Vase solutions containing sucrose result in changes to cell walls of sandersonia (Sandersonia aurantiaca) flowers

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Abstract

It is well established that vase solutions containing sugar can improve the vase-life of many cut flower crops. Since cut sandersonia flowers supplied with 2% sucrose are firmer during wilting compared to water-fed controls, we have examined whether the effects of sucrose treatment extend to alterations in cell wall structure in the floral tissues, which may influence the wilting-related flower softening. Mature but not fully opened individual flowers were removed from the stems of sandersonia plants and were fed continuously with either 2% sucrose solution or water for up to 10 days. Sucrose supplementation resulted in decreased amounts of chelator-soluble pectin and increased amounts of Na2CO3-soluble pectin per individual flower, and also changed the molecular size profiles of both these pectin fractions compared to the water-fed controls. The molecular size differences were obvious after 3 days in vase solutions, and diminished with subsequent vase time. Senescence-related galactose loss was delayed in sucrose-fed flowers but there was no difference in the levels of β-galactosidase activity present in these flowers compared to controls. The observed differences in cell wall pectins due to sucrose feeding were not reflected in differences to the overall firmness of pre-senescent flowers (up to day 3). High levels of galactose persisted into the wilting phase when sucrose-fed flowers were firmer than water-fed controls. We conclude that while sucrose induced significant quantitative and qualitative differences in pectin fractions and galactose content, firmness of floral tissue, particularly during senescence, was not governed by these events alone. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Cell wall; Flower; Galactose; Pectin; Postharvest; Vase-life; Sucrose

1. Introduction

Petal senescence is distinguished by visual features such as wilting, loss or change in colour, in-rolling, browning and abscission. Underlying
to the initiating factor(s) for flower senescence, and whether the pathways are completely shared for senescence of attached (natural) and cut flowers.

It is well known that supplying sugar (as glucose or sucrose solutions) increases the longevity of many cut flowers. The basis for this improvement is not fully understood but it is likely that sucrose supplementation is effective at a number of levels. While sucrose can act as a source of nutrition for tissues approaching carbohydrate starvation, it may also act as an osmotically active molecule thereby having a role in flower opening and subsequent water relations (Kuiper et al., 1995). There is increasing understanding of the way sugar status and metabolism provides signals that influence changes in plant gene expression (Smeekens, 2000; Coruzzi and Zhou, 2001). Exogenously supplied sugars may also participate in these signal pathways, intervening at numerous levels despite the nutritional, hormonal or overall developmental status of the flower tissue. Along these lines, the effect of sucrose in prolonging the vase-life of sweet pea flowers has been suggested to be through an influence on ethylene production (Ichimura and Suto, 1999), although the mechanisms of this are yet to be defined.

**Sandersonia aurantiaca** (Hook.) is a liliaceous monocotyledon with long stems bearing ten or more individual bell-shaped flowers. The corolla is formed from a single whorl of fused tepals. Factors affecting vase-life of the whole stems and individual flowers have been well-characterised, particularly in relation to the progression of ethylene-independent senescence (Eason and Webster, 1995; Eason and de Vrée, 1995; Eason et al., 2000a). Eason et al. (1997) reported a vase-life extension of up to 2 days for flowers on cut sandersonia stems in response to sucrose feeding. There was an increase in sandersonia flower firmness (resistance to compression) and a delay in the onset of wilting. Individual flowers gained one extra day of vase life when supplied with sucrose (Eason et al., 2000b). We have previously reported that significant alterations occur to cell wall polymers of sandersonia flowers at the onset of tepal wilt (O’Donoghue et al., 2002). We have now investigated whether sucrose feeding may be inducing changes to the cell wall resulting in retention of firmness, using individual sandersonia flowers, detached from the main stem, as model systems.

### 2. Materials and methods

Sandersonia tubers were grown under plastic according to the method described by Eason and Webster (1995). Individual flowers were cut from stems of the parent plant when the flowers were yellow with green, turned-back tips. Flowers were placed either in water or sucrose solution (2%, w/v) for up to 10 days (random allocation to treatments). Light and temperature conditions during the experiment were as described by Eason et al. (1997). Solutions were changed every second day. Flower development was monitored each day and flowers were given a rating according to the subjective numerical criteria described by Eason and Webster (1995). According to these criteria flowers were picked at stage 5, tip tissue around the aperture turned yellow by stage 6, and at stage 7 mature flowers were wholly orange and fully open. The onset of senescence was marked by fading colour (stage 8) and wilting (stage 9) of the aperture tissue. The bell tissue was wilted and faded by stage 10 and brown spots appeared (stage 11). Samples were collected after 0, 3, 5, 6, 7 and 10 days in vase solutions. There were four replicates of each treatment per collection day, with ten flowers per replicate.

Flower firmness was assessed by resistance to compression using an Instron Universal Testing Machine (model 4301), following the method described by Eason et al. (1997). Firmness was expressed in MPa. Data were collected from flowers at day 0, 3, 5, 6 and 10 only.

The stem, nectaries and reproductive tissues of each flower were removed and the remaining tepal tissue was weighed and either frozen in liquid N₂ or freeze-dried. Dry matter and water content per flower were calculated for the freeze-dried material. Ethanol-insoluble residue (EIR) was prepared from frozen tepal tissue according to the method of Huber (1992), including the use of Tris-buffered phenol to inactivate wall-bound en-
zymes. Tissues from all four replicates at each time-point were pooled for EIR production, and the following analyses were performed using subsamples of these EIR preparations.

Neutral sugars in the EIR were hydrolysed with 2 N trifluoroacetic acid, then derivatised to the corresponding alditol acetates and quantified by gas chromatography, using a BPX-70 column (Blakeney et al., 1983). Allose was used as an internal standard. Total uronic acids in EIR were assayed using the method of Ahmed and Labavitch (1977), with detection using the method of Blumenkrantz and Asboe-Hansen (1973).

To characterise soluble pectins, EIR was extracted with 50 mM trans-1,2-cyclohexanedi-amine-N,N,N,N,N-tetraacetic acid (CDTA) in 50 mM Na–acetate, pH 6.0, followed by 50 mM Na2CO3 containing 20 mM NaBH4 (ratio of 0.5 ml extractant per 1 mg EIR). Extractions continued for 4 h at room temperature, with constant shaking. Aliquots of the filtered CDTA and Na2CO3 extracts were passed through a Superose 6HR size exclusion column (10 mm × 300 mm, Pharmacia Biotech) in order to identify molecular size distributions of the extracted pectins. The column was operated in a 30 mM Na–acetate, pH 6.5 buffer containing 20 mM NaCl and 10 mM EDTA, with a flow rate of 0.5 ml min−1. Fractions of 0.5 ml were collected. The uronic acid and total carbohydrate contents of all extracts and all column fractions were quantified using the methods of Blumenkrantz and Asboe-Hansen (1973) and Dubois (1956), respectively.

β-Galactosidase activity was assayed in freeze-dried tepal tissue using p-nitrophenylgalactopyranoside as substrate and following the method of Tanimoto and Igari (1976) with modifications described by O’Donoghue et al. (1998). Activity was expressed as units flower−1, where 1 unit = 1 μmol nitrophenol released h−1.

3. Results

3.1. Physical characteristics

Sucrose supplementation delayed the onset of visual senescence symptoms after flowers had reached full maturity (stage 7, Fig. 1A), providing an extension of one day of vase life, similar to that described by Eason et al. (2000b). No treatment-related differences in flower firmness were measured until the day 6 timepoint when sucrose-fed flowers had greater compression strength than the controls (Fig. 1B). Dry matter was always higher in sucrose-fed sandersonia flowers, and more water was retained in these flowers at days 6 and 7, compared to the water-fed controls (Fig. 2). EIR recovery in the pooled replicates was 15.8 mg flower−1 at day 0, decreasing at every timepoint throughout the 10-day period for water-treated control flowers (day 3, 14.7; day 5, 13.8; day 6, 13.0; day 7, 12.9; day 10, 12.3 mg flower−1). In contrast, sucrose-fed flowers had elevated levels of EIR per flower compared to those at day 0, for up to 6 days (day 3, 16.7; day 5, 16.6; day 6, 16.4; day 7, 15.5; day 10, 12.6 mg flower−1).

Fig. 1. Physical characteristics of sandersonia flowers supplied with water or 2% sucrose solution after harvest. (A) Developmental stage, by visual assessment corresponding to numerical-based descriptors. (B) Compression strength (MPa) of whole flowers. Values are the average of four replicate collections. Bars on each graph indicate the LSD (p = 0.05) between treatment means.
Fig. 2. Weight characteristics of tepal tissues of sandersonia flowers supplied with water or 2% sucrose solution after harvest. (A) Dry matter (mg flower⁻¹). (B) Water content (mg flower⁻¹). Values are the average of four replicate collections. Bars at the top of each graph indicate the LSD (p = 0.05) between treatment means.

3.2. Quantitative analysis of cell walls

The main non-cellulosic neutral sugar in sandersonia flowers at day 0 was galactose, followed by xylose, arabinose and glucose (Table 1). Galactose, arabinose and glucose decreased significantly in control flowers over the 10-day period, with the major loss of galactose occurring between day 3 and day 5. Sucrose feeding of sandersonia flowers changed the pattern of non-cellulosic neutral sugar loss during vase-life. Rhamnose, xylose, mannose and glucose accumulated to varying extents in sucrose-fed flowers, while day-0 levels of galactose and arabinose were maintained for at least 6 days. By day 5 there were significantly higher amounts of galactose, glucose, arabinose and rhamnose in sucrose-fed flowers than in water-fed controls. A similar situation occurred for xylose and mannose content at day 6. β-Galactosidase activity increased on a per flower basis at day 5 and again at day 6 for both water and sucrose-fed flowers (Fig. 3).

Total pectin (mg uronic acid flower⁻¹) of sandersonia supplied with water steadily increased from day 0 to 10 (Fig. 4A). In sucrose-fed flowers, however, pectin accumulation was erratic, dropping below control levels at days 5 and 10, and increasing beyond control levels at days 6 and 7. Sucrose-fed flowers initially had less chelator (CDTA)-soluble pectin and more Na₂CO₃-soluble pectin flower⁻¹ than the water-fed controls (Fig. 4), but by day 6 (CDTA) and day 5 (Na₂CO₃) there were no treatment-related differences in the amount of these pectins (Fig. 4B, C).

Table 1

<table>
<thead>
<tr>
<th>Days</th>
<th>Treatment</th>
<th>Rha (mg flower⁻¹)</th>
<th>Fuc (mg flower⁻¹)</th>
<th>Ara (mg flower⁻¹)</th>
<th>Xyl (mg flower⁻¹)</th>
<th>Man (mg flower⁻¹)</th>
<th>Gal (mg flower⁻¹)</th>
<th>Glc (mg flower⁻¹)</th>
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<td>0</td>
<td>Water</td>
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<td>0.09 (0.01)</td>
<td>0.63 (0.09)</td>
<td>0.78 (0.15)</td>
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<td>Sucrose</td>
<td>0.59 (0.07)</td>
<td>0.06 (0.01)</td>
<td>0.46 (0.09)</td>
<td>0.74 (0.17)</td>
<td>0.76 (0.09)</td>
<td>1.15 (0.19)</td>
<td>0.52 (0.07)</td>
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<td>3</td>
<td>Water</td>
<td>0.46 (0.08)</td>
<td>0.06 (0.01)</td>
<td>0.46 (0.09)</td>
<td>0.74 (0.17)</td>
<td>0.76 (0.09)</td>
<td>1.15 (0.19)</td>
<td>0.52 (0.07)</td>
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<td>Sucrose</td>
<td>0.59 (0.07)</td>
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<tr>
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<td>Water</td>
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<td>Sucrose</td>
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<td>0.81 (0.08)</td>
<td>0.55 (0.04)</td>
<td>0.60 (0.08)</td>
<td>0.64 (0.01)</td>
</tr>
</tbody>
</table>

Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.

* Standard error of the mean of three observations.
an initial reduction in the amount of the largest polymers (day 3). Distributions at days 5 and 6 were substantially similar to that of day 0, but by day 7 these pectins were proportionately enriched in the largest-sized polymers and this trend continued at day 10. However, sucrose-fed flowers had CDTA-soluble pectins with a much higher level of largest-sized uronic acid-containing polymers by day 3, a complete opposite to the controls. The molecular size profile of CDTA-soluble pectins in sucrose-fed flowers at day 5 was sub-

3.3. Molecular size analysis of pectins

The molecular size distributions of pectins solubilised from EIR using CDTA and Na₂CO₃ were assayed on at least three separate extractions, using size exclusion chromatography on Superose 6HR. Molecular size profiles (% distribution basis) of uronic acid and total carbohydrate present in the CDTA and Na₂CO₃ extracts at day 0 are shown in Figs. 5A–8A. Difference plots (Figs. 5B–8B) are used to highlight the areas of change associated with molecular size distributions of pectins solubilised from flowers supplied with water or sucrose solution after harvest. In these plots, areas above/below the zero line indicate increases and decreases, respectively, of the relative amounts of polymers of a specific size, compared to what was present at day 0.

The uronic acids of pectins solubilised by CDTA from sandersonia flowers at harvest (day 0) were of a very broad size range (Fig. 5A). The peak shape indicated that most were very large polymers of a size close to the exclusion limit of the column, but as well there was a small proportion eluting very close to the inclusion volume of the column. Fig. 5B shows the relative changes to the CDTA-soluble pectin population relative to that present at day 0. For control sandersonia flowers supplied with water for 10 days, there was
stantially similar to day 3 of the control, but distributions on subsequent days were of the same pattern as the corresponding controls.

The size distributions of carbohydrates associated with CDTA-soluble pectins are shown in Fig. 6. At day 0, there were three distinct size groupings of total carbohydrate, including a group that eluted at the inclusion limit of the column. Since this peak also contained uronic acid it may contain a population of small, branched, water-soluble pectic oligomers. In the days after harvest, the distribution of carbohydrate in the CDTA-soluble extract from control sandersonia changed to being proportionately enriched in polymers of large size, with a corresponding loss in polymers of the middle and smallest size ranges. The molecular size distribution of total carbohydrate in CDTA-soluble extracts of sucrose-fed sandersonia differed from controls at days 3 and 5 but in subsequent days the size distributions for both were similar, with the exception that there was a slightly higher proportion of the oligomeric-type fragments in the sucrose-fed flowers than the controls.

There were two size groupings of Na₂CO₃-soluble pectins at day 0 (based on uronic acid distribution, Fig. 7). After 3 days in water, there were relatively less mid-sized polymers and more of the largest sized uronic acid-containing polymers, a situation that was reversed in subsequent days. There was a transient loss of a specific group of mid-size range polymers at day 6, indicating that this group was likely to be made up of a number of polymer types of differing uronic acid backbones and neutral sugar branching patterns that are poorly separated on Superose 6HR. In sucrose-fed flowers there was a general pattern of relative loss of large-sized polymers and relative enrichment of a mid-sized group from day 3.

Fig. 5. Molecular size distribution of uronic acids associated with CDTA-soluble pectin extracted from EIR of sandersonia flowers supplied with water or 2% sucrose solution after harvest. (A) Size profile (% distribution basis) at day 0. Tick marks at the top indicate the elution position of dextran size markers in the order 5 × 10³, 500, 73, 40, 9.3 kDa and glucose. (B) Difference plots for the following days compared to the distribution at day 0. Areas above/below the zero line indicate increases/decreases in the relative amounts of uronic acid-containing polymers of a specific size compared to what was present at day 0.

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**Fig. 5. (Continued)**
Fig. 8 indicates the molecular size distribution of total carbohydrate (primarily neutral sugar-containing branches associated with the uronic acid backbones) of Na₂CO₃-soluble pectins. The largest proportion of total carbohydrate was associated with very large polymers in sandersonia flowers at day 0. In following days both the control and sucrose-fed flowers had gradually less total carbohydrate associated with these largest polymers, and more associated with the mid-sized group.

4. Discussion

Sucrose-fed sandersonia flowers accumulated more dry matter, retained more tepal-bound water and were firmer during wilting than control flowers supplied with water only. We found that supplying extra sugar had effects beyond simply increasing the carbohydrate supply for nutrition and metabolism, since there were alterations to the nature of cell wall polymers in pre-senescent sandersonia which lasted for up to 5 days after picking. Cell wall alterations do not appear to be directly correlated to the sucrose-related delays in the visual symptoms of senescence, but do provide an insight into the wider effects of sucrose supplementation in cut flowers.

Alterations in quantity and molecular size of CDTA- and Na₂CO₃-soluble pectins indicate that sucrose feeding changes polymer synthesis and/or polymer breakdown. These changes might occur at a number of levels — the availability of sugar residues for polymer assembly, activation of wall synthesis enzymes and/or control of signal transduction pathways leading to an alteration of expression of genes encoding cell wall synthases. It is interesting that in pre-senescent flowers, there is a distinct difference in the solubilisation of CDTA- and Na₂CO₃-soluble polymers. Highly charged, linear pectins are either less soluble or are synthesised in reduced quantities in sucrose-fed flowers, whereas branched pectins are either more soluble (perhaps because of a change in the branching pattern) or are synthesised in increased quantities.

Increases in β-galactosidase activity and reduction of cell wall galactose content are closely associated with senescence of plant systems, in-
Fig. 7. Molecular size distribution of uronic acids associated with Na$_2$CO$_3$-soluble pectin extracted from EIR of sandersonia flowers supplied with water or 2% sucrose solution after harvest. (A) Size profile (% distribution basis) at day 0. (B) Difference plots for the following days compared to the distribution at day 0. Dextran size markers and difference plot descriptions are as for Fig. 5.

Excluding ripening fruit (Gross and Sams, 1984; Redgwell et al., 1997) and there are indications that this also occurs in flower petals e.g. carnations (de Vetten and Huber, 1990) and daylily (Panavas et al., 1998). There is also a substantial increase in β-galactosidase activity in sandersonia flowers at the onset of wilting during on-plant senescence, accompanied by significant mobilisation of galactose from cell wall polymers (O’Donoghue et al., 2002). By far the majority of cell wall galactose in the tepal tissues of sandersonia flowers resides in a fraction that is insoluble in water, chelator or 6 N NaOH, accumulating in this fraction until the flowers are fully open, and decreasing substantially as wilting progresses (O’Donoghue et al., 2002). The prolonged retention of at-harvest galactose levels in cell walls of sucrose-fed sandersonia flowers raises questions as to whether this is due to differences in the way galactose is incorporated into cell wall polysaccharides (e.g. linkages, branching patterns) or whether sucrose is also inducing changes in the amount and type of enzymes involved in cell wall modification, particularly galactose metabolism. The effects on galactose metabolism exclude changes in β-galactosidase activity since the onset of the activity of this enzyme was unaffected by sucrose feeding.

After day 3, the amount and molecular size distributions of CDTA- and Na$_2$CO$_3$-soluble pectins of sucrose- and water-fed flowers were quite similar, suggesting that loss of firmness during wilting was not connected to the status of these polymers. However, sucrose-fed flowers had consistently higher galactose levels than controls, and so the involvement of galactose-containing polymers in firmness of wilting tepals cannot be ruled out.

The wider goal of our cell wall research in sandersonia flowers is to understand the foundation and modification of texture in petal tissue in response to senescence signals. We have shown that sucrose can have a wider effect on cut flower petal tissue than simply alleviating nutritional stress, with an array of effects at the cell wall level. In this sense, cut flower senescence provides a well-regulated platform for investigating the complex pathway of sucrose signalling in non-
photosynthetic plant tissue. Our results have reiterated other findings from transgenic studies with cell wall hydrolyses in fruit, i.e. that the plant cell wall as a unit can adjust in response to alterations in components and modifying enzymes, but the outcome of such alterations cannot yet be easily interpreted in a logical ‘cause and effect’ pattern. We are continuing our research in this area.

Acknowledgements

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References


