyophilized partially-purified peroxidase dissolve in 55 μl 10 mM MES buffer pH 6.0 containing 5 mM CaCl2 and 0.06% H2O2. Once sealed, the container with samples was incubated at 20 °C for 24 h.

**Tensile measurements**

Following incubation, mounted exocarp strips were selected at random (using pseudo-random numbers) and placed between the pneumatic jaws of an ‘Instron’ universal material testing instrument (model 4301, High Wycombe, UK). The pneumatic jaws of the Instron were pre-set to give an original gauge length of 20 mm. In order to measure the tensile properties of the exocarp strips, both sides of the acetate templates bordering the cut-out were carefully cut with sharp scissors. The strips were then stretched by the Instron in order to measure the tensile properties of the exocarp strips, both sides of the acetate templates bordering the cut-out were carefully cut with sharp scissors. The strips were then stretched by the Instron at a rate of 1 mm minute−1 until tissue failure. Measurements of increasing load (N) and displacement (mm) were used to calculate stress (force/length) and strain (length/length), where stress is force normalized to the cross-sectional area of the exocarp strip and strain is displacement divided by original gauge length. Exocarp stiffness (Young’s modulus), or the resistance to deformation, was automatically calculated as the slope of stress versus strain at the steepest part of the curve (Fig. 1). Preliminary experiments showed that none of the above measurements were correlated to tissue thickness (data not presented). This was probably because thicker tissue had proportionally more, thinner-walled parenchyma cells that would have little effect on the mechanical properties of the exocarp strips. Therefore, the main determinant of exocarp stress measurements is probably cell wall stress in the outer layers of the exocarp. The mean tissue thickness (0.5 mm) was used in all measurements of stress calculated automatically by the Instron. Both the principle and the tensile parameters measured by the Instron were considered to be appropriate for material that has the same growth history and rate of growth at harvest. A similar methodology was previously used to assess the rheological properties of onion skins (Hole et al., 2000).

**Boiled peroxidase application to exocarp strips**

To establish whether changes to the mechanical properties of the exocarp strips on the application of partially-purified ‘wall-bound’ peroxidase were indeed enzymatic, a test was made using boiled extract to denature the protein component of the ‘wall-bound’ fraction. Thirty exocarp strips were prepared from each of five fruit (−35 dpa), as above. The following solutions were applied in 5 μl aliquots to two exocarp strips per solution: (i) 10 mM MES buffer pH 6.0 containing 5 mM CaCl2; (ii) lyophilized partially-purified ‘wall-bound’ extract dissolved in 55 μl 10 mM MES buffer pH 6.0 containing 5 mM CaCl2; (iii) boiled (30 min) lyophilized partially-purified ‘wall-bound’ extract dissolved in 55 μl 10 mM MES buffer pH 6.0 containing 5 mM CaCl2; (iv) lyophilized partially-purified ‘wall-bound’ extract dissolved in 55 μl 10 mM MES buffer pH 6.0 containing 5 mM CaCl2. The mechanical testing procedure was conducted on an Instron as previously described.

**Statistical analysis of tensile measurements**

For each age group (except ~10 dpa) there were five randomly selected proximal position fruit harvested from different plants, with eight exocarp strips excised from each fruit. Each set of eight strips was divided randomly into two sets of four strips and each set of four strips was used as a complete replicate block for the four treatments. As far as possible, the set of four strips in a replicate block were manipulated and measured at the same time and in a similar way to minimize non-treatment variability. The data were analysed by a conventional factorial analysis of variance, with 10 complete replicate blocks for each age group 20–60 dpa. The 10 dpa age fruits were treated in a similar way except that only one set of four strips could be excised from an individual fruit, therefore 10 fruit were used to give 10 complete replicates of each treatment.

**Results**

**Protein purification**

Wall-bound peroxidase isozymes extracted by ionic elution of washed cell walls that would have been substantially free of contamination from readily soluble protein, was further purified by bentonite. This partition led to a purification factor of 12.6 (Table 1), and was determined by the increase in specific activity. SDS–PAGE, demonstrated the removal or reduction of the major contaminating proteins detected by silver staining (indicating protein quantities of less than 1 ng), whilst retaining peroxidase activity (Fig. 2). The four peroxidase isozymes present in both the salt-extractable ‘wall-bound’ fraction and the bentonite-purified ‘wall-bound’ fraction could only be visualized using peroxidase activity stains. The protein level in each of the four peroxidase isozymes was below the detection level of both Coomassie R-250 (Andrews et al., 2000) and silver staining protocols.

**Tensile measurements**

Changes in the mechanical properties of tomato fruit exocarp strips reported here, resulted from the addition of
1.26×10^6 units (specific activity=0.138 units mg⁻¹ protein) of peroxidase activity per exocarp strip. The amount of activity present in an exocarp strip (weighing 10 mg) excised from a typical mature fruit from which the applied peroxidase was purified would be 2.87×10^3 units (specific activity=5.58×10⁻⁵ units mg⁻¹ protein), approximately 44-fold less than the activity applied.

Tensile measurements demonstrated significant changes in the mechanical properties of the tomato fruit exocarp with increasing fruit age. There was a significant (P <0.001) increase in exocarp tissue stiffness (Young’s modulus, Fig. 3A), stress at maximum load (Fig. 3B), and a significant (P <0.001) decrease in strain at maximum load (Fig. 3C). The application of partially-purified peroxidase to exocarp strips significantly (P <0.001) increased the stiffness of the tissue at all ages (Fig. 3A). There was no significant interaction (P >0.05) between the effect of peroxidase and fruit age. Figure 3B, shows that added peroxidase significantly increased (P <0.001) stress at maximum load for fruits older than approximately 30 dpa, the effect was less marked on younger fruits. Figure 3C shows that there was no significant effect (P >0.05) of peroxidase on the strain at maximum load of exocarp strips from fruit <60 dpa. There were no significant changes (P >0.05) in mechanical properties of exocarp strips treated with boiled partially-purified peroxidase (Table 2), showing that the stiffening activity was the consequence of enzymic activity.

In general, the effects of added hydrogen peroxide on the mechanical properties of tomato fruit exocarp strips were less consistent than the effects observed following the addition of partially-purified peroxidase to supplement endogenous peroxidases. Figure 4A, shows that hydrogen peroxide significantly (P <0.05) increased the stiffness of exocarp strips, when averaged for all age groups. However, the effect of hydrogen peroxide was dependent on fruit age (P <0.001); only tissue from fruits aged 30 and older become stiffer following the addition of hydrogen peroxide. Overall, there was no significant effect (P >0.05) of hydrogen peroxide on the stress or strain at maximum load (Fig. 4B, C) for any age of fruit. Furthermore, there appeared to be no significant interaction (P >0.05) between the effects of adding partially-purified peroxidase and hydrogen peroxide. The effect of peroxidase was not dependent on adding hydrogen peroxide and vice versa (data not shown).

**Discussion**

Like most biological processes, fruit growth is unlikely to be controlled by one single parameter. Potential fruit size has been shown to depend on the cell number within the

<table>
<thead>
<tr>
<th>Sample</th>
<th>Original fresh weight (OFWT) (mg×10³)</th>
<th>Volume (ml)</th>
<th>Total protein concentration (mg)</th>
<th>Total peroxidase activity (10⁶ units)</th>
<th>Specific activity (10⁶ units mg⁻¹ protein)</th>
<th>% Recovery</th>
<th>Purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysed salt-extractable 'wall-bound' fraction</td>
<td>475</td>
<td>772</td>
<td>515</td>
<td>1366</td>
<td>2.65</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Post-bentonite 'wall-bound' fraction</td>
<td>475</td>
<td>110</td>
<td>9.13</td>
<td>305</td>
<td>33.4</td>
<td>22.4</td>
<td>12.6</td>
</tr>
</tbody>
</table>

Table 1. Partial purification of peroxidase from the salt extractable ‘wall-bound’ fraction from mature tomato fruit exocarp

Fig. 2. SDS-PAGE analysis of peroxidase. Lanes marked 1 and 2, dialysed salt-extractable ‘wall-bound’ proteins. Lanes 3 and 4 are dialysed salt-extractable ‘wall-bound’ proteins following purification with bentonite. Lanes 1 and 3, proteins were fully denatured (100 °C for 5 min) and silver stained only. Lanes 2 and 4, proteins were not heat-treated, gels were stained for peroxidase activity with 4-chloro-1-naphthol, followed by silver staining. All lanes were loaded with 15 μl of sample buffer, each containing 7.5 μl of sample (Table 1).
developing ovary, whilst final fruit size depends on the expansion of these cells (Ho, 1992). Consequently genes shown to affect cell number within the tomato ovary, may have a direct correlation with final fruit size (Frary et al., 2000; Nesbitt and Tanksley, 2001). However, cell enlargement and final tomato fruit volume is largely determined by the accumulation of water (Ho, 1992). Therefore, one may predict that any restriction on turgor-driven expansion will affect final fruit size. Restrictions on turgor-driven expansion may be either physical, as that hypothesized for the fruit exocarp in the work presented here, or may be due to restrictions in the availability of water or photoassimilates, components that predominantly control turgor-driven growth (Ho, 1992; Bussières, 1993; de Koning, 1994).

Tensile measurements demonstrated a change in the mechanical properties of the tomato fruit exocarp through the course of tomato fruit growth (Fig. 3). The results showed that the extensibility of exocarp strips decreased with fruit age. Hence, as fruits matured an increased force (load) was required to cause the same degree of deformation as observed in exocarp strips from younger fruit. This increased resistance to deformation (stiffness) with fruit age, illustrates the potential of the fruit exocarp to restrict increases in tissue extensibility and also to regulate the rate of growth in maturing tomato fruit.

The potential of peroxidase to mediate changes in the mechanical properties of tissue has, until now, been largely speculative (Fry, 1986; Thompson et al., 1998; Brownleader et al., 1999; Hatfield et al., 1999; Andrews et al., 2000, 2002; Thompson, 2001). Here the potential of partially-purified, highly anionic peroxidases extracted from the cell walls of mature non-growing tomato fruit exocarp to change the mechanical properties of tomato fruit exocarp strips of any age is demonstrated (Fig. 3). These results showed that the maximum tissue extensibility was largely unaffected by the application of peroxidase, but stiffness and the load required to cause such tissue deformation was significantly increased following the application of peroxidase. The capacity of peroxidase to alter the mechanical properties of exocarp tissue supports the hypothesis that peroxidase mediates an increase in exocarp tissue stiffness over the course of fruit growth, restricting expansion in older fruits and, thus, controlling growth.

![Fig. 3. Mean tensile measurements of tomato fruit exocarp strips, excised from a range of fruit ages (10±60 dpa). (A) Young’s modulus (MPa). (B) Stress (MPa) at maximum load. (C) Strain at maximum load. Mean tensile measurement of replicate blocks with (open circles) and without (closed circles) the addition of partially-purified peroxidase. Error bars represent the standard error of the difference between means of each replicate block.](https://academic.oup.com/jxb/article/53/379/2393/512562)

**Table 2. Tensile measurements made on tomato fruit exocarp strips (~35 dpa), following the application of partially-purified peroxidase and boiled partially-purified peroxidase** Measurements of stress, strain from which Young’s modulus is calculated were made at maximum load (N).

<table>
<thead>
<tr>
<th>Tensile measurement</th>
<th>Control</th>
<th>Partially purified peroxidase</th>
<th>Boiled partially purified peroxidase</th>
<th>s.e.d</th>
<th>Degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young’s modulus (MPa)</td>
<td>70.2</td>
<td>81.6</td>
<td>72.8</td>
<td>2.780</td>
<td>17</td>
</tr>
<tr>
<td>Stress at maximum load (MPa)</td>
<td>8.98</td>
<td>9.52</td>
<td>8.93</td>
<td>0.679</td>
<td>18</td>
</tr>
<tr>
<td>Strain at maximum load (mm mm⁻¹)</td>
<td>0.155</td>
<td>0.130</td>
<td>0.154</td>
<td>0.013</td>
<td>18</td>
</tr>
</tbody>
</table>
The extraction and purification procedures employed have ensured that peroxidase is the main component of the partially-purified extract although the possibility cannot be ruled out that other enzymes may contribute to changes in stiffness. Furthermore, the response to hydrogen peroxide (Fig. 4) is consistent with peroxidase activity being responsible for the changes to the mechanical properties of the exocarp tissue. The application of hydrogen peroxide increased the stiffness of excocarp tissue excised from tomato fruits of 30 dpa and older. Thirty dpa is the fruit age at which the first of the three endogenous peroxidase isozymes (53, 48 and 43 kDa) previously implicated in the restriction of tomato fruit growth (Andrews et al., 2000), normally become detectable. The effect of adding partially-purified peroxidase and hydrogen peroxide were independent of each other. Therefore, the effect of one did not rely on the application of the other, indicating that endogenous hydrogen peroxide was not limiting, a result largely consistent with the lack of effect of added hydrogen peroxide (Fig. 4).

Tensile measurements strengthen the hypothesis that the fruit exocarp provides a physical constraint to turgor-driven growth. Furthermore, these observations suggest that peroxidases may play an intrinsic and important role in the regulation of tomato fruit growth through peroxidase catalysed stiffening of the fruit exocarp in the latter stages of fruit development, limiting further fruit growth.

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References


Fig. 4. Mean tensile measurements of tomato fruit exocarp strips, excised from a range of fruit ages (10–60 dpa). (A) Young’s modulus (MPa). (B) Stress (MPa) at maximum load. (C) Strain at maximum load. Mean tensile measurement of replicate blocks with (open circles) and without (closed circles) the addition of 0.06% hydrogen peroxide. Error bars represent the standard error of the difference between means of each replicate block.


