

## Simultaneous Transgenic Suppression of LePG and LeExp1 Influences Fruit Texture and Juice Viscosity in a Fresh Market Tomato Variety

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Tomatoes are grown for fresh consumption or for processing of the fruit. Some ripening-associated processes of the fruit can either contribute to or degrade attributes associated with both fresh and processing quality. For example, cell wall disassembly is associated with loss of fresh fruit firmness as well as with loss of processed tomato product viscosity. Several enzymes contribute to cell wall polysaccharide disassembly. Polygalacturonase (PG, poly[1,4- $\alpha$ -D-galactouronide] glucanohydrolase, EC 3.2.1.15) is among the most abundant polysaccharide hydrolases in ripening tomato fruit and is the major contributor to pectin depolymerization. Expansin (LeExp1) is also abundant in ripening fruit and is proposed to contribute to cell wall disassembly by nonhydrolytic activity, possibly by increasing substrate accessibility to other enzymes. Suppression of either *LePG* or *LeExp1* expression alone results in altered softening and/or shelf life characteristics. To test whether simultaneous suppression of both *LePG* and *LeExp1* expression influences fruit texture in additive or synergistic ways, transgenic *Lycopersicon esculentum* var. Ailsa Craig lines with reduced expression of either *LePG* or *LeExp1* were crossed. Fruits from the third generation of progeny, homozygous for both transgenic constructs, were analyzed for firmness and other quality traits during ripening on or off the vine. In field-grown transgenic tomato fruit, suppression of *LeExp1* or *LePG* alone did not significantly increase fruit firmness. However, fruits suppressed for both *LePG* and *LeExp1* expression were significantly firmer throughout ripening and were less susceptible to deterioration during long-term storage. Juice prepared from the transgenic tomato fruit with reduced *LePG* and *LeExp1* expression was more viscous than juice prepared from control fruit.

**KEYWORDS:** Tomato; *Lycopersicon esculentum*; fruit; texture; juice; ripening; cell wall; viscosity; polygalacturonase; expansin

### INTRODUCTION

The quality of fresh ripe tomato fruit is affected by environmental and genetic factors. The fruit ripening process results in biochemical changes that enhance fresh fruit quality, such as lycopene accumulation and development of flavor volatiles. However, the ripening process also initiates degradative processes, such as fruit softening and membrane breakdown, that ultimately lead to fruit senescence and limit the useful shelf life of the fruit. The process of fruit softening includes the changes in tissue texture that are, at least in part, consequences of alterations in the architecture of cell wall polysaccharides (1–3). Changes in cell wall architecture include modifications

and depolymerization of the polysaccharides as well as rearrangements of their associations. Enzymatic polysaccharide depolymerization is accomplished by a number of hydrolases in the cell wall, including polygalacturonases (PGs, poly[1,4- $\alpha$ -D-galactouronide] glucanohydrolase, EC 3.2.1.15), pectin methyl esterases, pectate lyases, and glycosidases, including galactosidases, arabinosidases, and mannosidases (1, 4). Associations and rearrangements between polysaccharide polymers may influence the access that hydrolases have to their substrates and also may alter hydration within the wall structure. The cell wall protein, expansin, is hypothesized to be involved with changes in polysaccharide associations and rearrangements during fruit softening, as well as in other developmental processes that involve the interactions between cellulose microfibrils and the interspersed hemicellulosic network in the cell wall (5–11).

Modification of the expression of a single cell wall hydrolase or expansin gene reduces, but does not eliminate, fruit softening

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suggesting that individual cell wall hydrolases or expansins are not independently responsible for fruit softening (8, 12, 13). Rather, it has been proposed that hydrolases and expansins may act cooperatively to carry out ripening-associated cell wall disassembly (7). This is consistent with the conclusion that coordinated expression of many genes is required to carry out multiple ripening-associated processes, including fruit softening (14). Thus, it is possible that altering the expression of multiple cell wall modifying functions simultaneously may increase their impact on fruit softening and texture.

PG activity in tomato fruit, encoded by the ripening regulated tomato fruit PG gene, *LePG*, is responsible for much of the depolymerization of pectic homogalacturonans once they are deesterified (2, 3), but *LePG* alone is neither necessary nor sufficient for most of the changes required for tomato fruit softening. Secondary characteristics related to postharvest quality of the fruit, however, are improved by reduced *LePG* expression (12, 15–19). Antisense suppression of *LePG* expression resulted in fruit that are more firm at the overripe stage when depolymerization of covalently bound pectins is reduced (20, 21). Expansins cause loosening of cell wall polymers but appear to have no hydrolytic or transglycolytic activity (22, 23). Reduced expression of a ripening-regulated expansin, *LeExp1*, in tomato reduced fruit softening as ripening commenced (8) although other expansins are expressed in ripening fruit (9–11). Because the altered expression of *LePG* and *LeExp1* has been proposed to differentially influence pectin and, potentially, hemicellulose polymers, the simultaneous suppressed expression of both genes in ripe fruit may act synergistically to influence overall softening and texture. Here, we investigated the changes in whole fruit firmness or texture in transgenic Ailsa Craig tomato cultivars engineered to simultaneously suppress the expression of *LePG* and *LeExp1*. Ailsa Craig transgenic lines were chosen because the consequences of individually suppressing *LePG* or *LeExp1* in this variety were well-characterized and the variety is one that has been used for production of fresh tomatoes (8, 16–19, 24–27). Crossing the previously characterized homozygous transgenic lines allowed for the examination of the effects of simultaneously reducing the expression of both *LeExp1* and *LePG*.

## MATERIALS AND METHODS

**Plant Material.** Tomato plants (*Lycopersicon esculentum* cv. Ailsa Craig) with reduced expression of *PG* (AC-PG) were kindly provided by D. Grierson (University of Nottingham). These plants had been transformed with an antisense *LePG* gene regulated by the CaMV 35S promoter and had been originally designated GR105, expressing 0.85% of the mean normal *LePG* activity in fruit (18). Tomato plants (*L. esculentum* cv. Ailsa Craig) with reduced expression of *LeExp1* (AC-Exp1) have been described, and the line no. 18–40 was used in this work (8). These plants had been transformed with a sense-suppression construct of the *LeExp1* gene regulated by the CaMV 35S promoter and expressed approximately 3% of the wild-type levels of *LeExp1* protein.

Transgenic lines with suppressed expression of both *LePG* and *LeExp1* were obtained by crossing AC-PG and AC-Exp1 lines. Pollen from AC-PG was introduced to emasculated unpollinated AC-Exp1 flowers. The hemizygous T<sub>1</sub> progeny of this cross was self-pollinated, and the T<sub>2</sub> progeny homozygous for both of the transgenes was identified by polymerase chain reaction (PCR) amplification of the endogenous and the transgenic *LePG* and *LeExp1* genes. Individuals doubly homozygous for both transgenes were confirmed by Southern blots using *LePG* and *LeExp1* probes. The T<sub>2</sub> progeny plants with neither transgene also were identified in this screen, and these azygous lines (AC) were used as controls in subsequent analyses. In the T<sub>2</sub> progeny, 1:16 individual plants were expected to be double transgenics

(AC-PG-Exp1) and 1:16 were expected to be azygous as each transgene segregated independently. To confirm the identity of the lines, the doubly homozygous T<sub>2</sub> (AC-PG-Exp1) and the azygous control (AC) individuals were self-pollinated and the T<sub>3</sub> progeny plants were analyzed by PCR.

Tomato plants (also in *L. esculentum* cv. Ailsa Craig) overexpressing *LeExp1* (AC + Exp1) have been described, and the line no. 17–87 was used in this work (8). These plants were transformed with a sense *LeExp1* gene regulated by the CaMV 35S promoter and expressed at least 300% of wild-type levels of *LeExp1*. Each of the transgenic plants used in these studies was homozygous for the transgenes.

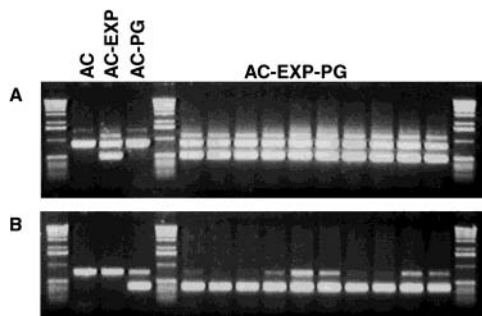
Plants were grown in typical greenhouse conditions during three seasons and were grown in field trials in the summers of 2000 and 2001 in Davis, California. Standard cultivation and fertilization practices were used. Blocks of 20 plants of each genotype were planted in three locations in the field. Fruits were tagged 3 days after anthesis or were staged according to standard color charts for tomato fruit ripeness. Fruits were harvested from all of the plants in each of the three replicate plantings at the mature green stage (28 days postanthesis) or at the indicated ripening stages. Fruits were harvested in multiple lots, and each lot contained at least 12 fruits of each genotype for comparisons. Mature green fruits were allowed to ripen off the vine for 18 days at 24 °C in trays lightly covered with plastic wrap. The time scale for the texture measurements of fruits ripened off the plant was adjusted to the time that each fruit reached the breaker stage ( $\pm 2$  days). Fruits harvested at the indicated ripening stages on the plant were stored at 24 °C in trays lightly covered with plastic wrap for 24 h before fruit firmness was measured.

**Genetic Analysis.** DNA was isolated from young leaves using Qiagen DNeasy Plant Mini Kit (Valencia, CA) and tungsten carbide beads to macerate the tissue. PCR screening for the *LeExp1* genes used the forward primer EXP-EF1, AATGTACAAAATACTCCTAATTGG, and the reverse primer EXP-EB1, ATTCTTTCCGATGAAAGTTTGGC. An intron in the endogenous *LeExp1* sequence allowed discrimination between the endogenous and transgenic *LeExp1* genes. These primers were used for PCR with Takara Taq polymerase (PanVera, Madison, WI) and 30 cycles at 66 °C annealing temperature. PCR screening for the *LePG* genes used the primers PG-IF1, CAGAACTCAGATTATTGTACTCG, and PG-IB1, CATGTATTCCGTTTGTGTAGTCATG, for the endogenous *LePG* gene and used the primers 35S, GCACAATCCCACTATCCTTCGC, and PG-anti, CTTCTCAAGCAAATCACCTTTTCA, for the *LePG* transgene. The four *LePG* primers were used simultaneously using Takara Taq polymerase and 30 cycles at 64 °C annealing temperature.

**Protein Analysis.** Cell wall proteins were extracted as previously described (11). A 20  $\mu$ g amount of total protein was separated on 12% polyacrylamide gel electrophoresis (PAGE), electroblotted to Immobilon-P membranes (Millipore, Bedford, MA), and reacted with polyclonal *LeExp1* or *PG* antibodies. Cross-reacting proteins were visualized using horseradish peroxidase-linked secondary antibodies and Western Lightning Chemiluminescence reagents (Perkin-Elmer, Boston, MA) and detected on film. *PG* activity was assessed by a gel diffusion assay (28).

**Whole Fruit Texture Measurements.** Total fruit firmness was measured by compression of the blossom end of the fruit using a TaXT2i Texture Analyzer (Texture Technologies, Scarsdale, NY) with a 2.5 cm diameter flat plexiglass probe. The fruits were compressed 2 mm at a test speed of 0.5 mm/s. Compression of each fruit was measured three times, and the average of the maximum force necessary to compress the fruit 2 mm was used. At least 30 fruits collected from multiple harvests of each genotype were measured. The textures of fruit that had been ripened in storage were measured daily, and the textures of fruit that was harvested at designated ripening stages were measured after 24 h. Fruits that were ripened in storage were kept at 24 °C on pressed paper fruit trays with a light covering of plastic film to reduce moisture loss.

**Fruit Storage Properties.** Fruits were harvested at the pink stage, and their firmness was measured 24 h later. Standard criteria were used to measure the extent of deterioration during storage. Twelve fruits of each genotype were stored in typical fruit shipping crates with pressed



**Figure 1.** PCR products from genomic DNA from control, transgenic parents, and progeny tomato lines. PCR using *LeExp1* primers Exp-EF1/Exp-EB1 (A) and the *LePG* primers PG-IF1/PG-IB1/35S/PG-anti (B) produced endogenous and diagnostic fragments from genomic DNA from the parental lines (AC-Exp1 and AC-PG) crossed to produce the T<sub>3</sub> homozygous progeny (AC-PG-Exp1). The primers produced only the endogenous DNA fragments from the control (AC). A standard set of 1 kb size markers is included (Gibco BRL, Rockville, MD).

paper trays. Plastic film covered the fruit lightly to reduce moisture loss over the 2 months of storage.

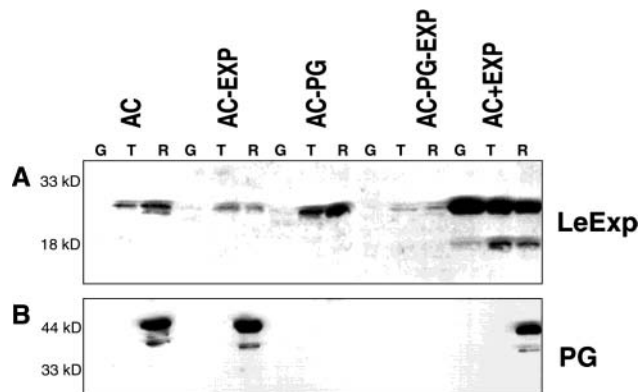
**Juice Preparation and Consistency Determination.** Field-grown tomato fruits were collected at the mature green/breaker, pink, and red ripe stages, weighed, and chopped into quarters. Juice was prepared from ca. 1 kg of chopped fruit samples by a laboratory-scale hot break method using a microwave to heat the fruit in a glass dish covered with plastic film (Litton Commercial microwave oven operating at 1194 W; Litton Microwave Cooking Products, Memphis, TN). Heating was carried out at full power for 6 min followed by heating at half power for an additional 6 min. Heated samples were cooled rapidly on ice. To compensate for evaporation during heating, deionized water was added to the samples. Juice was extracted twice using a benchtop finisher with a screen size of 0.033 in. (0.84 mm). Bostwick consistency determinations were made by measuring the distance that the juice front traveled in 30 s at 20 °C. Triplicate measurements were performed for each sample.

## RESULTS

### Transgenic Tomato Lines Suppressed for *LePG* and *LeExp1* Expression Were Generated by Cross-Pollination.

Transgenic *L. esculentum* cv. Ailsa Craig lines suppressed for both *LePG* and *LeExp1* expression were obtained by crossing two homozygous transgenic tomato lines; one was sense-suppressed for *LeExp1* expression (AC-Exp1) (18), and the second line was anti-sense suppressed for *LePG* expression (AC-PG) (18, 19). All of the T<sub>1</sub> progeny from this cross were hemizygous for each of the transgenes, as expected. As anticipated, two of 13 progeny (T<sub>2</sub>) of the self-pollinated T<sub>1</sub> plants were homozygous for both the *LePG* and the *LeExp1* transgenic sequences and one of 16 progeny was azygous for both of the transgenes.

All T<sub>3</sub> progeny coming from the self-pollination of the selected T<sub>2</sub> progeny had the appropriate transgene genotype (Figure 1). Using the primer pair EXP-EF1/EXP-EB1 and genomic DNA from the parental lines and all T<sub>3</sub> progeny, a 858 bp endogenous *LeExp1* sequence containing a 436 bp intron was amplified. Using the primer pair PG-IF1/PG-IB1 and genomic DNA from the parental lines and all T<sub>3</sub> progeny, the 752 bp endogenous *LePG* sequence was amplified from introns 7 and 8 of the genomic sequence. The primer pair EXP-EF1/EXP-EB1 also amplified a 422 bp fragment diagnostic for the *LeExp1* transgene from the parental AC-Exp1 and all AC-PG-Exp1 T<sub>3</sub> genomic DNA preparations. The primer pair PG-anti/35S amplified a ca. 350 bp fragment diagnostic for the *LePG* transgene from AC-PG and all AC-PG-Exp1 T<sub>3</sub> genomic DNA



**Figure 2.** Cell wall proteins from ripening control and transgenic fruit. Cell wall proteins (10 µg) from fruit at the green (G), turning (T), and ripe (R) stages from the control (AC), the parental lines (AC-PG and AC-Exp1), the homozygous suppressed lines (AC-PG-Exp1), and the line overexpressing *LeExp1* (AC + Exp1) were separated by SDS-PAGE, transferred to membranes for Western blots, and exposed to antibodies to *LeExp1* (A) and *LePG* (B) before detection.

preparations. Because all of the T<sub>3</sub> progeny from the selected homozygous T<sub>2</sub> AC-PG-Exp1 lines contained both of the diagnostic *LePG* and *LeExp1* fragments, we concluded that the T<sub>3</sub> individuals were homozygous for both the *LePG* and the *LeExp1* transgenes. Southern blots of genomic DNA from the parental and progeny lines confirmed this conclusion (data not shown).

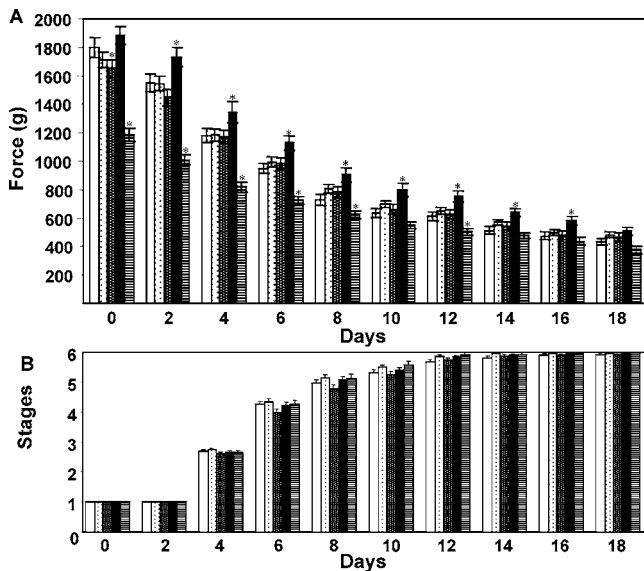
**Expansin and PG Protein Expression Are Reduced in Doubly Homozygous Ripe Fruit.** Cell wall-associated proteins prepared from fruits at the green, turning/breaker, and ripe stages were compared between the parental and the T<sub>3</sub> homozygous lines. As expected, both *LeExp1* and *LePG* proteins were abundant in the preparations from azygous control red ripe fruit and these proteins were much less abundant in the doubly homozygous (AC-PG-Exp1) fruits at all stages (Figure 2). *LePG* protein was not detected in the extracts from red ripe AC-PG fruit. The *LeExp1* protein was not detected in the extracts from AC-Exp1 fruit.

PG enzyme activity was measured in proteins solubilized by high salt extraction of fruit pericarp tissue from green, pink, and ripe fruits. The proteins extracted from red fruit from the AC-PG homozygous parent and the double homozygous AC-PG-Exp1 lines had less than 1% of the detectable PG enzyme activity in the proteins from the AC or AC-Exp1 fruit (data not shown).

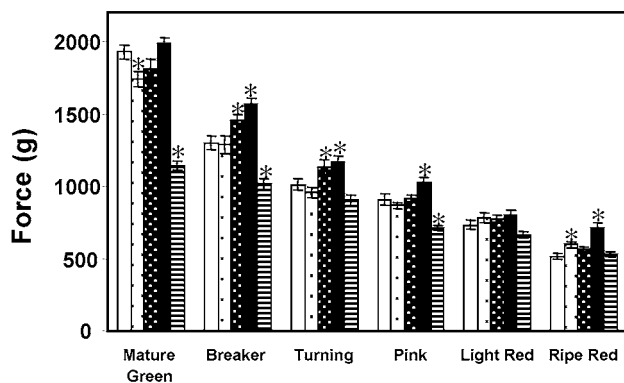
### Total Fruit Firmness Is Increased by Reduced Expression of *LeExp1* and PG.

Mature green fruits were harvested and allowed to ripen off the plant in storage. Fruit texture was measured daily, and color changes associated with ripening were followed in order to stage the progression of ripening (Figure 3). Field-grown control azygous AC fruits softened over 14 days following harvest, and transgenic AC-Exp1 and AC-PG fruits exhibited very similar softening profiles. However, fruit simultaneously suppressed for both *LeExp1* and *LePG* were significantly (>95%, Duncan's midrange test) firmer than controls at the early ripening stages and this significant increased firmness was observed throughout the entire ripening period. Fruits overexpressing *LeExp1* softened substantially more at all stages of ripeness. The softening of fruit from the AC + Exp1 lines was significantly (>95%, Duncan's midrange test) different from the control line, AC, until 12 days after the mature green stage. All fruits changed color from green to red at the same rate indicating that the general progress of ripening was not





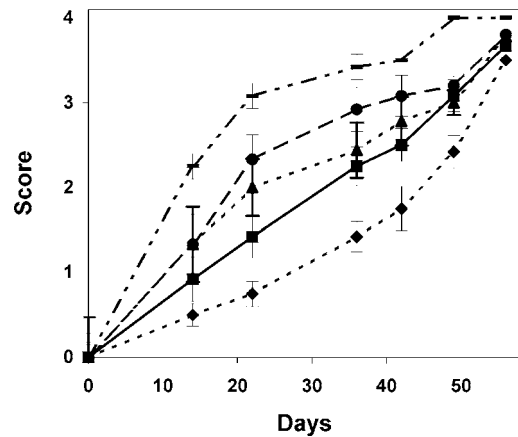
**Figure 3.** Firmness and ripening of fruit. Fruit grown in the field and picked at the mature green stage were allowed to ripen at 20 °C. The firmness (A) and ripening stages (B) of control AC (white bar) and transgenic [AC-Exp1 (light stipple), AC-PG (heavy stipple), AC-PG-Exp1 (black), and AC + Exp (hatched bar)] were measured. Firmness was measured as the force necessary to compress the blossom end of the fruit 2 mm. Ripening was assessed by the exterior color changes of the fruit (1, mature green; 2, breaker; 3, turning; 4, pink; 5, light red; and 6, red ripe). Error bars indicate standard errors of the means. Values that differ by Duncan's multiple range test (95%; SAS Statistical Package, v. 8.0, SAS Institute, Cary, NC) from the control, AC, at each time are indicated by an asterisk.



**Figure 4.** Firmness of fruit ripened on the plants in the field. Fruit were harvested at the indicated stages and held at room temperature for 24 h before measuring the force necessary to compress the blossom end of the fruit 2 mm. Fruit from AC-Exp1 (light stipple) and from AC-PG (heavy stipple) were compared to control AC (white bar) and homozygous AC-PG-Exp1 (black bar) and AC + Exp1 (hatched bar) fruit. Error bars indicate standard errors of the means. Values that differ by Duncan's multiple range test (95%; SAS Statistical Package, v. 8.0) from the control, AC, at each ripeness stage are indicated by an asterisk.

affected by suppression of *LeExp1* or *LePG* or overexpression of *LeExp1*. Results were similar in fruit grown in the greenhouse although the AC-Exp1 line was significantly firmer than azygous control fruit (data not shown), as previously reported (18).

To test whether fruits ripened on the plant exhibited similar softening characteristics to fruits allowed to ripen in storage, fruits were harvested from field-grown plants at specific stages of ripeness and total fruit firmness was measured (Figure 4). Fruits were tagged 3 days after anthesis and harvested 24

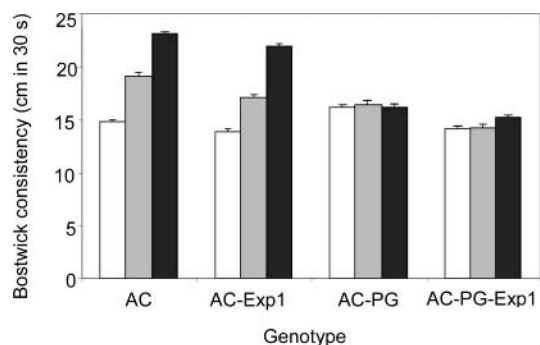


**Figure 5.** Fruit integrity during long-term storage at 20 °C. Fruit from control AC (▲), suppressed for *LeExp1* (AC-Exp1, ◆), suppressed for *LePG* (AC-PG, ●), suppressed for *LeExp1* and *LePG* (AC-PG-Exp1, ■), and overexpressing *LeExp1* (AC + Exp1, —) were compared. Twelve fruits of each genotype were picked at the pink stage and scored for tissue integrity at weekly intervals. Score description: 0, no deterioration, uniformly firm; 1, slight deterioration, some softness; 2, moderate deterioration, some firmness and wrinkles; 3, severe deterioration, portions of extreme softness; 4, very severe deterioration, wateriness, fungal infection, mostly decomposed (27). Error bars indicate standard errors of the means.

(mature green), 26 (breaker), 28 (turning), 30 (pink), 32 (light red), and 34 (red ripe) days later. Fruit staging was confirmed by color comparisons with standard tomato fruit ripeness charts. Azygous control AC fruits exhibited a decrease in firmness after the green stage. As compared to azygous fruit, the fruits from AC-Exp1 plants were less soft at the early stages of ripening, and AC-PG fruits were less soft at the latest ripening stages, as expected. AC-PG-Exp1 fruits were more firm at all ripening stages as compared to the azygous AC fruits, and the difference was statistically significant at the breaker, pink, and red ripe stages (>95%, Duncan's midrange test). At the red ripe stage, the AC-PG-Exp1 fruits were 20% firmer than control fruits. At all ripening stages, the AC + Exp1 fruits were more soft.

**Longevity of Fruit Integrity Is Improved by Reduced Expression of *LeExp1* and *PG*.** To assess the shelf life of control and transgenic tomatoes, fruits were harvested from the field at the pink stage and visual assessments were made of the overall integrity of the fruits during storage for 2 months at 20 °C (Figure 5). A standardized rubric of fruit deterioration was used to evaluate the quality of the fruits (27). Fruits from the AC + Exp1 transgenic line deteriorated very rapidly, within 2 weeks. The control AC and the AC-PG fruits showed substantial deterioration after 3–4 weeks. Fruits from the AC-Exp1 fruits did not deteriorate until 7 weeks of storage. Fruits from the AC-PG-Exp1 line deteriorated after about 6 weeks. The shelf life of the ripe tomato fruit was extended by 2–3 weeks by the reduced expression of *LeExp1*, but the reduced expression of *LePG* in addition to the reduced *LeExp1* expression did not improve the storage characteristics of these fruits.

**Juice from Ripening Tomatoes with Reduced Expression of *LeExp1* and *PG* Has Increased Viscosity as Compared to Control.** Juice was prepared from fruit harvested at the mature green/breaker, pink, and red ripe stages from plants grown in the field using procedures similar to those used for fresh tomato processing (Matoba and Barrett, personal communication). The Bostwick consistometer was used to measure the flow properties of the juices, an aspect of viscosity. The consistency of juice preparations decreased as expected as a



**Figure 6.** Consistency of juice prepared from ripening fruit. Bostwick consistency (cm in 30 s) of juice prepared from control and transgenic fruit at the green/breaker (white bar), pink (gray bar), and red (black bar) stages were measured. Data are averages of four measurements, and error bars represent standard deviations.

function of ripening stage of the fruit from the AC and AC-Exp1 lines, but the decrease was 5–10% less in material from the AC-Exp1 fruits at all stages, indicating that the AC-Exp1 material was more viscous and that the increased viscosity of the juice was maintained throughout ripening (**Figure 6**). Preparations from red AC-PG and AC-PG-Exp1 fruits flowed 25–30% less in the Bostwick consistometer than preparations from red AC and AC-Exp1 fruits, indicating that the AC-PG and AC-PG-Exp1 materials were even more viscous than material prepared from red AC and AC-Exp1 fruits (**Figure 6**). Using analysis of variance with Tukey's multiple comparisons test, the differences between preparations from AC-PG and AC-PG-Exp1 red fruits and preparations from AC red fruit were significant ( $p = 0.049$ ); the differences between the preparations from pink fruits were not significant; and the differences between preparations from AC-PG and AC-Exp1 mature green/breaker fruits were significant ( $p = 0.02$ ). Other comparisons were not significant. Changes in pH and Brix values during ripening were indistinguishable among all genotypes.

## DISCUSSION

Crossing two homozygous lines of transgenic Ailsa Craig tomatoes with suppressed expression of either *LeExp1* (8) or *LePG* (18, 19) resulted in a line of tomato with constitutively suppressed expression of both *LeExp1* and *LePG*, AC-PG-Exp1, and an azygous control line, AC, lacking both transgenes. The texture of the intact fruit from these plants was measured by their firmness, expressed as the force necessary to compress the whole fruit. Previous reports demonstrated that suppression of *LeExp1* alone in Ailsa Craig fruits from plants grown in greenhouses increased fruit firmness, particularly early in ripening (8), and that result was confirmed in this study. However, in tomato fruits grown in the field, suppression of either *LeExp* or *LePG* alone did not significantly increase fruit firmness. Rather, for Ailsa Craig fruits from plants grown in the field, simultaneous suppression of both *LeExp1* and *PG* was required to significantly reduce fruit softness during ripening. Firmness increased approximately 15% in fully ripe fruits by the simultaneous suppression of *LePG* and *LeExp1*. This increased firmness was evident at all stages of ripening from mature green fruits to the red ripe fruits, 21 days later, and the increased firmness was observed in fruits ripened on the plant or after harvesting at the mature green stage. The progression through ripening as measured by external color changes, however, was not altered by suppression of either *LeExp1* or *LePG* or both genes simultaneously.

In addition to alterations to fruit texture, suppression of *LeExp1* dramatically delayed fruit deterioration in long-term storage, confirming previous observations (27). Conversely, overexpression of *LeExp1* dramatically reduced fruit firmness and accelerated fruit deterioration. Simultaneous suppression of *LePG* and *LeExp1* expression delayed deterioration of the unsterilized fruit; however, reduced *LeExp1* expression alone was slightly more effective as a deterrent of tissue decomposition during long-term storage.

Although juice products are not typically prepared from the Ailsa Craig variety of tomatoes, the impact of *LeExp1* expression on the consistency of the juice prepared from this variety agreed with rheological properties of juice and paste products prepared from a processing hybrid variety (31). Detailed analysis of the consistency of products from fruits overexpressing *LeExp1* and from fruits suppressed for *LeExp1* expression suggests that the rheology of processed products may be altered by different mechanisms when *LeExp1* is abundant or when *LeExp1* is suppressed (32).

The expression of many genes increases during ripening, and the products of these genes may influence depolymerization or modifications of cell wall polysaccharides. The expression of *LePG* in ripening tomato fruits (2, 29, 30) is accompanied by increases in other proteins whose functions are to depolymerize or modify polysaccharides in the cell wall, such as pectin methylesterase (33), endo 1, 4,  $\beta$ -glucanase (34), xyloglucan endotransglycosylase (35),  $\beta$ -galactosidases (36, 37), and pectate lyases (38). One of the several expansins expressed during tomato fruit development (9), *LeExp1*, is expressed abundantly in ripening fruits (5), although it does not appear to function primarily as a hydrolase. Rather, expansin may function to loosen the associations between the structural polysaccharide polymers in the wall by disrupting hydrogen bonding between the cellulose microfibrils and the tightly associated xyloglucan polymers (23). Suppressed expression of *LeExp1* contributes primarily to increased fruit firmness at the earlier stages of fruit ripening (8), and changes that occur because of altered *LeExp1* expression may influence the susceptibility of pectic polysaccharides to hydrolases such as *LePG*. We have demonstrated that concurrently suppressing the expression of both *LeExp1* and *LePG* increases fruit firmness more than suppressed expression of either gene alone. Although fruits with suppressed expression of both *LeExp1* and *LePG* were firmer throughout ripening, fruits continue to soften significantly. Other hydrolases with polymer-modifying functions or other proteins with undescribed influences on polymer structure or intermolecular associations are most likely necessary for the complete process of fruit softening. Juice products prepared from fruit with reduced expression of both *LeExp1* and *LePG* display, however, the beneficial effects of increased viscosity.

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