Changes in Sugar-related Enzymes during Wilting of Cut Delphinium Flowers

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Summary

Abscission of sepal s from cut delphinium flowers is prevented by treatment with silver thiosulphate (STS). However, the sepal s still wilt and the pistils develop which reduce flower quality. To understand the process of wilting, unrelated to that initiated by ethylene, sugar uptake and metabolism in cut delphinium flowers were examined. STS-treated delphinium sepal s wilted 4 to 9 days after harvest accompanied by a decrease in fresh weight while the pistils continued to grow. A decrease in water content with a simultaneous reduction in soluble carbohydrate content suggested that wilting is attributable to a decrease in soluble carbohydrates as the osmoticum. The activities of cell wall and soluble acid invertase, sucrose synthase (SS), and mannitol dehydrogenase (MDH) were measured because these enzymes are related to sink mechanism in sucrose- and mannitol-translocating plants. Cell wall invertase activity and p-chloromercurybenzenesulphonic acid (PCMB)-sensitive sucrose uptake by the sepal s decreased to a very low level during wilting. When intact florets were supplied with ¹⁴C-sucrose, a change in sucrose distribution in sepal s corresponded with that in cell wall invertase. Therefore, the reduction in cell wall invertase activity that decreases the soluble carbohydrates as the osmoticum could be related to sepal wilting. SS activity that is known to be important in sink metabolism also decreased during sepal wilting, suggesting that SS was related to the wilting. MDH activity in pistils paralleled their rapid growth while cell wall invertase and SS activities remained consistently high except at harvest.

Key Words: cell wall invertase, delphinium, flower senescence, sepal, sucrose synthase.

Introduction

The process of flower senescence differs among species. The correlation between ethylene and flower senescence is such that, in ethylene-sensitive species, ethylene accelerates flower and corolla abscission (Brown, 1997) and induces flower wilting (O’Neill and Nadeau, 1997; O’Neill, 1997). In numerous other species, however, neither exogenous ethylene nor ethylene inhibitors has an effect on petal wilting (Woltering and van Doorn, 1988). For example, aminooxycetic acid (an ethylene biosynthesis inhibitor) and ionic silver (an inhibitor of ethylene action) do not influence the time of petal wilting in several *Hemerocallis* species (Lukaszewski and Reid, 1989). Utilizing *Hemerocallis* to study petal senescence, Panavas et al. (1998) reported that abscisic acid (ABA) signaling, cell-to-cell attachment, membrane permeability and degradative enzymes are involved in the process. However, the relationship between sugar metabolism and petal wilting that is apparently unrelated to ethylene action has not been studied in detail.

Delphinium is one of the more popular ornamental plants but its cut flowers are sensitive to ethylene, which results in abscission of sepal s (Woltering and van Doorn, 1988). Therefore, cut stems are dipped in STS to prevent sepal abscission. However, even with the STS-treatment, floret quality deteriorates because sepal s wilt and pistils continue to enlarge. This senescent phenomenon is attributed to factors other than ethylene because ethylene action is inhibited by STS. Ichimura and Hiraya (1999) reported that sucrose was effective in delaying the wilting of cut sweet peas that were treated with STS, which indicates that sugar is involved in wilting that is unrelated to ethylene. Sugar delays wilting in some flowers that are insensitive to ethylene, so it may be important in maintaining adequate osmotic pressure in petals (van Doorn and Stead, 1994). However, there are few studies on the role of sugar-related enzymes on flower wilting, whereas their roles during floral organ development in lily have been reported (Ranwala and Miller, 1998).

Ichimura et al. (2000) reported high sucrose and mannitol content in delphinium leaves, indicating that these two carbohydrates are translocatable. Sucrose is primarily metabolized by acid invertase (AI) and/or sucrose synthase (SS) whose activities supposedly contribute to sink strength (Black et al., 1995), and is then phosphorylated by hexose kinase for further metabolism (Kanayama et al., 1997; Kanayama et al., 1998). Godt and Roitsch (1997) suggested that an important function
of cell wall invertase is to establish and maintain sink metabolism in tomato plants, whereas D’Aoust et al. (1999) subsequently reported that SS participates in the regulation of sugar accumulation into the sink tissue. Fellman and Loescher (1987) reported that mannitol and sucrose are utilized in sink tissue of celery. Mannitol is oxidized to mannose by NAD-dependent mannitol dehydrogenase (MDH) for further metabolism in sink tissue (Stoop and Pharr, 1992; Stoop et al., 1995). Thus, Stoop and Pharr (1993) suggested that MDH is a key enzyme in mannitol metabolism in sink tissue in plants that synthesize mannitol.

In this study, we tested the hypothesis that sepal wilting and pistil development are related to sink strength in each organ by examining the sugar content, sucrose-, and mannitol-related enzyme activities, and sugar uptake capacity by cut delphinium flowers after STS treatment.

Materials and Methods

Plant material

Delphinium L. ‘Clear Springs Mix’ was grown under natural day length. Fully expanded normal florets with a diameter of 4 cm or more were detached from plants and their cut ends dipped in STS (0.1 mM Ag+) for 3 hr at room temperature. They were then transferred to distilled water at 25°C and kept at approximately 56% relative humidity under constant light at 20.5 μmol·m⁻²·sec⁻¹ supplied by white fluorescent lamps. Fresh and dry weights of three representative florets were measured 0, 4, 9 and 14 days after treatment with STS. On each sampling date, sepal and pistils were excised, frozen in liquid nitrogen, and stored at -80°C for enzyme and sugar analyses.

Enzyme extraction and assays

Crude enzyme for acid invertase and SS activities was extracted and assayed according to a modified method of Miron and Schaffer (1991). Approximately 0.5 gFW frozen tissue was homogenized in a mortar in 3 volumes of 50 mM Hepes-NaOH (pH 7.5) containing 1 mM MgCl₂, 10 mM KCl, 1 mM EDTA, 2.5 mM DTT, 3 mM diethyldithiocarbamic acid and 1 % (w/v) polyvinylpolypyrrolidone (PVPP). After centrifugation at 10,000 × g for 15 min, the supernatant was desalted with NAP-10 column (Amersham Pharmacia Biotech, Sweden). An aliquot of the desalted crude soluble enzyme was assayed for soluble invertase and SS activity. The insoluble pellet was washed twice in 3-ml suspension of extraction buffer without PVPP and recentrifuged at 10,000 × g for 15 min. The pellet was resuspended in the extraction buffer containing 1 M NaCl without PVPP and the solubilized cell wall invertase was assayed. MDH activity was extracted and assayed according to the method of Stoop and Pharr (1993). All procedures of the enzyme extraction were carried out at 4°C or on ice.

The crude enzyme was prepared from three independent extractions of several florets.

Determination of soluble carbohydrate content

Soluble sugar content was determined by the method of Suzuki et al. (2000). The sugars were prepared from four independent extractions of several florets.

Distribution of radioactivity

Three representative florets were harvested and their peduncles were placed in 5 ml of 0.35 μM [U-¹⁴C] sucrose (Amersham Biosciences) or 3.75 μM [1-¹⁴C] mannitol (Amersham Biosciences) at 7.4 kBq·ml⁻¹ and kept at 25°C under constant light at 20.5 μmol·m⁻²·sec⁻¹ supplied by white fluorescent lamps. After 3 hr, florets were cut into sepal, pistils and the others. Each organ was extracted with hot ethanol and radioactivity in the extract determined with a scintillation counter. Nearly all radioactive compounds were recovered.

Sucrose uptake into sepal tissue

¹⁴C-sucrose uptake into sepal tissue was measured by the method of Orosa-Anim et al. (1996) in the presence and absence of 2 mM p-chloromercuribenzenesulfonic acid (PCMB), except that 1-mm strips (0.25 gFW) of sepal was used. After a 60-min incubation in 5 ml of 0.71 μM [U-¹⁴C] sucrose (Amersham biosciences) at 1.48 kBq·ml⁻¹, the strips were extracted with hot ethanol; radioactivity in the extract was determined with a scintillation counter. The experiments were replicated three times using sepal strips prepared from several florets. The ¹⁴C-sucrose uptake was found to be linear over 60 min (Fig. 1). Nearly all radioactive compounds were recovered. The PCMB-sensitive uptake was calculated by subtracting the uptake obtained in the presence of PCMB from the total uptake in the absence of PCMB.

![Graph showing ¹⁴C-sucrose uptake into delphinium sepal tissue slices.](image)
Results

Sepal wilting and pistil growth

The STS-treated sepals did not abscise during incubation but the flower quality deteriorated (Fig. 2). The sepals and pistils were presentable for the first 4 days after harvest and then the sepals began to wilt and the pistils enlarged. The wilted sepals and the enlarged pistils became very conspicuous 9 days after harvest. Thus, the quality of STS-treated cut delphinium florets deteriorated notably from 4 to 9 days after harvest.

The fresh weight of sepals remained unchanged during the first 4 days but rapidly decreased between 4 and 9 days; their dry weight changed little until 9 days after harvest (Fig. 3A). These changes indicate that sepals lost considerable water 4 to 9 days after harvest, while wilting (Table 1). Soluble carbohydrate content in sepals was determined during the same period in order to know the relationship between sugar and water contents during wilting. Hexose and mannitol contents were high whereas sucrose level was very low as shown in Table 1. Ichimura et al. (2000) reported a similar result in delphinium sepals. Total soluble carbohydrates per floret decreased approximately 50% from 4 to 9 days after harvest. This reduction closely paralleled the rate of water loss. Contrarily, the fresh and dry weights of the developing pistils increased significantly between 4 and 9 days after harvest (Fig. 3B).

Enzyme activity

The activities of soluble and insoluble invertase are

![Graph A](https://via.placeholder.com/150)

![Graph B](https://via.placeholder.com/150)

Fig. 3. Changes in fresh (●) and dry (○) weight of sepals (A) and pistils (B) of delphinium florets. Values are means ± SE (n=3).

Table 1. Soluble carbohydrate and water content during wilting of delphinium sepals.

<table>
<thead>
<tr>
<th>Days after harvest</th>
<th>(H_2O^)</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Mannitol</th>
<th>Total sugar (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>425.5</td>
<td>0.33 ± 0.00 (^b)</td>
<td>2.62 ± 0.48</td>
<td>2.46 ± 0.30</td>
<td>1.68 ± 0.25</td>
<td>7.09</td>
</tr>
<tr>
<td>9</td>
<td>211.1</td>
<td>0.05 ± 0.00</td>
<td>1.49 ± 0.18</td>
<td>0.93 ± 0.11</td>
<td>1.15 ± 0.31</td>
<td>3.62</td>
</tr>
</tbody>
</table>

\(^a\) Water content was calculated from the mean values at 4 and 9 days in Fig. 3A.

\(^b\) Total sugar is the sum of sucrose, glucose, fructose and mannitol content.

\(^\) Values are means ± SE (n=4).
weight in this study because activities per mg soluble protein showed similar patterns.

**Distribution of radioactivity**

The ability of sepals and pistils to absorb sucrose and mannitol through the peduncles was investigated using intact florets (Fig. 5). The distribution of $^{14}$C-sucrose in sepals decreased from 71% to 17% between 4 and 9 days after harvest when the sepals were wilting. However, sucrose distribution in pistils increased gradually 4 to 14 days after harvest. The distribution of $^{15}$C-mannitol in sepals changed little compared with that of $^{14}$C-sucrose.

**Table 2.** PCMB$^{S}$ - sensitive sucrose uptake into sepal tissue of delphinium.

<table>
<thead>
<tr>
<th>Days after harvest</th>
<th>PCMB$^{S}$</th>
<th>Sucrose uptake</th>
<th>PCMB$^{S}$ - sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$^{14}$C-sucrose ($\mu$mol $\cdot$ gFW$^{-1} \cdot$ hr$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.255 ± 0.028$^{2}$</td>
<td>0.142 ± 0.007</td>
<td>0.113 ± 0.011</td>
</tr>
<tr>
<td>11</td>
<td>0.223 ± 0.007</td>
<td>0.194 ± 0.025</td>
<td>0.029 ± 0.018</td>
</tr>
</tbody>
</table>

$^{2}$p-chloromercuribenzenesulfonic acid.
$^{2}$Values are means ± SE (n=3).

**Sucrose uptake into sepal tissue**

PCMB$^{S}$ is a nonpermeant sulphydryl reagent that can be used as a potent inhibitor of cell wall invertase (Renault et al., 1992) and sugar transporter (Ofosu-Anim et al., 1996). PCMB$^{S}$-sensitive sugar uptake, which is supposed to be cell wall invertase and transporter-mediated, decreased to 25% in sepals during wilting (Table 2).
Discussion

Sepal wilting which became noticeable between 4 and 9 days after harvest was accompanied with a loss of nearly 50% in fresh weight and a concurrent decrease in soluble carbohydrate per floret (Fig. 3A, Table 1). Soluble carbohydrates are the most important osmotica for adjustment of turgor pressure (Yakushiji et al., 1996; Pfeiffer and Kutschera, 1996), and thus wilting can be attributed to a decrease in water potential of the cells.

The decline in activity of cell wall invertase, which hydrolyzes sucrose in the apoplast (Weil and Rausch, 1990), was also accompanied by the loss of soluble carbohydrates. Godt and Roitsch (1997) proposed that cell wall invertase is involved in maintaining sink strength by supplying hexose to its transporter in plasma membrane. The change in sucrose distribution in sepal also corresponded with that in cell wall invertase activity in this study (Fig. 4B, 5A). This significant decrease in distribution was not found in mannitol (Fig. 5B) but in sucrose. Furthermore, the velocity of PCMB-sensitive sucrose uptake after wilting was probably too slow to maintain adequate osmotic pressure. Therefore, a decrease in cell wall invertase activity could be related to the lowering of osmotic pressure in cells of wilting sepal. SS activity also decreased rapidly during the wilting of sepal although the activity after wilting was still as high as that at harvest. Because SS plays a key role in sink strength (D’Aoust et al., 1999), the enzyme as well as cell wall invertase could be related to the wilting of sepal.

The fluctuation in soluble invertase activity that was much higher than cell wall invertase activity did not correspond to sepal wilting and pistil growth. If most sugars accumulate in vacuoles of sepal cells, the high activity of soluble invertase could account for the low sucrose concentration as in carnation (Woodson and Wang, 1987) and tomato fruit (Balibrema et al., 1996).

MDH activity was not detected in sepal, suggesting that mannitol metabolism is slow in delphinium sepal (Ichimura et al., 2000). In contrast, MDH activity was detected in pistil although it was still lower than invertase and SS activities. MDH activity in pistil increased between 4 to 9 days after the harvest of florets when the fresh and dry weights of the pistils increased. Stoop and Pharr (1993) reported that MDH converted mannitol to hexose that was utilized to support sink growth. Therefore, it is possible that MDH as well as cell wall invertase and SS are related to pistil growth in delphinium.

Acknowledgement

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Literature Cited


デルフィニウム切り花の萎れに伴う糖代謝酵素活性の変動

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摘要

デルフィニウムの花束は、電気泳動の結果で確認される。エチレン生成刺激を用いて花束を処理した結果、糖代謝酵素活性に変動が観察された。特に、切り花の萎れに伴う糖代謝酵素活性の変動が観察された。それにより、切り花の糖代謝酵素活性に変動が観察された。