Endogenous carbohydrate status affects postharvest ethylene sensitivity in relation to leaf senescence and adventitious root formation in *Pelargonium* cuttings

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Received 16 June 2007; accepted 1 October 2007

**Abstract**

This study investigated the role of ethylene action in postharvest leaf senescence of two cultivars of pelargonium (*Pelargonium × hortorum* L. H. Bailey ‘Patriot Bright Red’ and ‘Patriot White’) cuttings to determine whether endogenous carbohydrate status interacts with ethylene action in relation to leaf senescence and adventitious root formation. In the first experiment, ethylene or receptor-directed ethylene-action blocker, 1-MCP, was applied to cuttings harvested early in the morning, when carbohydrate status is relatively low. Cuttings were stored in sealed bags at 20 ± 1 °C for 3 days in darkness. Ethylene promoted chlorophyll breakdown and leaf senescence during propagation, whilst application of 1-MCP decreased those responses. In the second experiment, application of ethylene had no significant effect on the leaf senescence of cuttings that were harvested at the end of the day, when carbohydrate status was relatively high. In contrast, ethylene application to cuttings with low carbohydrate status (harvested from stock plants kept in darkness for 9 h) resulted in substantially higher leaf senescence. Highly significant positive correlations were calculated between the total chlorophyll content of the oldest leaf after 4 days of initial propagation and sucrose, total sugars, and total non-structural carbohydrate concentrations in the whole cutting, irrespective of postharvest ethylene concentration during storage. Adventitious root formation was inhibited by 1-MCP, whereas it was promoted by exogenous ethylene only when applied to cuttings with a higher carbohydrate status. For those cuttings, ethylene concentration was positively correlated with final root number. These results indicate that ethylene action is involved in postharvest leaf senescence, and that ethylene sensitivity decreases with the increase in preharvest endogenous carbohydrate status of the cuttings. In addition, when the endogenous carbohydrate status is high, ethylene sensitivity promotes adventitious root formation without triggering rapid leaf senescence. These results collectively suggest that preharvest endogenous carbohydrate status interacts with postharvest ethylene action to regulate leaf senescence and adventitious root formation in pelargonium cuttings.

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**Keywords:** Ethylene action; Geranium; 1-Methylcyclopropene (1-MCP); Rooting response; Storage; Sugars

1. **Introduction**

Pelargonium (*Pelargonium × hortorum* L. H. Bailey), also known as geranium, is produced worldwide as an ornamental flowering plant. The European and North American pelargonium production industry relies on the import of vegetative leafy stem cuttings produced in low latitude regions of the world. The success of the pelargonium industry has become increasingly dependent on the importation of high-quality cuttings. Transportation occurs for 2–4 days often under unfavorable conditions, such as temperature extremes, darkness, and exposure to ethylene. The potential to initiate and grow roots, whilst maintaining the functionally active green leaves, are the important quality characteristics of leafy stem cuttings. However, rapid senescence of mature leaves and impairment in subsequent root formation frequently occur as a result of extremes in postharvest shipping environment (Purer and Mayak, 1989; Šerek et al., 1998).

Leaf senescence is an orderly succession of physiological and molecular events that disassemble and degrade cellular components. Although the underlying molecular programs of leaf senescence triggered by age-mediated factors and exter-
nal factors (e.g., phytohormones, temperature, and darkness) are different (Quirino et al., 2000), chlorophyll loss is typically associated with leaf senescence process induced by any of those factors. Chlorophyll breakdown usually starts at the leaf margin and progresses to the interior of the leaf lamina. The products of cellular degradation in the senescing leaves are translocated as nutrients to younger organs or active sinks. Wounding resulting from harvesting the cutting stimulates ethylene production, which can promote leaf senescence (Abeles et al., 1992). Some research reports implicate ethylene as an important factor in postharvest leaf senescence of pelargonium cuttings (Purer and Mayak, 1989; Arteca et al., 1996). Leaf yellowing of pelargonium cuttings during short-term storage can be promoted by external ethylene application (Purer and Mayak, 1989; Mutui et al., 2005) and reduced by the application of ethylene-action inhibitors such as silver nitrate, silver thiosulfate, and 1-methylcyclopropene (1-MCP) (Carow and Bahnemann, 1980; Paton and Schwabe, 1987; Purer and Mayak, 1989; Serek et al., 1998; Blankship and Dole, 2003). However, investigations by Kadner et al. (2000) suggested that ethylene action was only partially responsible for postharvest storage induced leaf senescence in pelargonium cuttings (Kadner and Druege, 2004).

Carbohydrates have been identified as key compounds in the regulation of harvest-induced senescence in broccoli (Brassica oleracea) florets (Couce et al., 2003). Druege et al. (2004) already found that survival rate of pelargonium cuttings limited by leaf senescence was positively correlated with glucose levels in cutting tissues at the time of insertion. Carbohydrates are necessary not only to fuel cellular carbon, energy metabolism, and osmotic functions, but also to modulate gene expression and induce signal transduction (Koch, 1996; Sheen et al., 1999; Smeekens, 2000). Recent studies suggest that carbohydrates interact with the ethylene signal transduction pathway (Zhou et al., 1998; Leon and Sheen, 2003; Rolland et al., 2006), and glucose enhances the degradation of the ethylene-insensitive 3 (EIN3), a key transcriptional regulator in ethylene signaling (Yanagisawa et al., 2003). Recently, we have shown that diurnal carbohydrate dynamics affect postharvest ethylene sensitivity with regard to the leaf abscission in portulaca (Portulaca grandiflora) and lantana (Lantana camara) unrooted leafy stem cuttings (Rapaka et al., 2007a, b).

Adventitious root formation in leafy stem cuttings involves a complex interaction of numerous physiological processes. It predominantly relies on nutrients such as nitrogen and carbon (Druege et al., 2004; Rapaka et al., 2005) as well as the interactions between plant hormones auxin, cytokinins, and ethylene (Kevers et al., 1997). In pelargonium cuttings, endogenous carbohydrate status at harvest substantially influences subsequent rooting, especially following short-term storage or shipment of cuttings (Druege et al., 2004; Rapaka et al., 2005). Furthermore, pre-storage sugar loading improved the post-storage rooting response in pelargonium cuttings (Paton and Schwabe, 1987). Postharvest ethylene action also affects subsequent rooting response in pelargonium cuttings. External ethylene application during storage promoted root formation of cuttings, whilst blocking of ethylene action by application of 1-MCP inhibited rooting (Serek et al., 1998; Kadner and Druege, 2004; Mutui et al., 2005). Therefore, it is possible that preharvest carbohydrate status and postharvest ethylene interact to regulate adventitious rooting in shoot-tip cuttings.

We investigated the role of ethylene action and its interplay with endogenous carbohydrate status in postharvest storage induced leaf senescence and adventitious root formation in pelargonium cuttings. Cuttings were either harvested at 8 a.m. or 4 p.m., which represents the lowest or highest carbohydrate export capacity of source-leaves during the day, respectively (Rapaka et al., 2007a). A short-term preharvest dark treatment was used to obtain cuttings with different initial carbohydrate levels without triggering potential adaptation problems, which are caused by different light levels at stock plant production and subsequent propagation (Forschner and Reuther, 1984; Druege et al., 2004; Rapaka et al., 2005). Cuttings were either treated with ethylene or 1-MCP at the beginning of the postharvest storage treatment. Preharvest carbohydrate levels, ethylene concentrations during storage, and chlorophyll content during propagation were analyzed and leaf senescence and rooting response were determined.

The first experiment was focused on elucidating the role of ethylene action in leaf senescence of pelargonium cuttings, which were presumed to have low carbohydrate levels, and also considered the rooting response. The second experiment was focused on determining whether endogenous carbohydrate status interacts with ethylene action to regulate leaf senescence and adventitious root formation.

2. Material and methods

2.1. Plant material

Rooted cuttings of pelargonium ‘Patriot Bright Red’ and ‘Patriot White’ were obtained from Oglevee Ltd. (Connellsville, PA, USA). ‘Patriot Bright Red’ is moderately sensitive and ‘P. White’ is very sensitive to postharvest storage induced leaf senescence (Oglevee Ltd., personal communication). The cuttings were transplanted on 7 April 2006 into 72-cm-diameter plastic pots (6 L) containing a commercial peat moss mix (Middle weight Mix #3-B, Fafard Company, Anderson, SC, USA) and were maintained as stock plants with natural photoperiods in a greenhouse at Clemson University, SC, USA. The stock plants were irrigated manually and provided with fertilizer at every watering with 250 mg L\(^{-1}\) N using Peters Excel (15% N + 5% P\(_2\)O\(_5\) + 15% K\(_2\)O + 5% Ca + 2% Mg + micronutrients; Scotts-Sierra Horticultural Products Company, Marysville, OH, USA). Greenhouse environment was controlled by climate-control computer (Argus Controls Environmental Systems, White Rock, BC, Canada), and the heating/ventilation set points during day and night were 18.3/22.2 °C from April to June and 18.3/26.6 °C from July to September, providing an average air temperature of 22.2 °C and 25.4 °C and an average relative humidity (RH) of 73.7% and 79.0%, respectively. The average photosynthetic photon flux measured outside the greenhouse was 878 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) (400–700 nm) per 14 h day length. Plants were shaded with retractable curtains when radiation outside the greenhouse exceeded 500 W m\(^{-2}\). The daily light integral (DLI)
was continuously measured using greenhouse weather tracker (Spectrum Technologies, Inc., East - Plainfield, IL, USA) and average DLI measured inside the greenhouse was 12 mol m\(^{-2}\).

2.2. Experiment 1: effect of postharvest ethylene and 1-MCP application in early morning (presumably low carbohydrate) cuttings

Terminal stem cuttings with four leaves, of which at least one was fully developed, were harvested from stock plants of both cultivars at 8 a.m. on 12 June and 4 September 2006 (Harvests I and II, respectively). On each harvest date, 72 cuttings were severed from the stock plants of each cultivar with sterilized knives and divided into three replicated lots, each consisting of 24 cuttings. From each replicated lot, six cuttings were immediately inserted in the rooting medium to study the leaf senescence and subsequent rooting without storage (nonstored control) and the other 18 cuttings were randomly placed in three sealed polyethylene bags (volume: 0.946 L) containing a moist paper towel as previously described (Rapaka et al., 2007a), six cuttings per bag, for short-term storage.

2.2.1. Postharvest treatment

One of the three bags with cuttings remained chemically untreated (stored control), whilst the other two bags were either treated with 1-MCP or ethylene at the beginning of storage. 1-MCP was applied at a concentration of 2 mg L\(^{-1}\) by using a commercial powder formulation (EthylBloc; FlorAlife Inc., Walterboro, SC, USA) as previously described (Rapaka et al., 2007a). For ethylene application, the bags were injected with ethylene (Scott Specialty Gases, Plumsteadville, PA, USA) to create an initial concentration of 1 \(\mu\)L L\(^{-1}\) (as previously described). The cuttings from each treatment (2 preharvest \(\times\) 2 postharvest = 4 treatments) were stored for 72 h at 20 ± 1 °C in darkness to simulate the shipping environment. Ethylene concentration in the headspace of the bags was measured during storage. At the end of the storage period, cuttings were retrieved from the bags and inserted in the rooting media to study leaf senescence and subsequent rooting.

2.2.2. Preharvest treatment

Half of the stock plants of both cultivars were covered with a woven black-out cloth (Ludwig Svensson, Charlotte, NC, USA) (dark treatment), whilst the other half were exposed to full sunlight (light treatment) on the harvest day. The shade curtains in the greenhouse were also kept open on the harvest day. Terminal stem cuttings (as previously described) were harvested from stock plants of both cultivars and treatments (light and dark) at 4 p.m. on 31 July and 14 August 2006 (Harvests I and II, respectively). The DLI received by the plants under the light treatment on the harvest day was 13 and 14 mol m\(^{-2}\) for Harvests I and II, respectively. The leaf temperature during the experiment was measured with a microprocessor thermometer (HH21, Omega Engineering, Inc. Stamford, CT, USA) and temperature of the stock plants under the dark and light treatments averaged 27.3 °C and 27.8 °C, respectively, for Harvest II (not measured for Harvest I). On each harvest date, 63 cuttings were harvested from each of the two light treatments and cultivars and divided into three replicated lots, each consisting of 21 cuttings. From each replicated lot, three cuttings were used for immediate carbohydrate analysis, six cuttings were inserted in the rooting medium to study leaf senescence and subsequent rooting without storage, and the other 12 cuttings were randomly placed in two sealed polyethylene bags (volume: 0.946 L) containing a moist paper towel as previously described (Rapaka et al., 2007a), six per bag, for short-term storage.

2.3. Postharvest treatment

One of the two bags remained chemically untreated, whilst the other bag was injected with ethylene to create an initial concentration of 1 \(\mu\)L L\(^{-1}\) (as previously described). The cuttings from each treatment (2 preharvest \(\times\) 2 postharvest = 4 treatments) were stored for 72 h at 20 ± 1 °C in darkness to simulate the shipping environment. Ethylene concentration in the headspace of the bags was measured during storage. At the end of the storage period, cuttings were retrieved from the bags and inserted in the rooting media to study leaf senescence and subsequent rooting.

2.4. Determination of leaf senescence and adventitious root formation

Immediately after harvest and short-term storage, six cuttings per treatment and replication (\(n = 3\)) were inserted into commercial inert foam rooting wedges (Oasis, Smithers-Oasis North America, Kent, OH, USA) and propagated on mist benches for 21 days in a greenhouse. No plant hormones or fertilizers were applied. The mist system was operated for 6 s every 6 min during the initial 24 h, and then only during normal daylight hours for the remainder of the propagation period. Greenhouse heating/ventilation temperature set points of 23/26 °C provided an average air temperature of 27 °C, and the mist bench heating set point was 25 °C. The cuttings were shaded using retractable curtains when the radiation outside the greenhouse exceeded 250 W m\(^{-2}\). The average DLI measured inside the propagation house was 7 mol m\(^{-2}\) and RH averaged 72%. The number of leaves that decayed due to chlorosis, abscission, and secondary infection with Botrytis was recorded. After 21 days in propagation, the number of root tips emerging outside the foam rooting wedges was recorded.

2.5. Analyses of carbohydrates, ethylene, and chlorophyll

To study the initial carbohydrate status of cuttings, carbohydrate concentrations in leaf lamina, petiole, and stem were separately analyzed immediately after harvest. For analysis, leaves, petioles, and stems of three cuttings per treatment and replication (\(n = 3\)) were pooled, immediately frozen in liquid nitrogen, and stored at −70 °C until lyophilization. The samples were freeze dried at −40 °C and then ground. Soluble sugars from 50 mg of tissue were extracted with 12 methanol:5
chloroform:3 water (MCW; by volume) as described by Miller and Langhans (1989). Mannitol (1 mg) was added as an internal standard during the extraction process. The tissue extracts were evaporated to dryness in vacuo at 40 °C, and the residue was dissolved in 1 mL high purity water (18.2 MΩ cm−1, NANOpure Diamond, Barnstead International, Dubuque, IA, USA). Glucose, fructose, and sucrose were separated using a Dionex DX-300 High Performance Liquid Chromatography system with a 4 mm × 250 mm CarboPac™ column and detected with an electrochemical detector (Dionex, Sunnyvale, CA, USA). Quantification of the above individual sugars was based on the calibration curves obtained from their respective standards. Starch in dried tissue residue, following soluble sugar extraction, was determined using enzymatic hydrolysis of starch using amyloglucosidase (from Rhizopus mold, EC 3.2.1.3; Sigma chemicals, St. Louis, MO, USA) into glucose. For ethylene analysis, gas samples of 1 mL were taken from the bags as described previously (Rapaka et al., 2007a) at every 24 h interval during the 72-h storage period. Ethylene was analyzed using gas chromatograph (Shimadzu GC-9A, Kyoto, Japan) equipped with a flame-ionization detector and a phenyl isocyanate/Porasil C column. For chlorophyll analysis, basal 1-cm sections from the left and right sides of the oldest leaf (lamina) were excised on day 4 during propagation and used for the analysis. Extraction was carried out in dimethyl sulfoxide (Sigma–Aldrich Inc., St. Louis, MO, USA) (Hiscox and Israelstam, 1979) at 65 °C for 1.5 h. The absorbance was measured at 645 and 663 nm using a spectrophotometer (Genesys™ 20, Thermo Fisher Scientific, Inc., Madison, WI, USA). Chlorophyll content was determined using the equation: 
\[ \text{chl}_{a+b} = (20.2 \times A_{645} + 8.02 \times A_{663}) \times \text{tissue weight (g)} \]
(Aronn, 1949).

2.6. Statistics

Data were analyzed with ANOVA/MANOVA and Regression modules of the Statistica 7.1 software program (StatSoft Inc., 2005). Harvest date had no influence on results, therefore the two dates were pooled. If significant effects were found between treatments or time of sampling during storage, the mean values were compared using the Tukey’s test with significance
level of at least \( P \leq 0.05 \). Linear regressions and correlations were calculated between preharvest carbohydrate concentrations, postharvest ethylene concentration in sealed bags during storage, and chlorophyll content in the oldest leaf during propagation, number of leaves senesced per cutting during 14 days of propagation, and the number of subsequently formed adventitious roots. For analysis of linear regressions, replications were considered individually to account for all variation.

3. Results

3.1. Effect of postharvest ethylene and 1-MCP application

In experiment 1, analysis of ethylene concentration in the sealed bags during storage indicated that cuttings of both cultivars produced ethylene that accumulated in the bags (Fig. 1). However, application of 1-MCP substantially increased ethylene production. The ethylene concentration in the bags of control cuttings was lower than those concentrations measured in the bags of ethylene-treated cuttings at 24 and 48 h during storage. However, at 72 h of storage, ethylene concentrations in bags of control cuttings and ethylene-treated cuttings were similar. No leaf decay due to senescence was observed during the 14 days of propagation of nonstored cuttings in both cultivars (Fig. 2A). The total chlorophyll content measured at day 4 of propagation was lower in both stored control and ethylene-treated cuttings compared to those of nonstored cuttings in both cultivars (Fig. 2B). However, at least for ‘P. White’, more leaves senesced during propagation when stored cuttings were treated with ethylene compared to control cuttings (Fig. 2A). In contrast, in 1-MCP-treated cuttings, the total chlorophyll content at day 4 and leaf senescence during the 14 days of propagation were similar to those of nonstored cuttings in both cultivars. However, root number in both cultivars was significantly lower in stored cuttings treated with 1-MCP than in any other treatment (Fig. 2C). Control and ethylene-treated cuttings formed a similar number of roots as nonstored cuttings.

3.2. Effect of preharvest endogenous carbohydrate status and postharvest ethylene application

In experiment 2, the effect of preharvest dark treatment on endogenous carbohydrate status in leaf lamina, petiole, and stem are presented in Fig. 3. In leaves, starch was the most abundant carbohydrate in cuttings taken from plants exposed

Fig. 3. Effect of preharvest short-term dark treatment on carbohydrate concentrations in (A and B) leaves, (C and D) petioles, and (E and F) stems of pelargonium cuttings of two cultivars. Different letters indicate significant differences \( (P \leq 0.05) \) within specified carbohydrate fractions \( (n = 6) \). Values are expressed on a dry-mass basis. ND: not detected.
to light, whereas starch was essentially depleted in cuttings taken from plants in darkness (Fig. 3A and B). Leaf sucrose was the second largest carbohydrate fraction and concentrations were also lower in cuttings harvested from plants in darkness. Leaf glucose and fructose concentrations were relatively low and were marginally higher only in plants of ‘P. Bright Red’ when exposed to light preceding harvest. In both cultivars, sucrose in petioles and stems was significantly lower in cuttings taken from stock plants in darkness compared to those taken in light (Fig. 3C–F). Starch concentrations were relatively low in petioles and were reduced to trace amounts in cuttings taken from stock plants in darkness. Stock plant darkness had less of an effect on glucose and fructose concentrations in petioles and stems. However, stem fructose in ‘P. Bright Red’ and stem starch in both cultivars were not detected. In both cultivars, whole cutting (leaves + petioles + stem) total sugar (TS = glucose + fructose + sucrose) concentration and total non-structural carbohydrate (TNC = TS + starch) concentration were significantly higher in the cuttings taken from the light treatment. The TS concentrations for cuttings taken in light compared to darkness were 39.1 and 20.1 g kg$^{-1}$ dry-mass, respectively, for ‘P. Bright Red’ and 40.9 and 26.3 g kg$^{-1}$ dry-mass, respectively, for ‘P. White’ (for both cultivars differences significant at: $P < 0.001$, $n = 6$). Similarly, the TNC concentrations for cuttings taken in light compared to darkness were 59.4 and 20.4 g kg$^{-1}$ dry-mass, respectively, for ‘P. Bright Red’ and 74.1 and 26.7 g kg$^{-1}$ dry-mass, respectively, for ‘P. White’ (for both cultivars differences significant at: $P < 0.001$, $n = 6$).

The light and dark treatments provided to stock plants did not apparently influence postharvest ethylene production in either cultivar, since ethylene concentrations in sealed bags containing untreated (control) cuttings were similar during storage (Fig. 4). Similarly, in the bags treated with exogenous ethylene, no differences in ethylene concentrations were observed between preharvest light and dark treatments. However, throughout the storage period, the ethylene concentrations in bags containing control cuttings were lower than bags that received the ethylene application.
Preharvest dark treatment had no effect on leaf senescence during propagation in nonstored cuttings or stored, control cuttings (Fig. 5A). However, leaf senescence was substantially higher when cuttings harvested following darkness were stored with an exogenous application of ethylene. Chlorophyll content of stored control cuttings harvested in the preharvest light treatment was similar to nonstored cuttings (Fig. 5B). The application of ethylene resulted in only marginal decrease in chlorophyll content when cuttings were harvested in light (difference statistically significant in ‘P. White’, Fig. 5B). In contrast, in stored cuttings harvested following darkness had significantly lower chlorophyll content, regardless of ethylene application.
Therefore, among different postharvest storage treatments, the most significant leaf decay occurred only when cuttings were harvested following dark treatment and treated with ethylene exogenously.

The preharvest dark treatment did not influence subsequent rooting of nonstored cuttings or stored, control cuttings (Fig. 5C). Storage reduced rooting only in ‘P. Bright Red’ cuttings, and the magnitude of that response depended on postharvest treatments. Root number of both cultivars was actually greater when light-harvested cuttings were treated with ethylene at the beginning of storage compared to stored, control cuttings (Fig. 5C). Furthermore, in ‘P. Bright Red’, the root number of those ethylene-treated cuttings harvested from light treatment was significantly higher among all different storage treatments.

Correlations were calculated between preharvest carbohydrate status and postharvest ethylene concentration in sealed bags (mean during 3 days storage period) as independent variables and total chlorophyll content in the oldest leaf at day 4 during propagation (Figs. 6 and 7A and B), number of leaves senesced per cutting during 14 days of propagation, and number of adventitious roots formed (Fig. 7C and D) as dependent variables. In nonstored cuttings, no correlations were calculated between preharvest carbohydrate status and total chlorophyll content in the oldest leaf because cuttings taken from the light and dark preharvest treatments had similar chlorophyll content (Fig. 5B). In stored cuttings, preharvest carbohydrate status in different cutting tissues was often positively correlated with total chlorophyll content, even when data were pooled irrespective of postharvest ethylene application at storage (Table 1). In both cultivars, these relationships existed with sucrose, TS, and TNC in leaves, petioles, and stems. In addition, whole cutting carbohydrate concentration was significantly correlated with total chlorophyll content (Fig. 6A–D).

In ‘P. Bright Red’, the regression slope was marginally higher for stored, control cuttings compared to ethylene-treated cuttings over the same broad range of TS (slopes: 0.038 and 0.031, respectively) (Fig. 6A) as well as TNC (slopes: 0.019 and 0.015, respectively) (Fig. 6C). However, there was no correlation between postharvest ethylene concentration and chlorophyll content.

**Table 1**

Correlation coefficients (r) between preharvest (at excision) carbohydrate concentrations in different cutting tissues or postharvest ethylene concentration in sealed bags (mean during 3 days of storage period) as independent variables and the chlorophyll a+b content in the oldest leaves on day 4 of propagation and number of leaves senesced per cutting during 14 days of propagation as dependent variables (n=24) in stored pelargonium cuttings of ‘Patriot Bright Red’ (PBR) and ‘Patriot White’ (PW).

<table>
<thead>
<tr>
<th>Characteristics</th>
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<th>Chlorophyll content</th>
<th>Leaf senescence</th>
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<td></td>
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<td>PW</td>
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<td>Glucose</td>
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<td>Stem</td>
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Data were pooled for all preharvest light and storage treatments. Total sugars: glucose + fructose + sucrose; total non-structural carbohydrates: total sugars + starch; n.c., not calculated because carbohydrates were not detected; n.s., not significant.

* P ≤ 0.05.
** P ≤ 0.01.
*** P ≤ 0.001.
content when ‘P. Bright Red’ cuttings taken from different preharvest light treatments were pooled (Table 1) or separated (data presented for cuttings taken in preharvest light treatment, Fig. 7A). In ‘P. White’, stored control cuttings had higher chlorophyll content than cuttings treated with ethylene with the same preharvest TS (Fig. 6B) or TNC levels (Fig. 6D). Postharvest ethylene concentration was negatively correlated with chlorophyll content when ‘P. White’ cuttings taken from different preharvest light treatments were pooled (Table 1). However, when separately regarded, a significant negative correlation was calculated only in the cuttings taken from preharvest light treatment (Fig. 7B), which had relatively higher carbohydrate levels, and not in cuttings taken from preharvest dark treatment (data not shown).

In stored cuttings, the preharvest carbohydrate status in different cutting tissues as well as in whole cutting was negatively correlated with the number of leaves senesced per cutting during initial 14 days of propagation, irrespective of postharvest ethylene application at storage (Table 1). Alternatively, postharvest ethylene concentration was positively correlated with number of leaves senesced when cuttings taken from different preharvest light treatments were pooled (Table 1). However, when separately regarded, a significant positive correlation was only calculated in the cuttings taken from preharvest dark treatment (r = 0.86, P < 0.001, n = 12) in ‘P. White’. In ‘P. Bright Red’, significant positive correlations were calculated in the cuttings taken from both preharvest treatments, but the steepness of the regression slope over the same broad range of ethylene concentration was substantially higher for the cuttings taken from preharvest dark treatment (slope: 2.2, r = 0.85, P < 0.001, n = 12) when compared to those taken from light treatment (slope: 0.44, r = 0.63, P = 0.05, n = 12).

Cuttings harvested from light and dark preharvest treatments developed a similar number of roots, and thus no correlations with preharvest carbohydrate status were calculated for non-stored cuttings or stored, control cuttings. However, subsequent root number was positively correlated with preharvest carbohydrate status in stored ethylene-treated cuttings (for ‘P. Bright Red’ r = 0.61 and for ‘P. White’ r = 0.69 with whole cutting TNC, n = 12 and significant at P ≤ 0.05). The postharvest ethylene concentration was positively correlated with subsequent root number in stored cuttings taken from preharvest light treatment which had relatively higher carbohydrate levels (Fig. 7C and D). Although the regressions are weak, it only becomes obvious that rooting response was generally higher for cuttings treated with exogenous ethylene (19 and 18 roots in ‘P. Bright Red’ and ‘P. White’ on an average, respectively) when compared to untreated-controls (13 and 12 roots in ‘P. Bright Red’ and ‘P. White’ on an average, respectively) (Fig. 7C and D). In the cuttings taken from preharvest dark treatment, which had relatively lower carbohydrate levels, postharvest ethylene concentration and root number were not correlated (data not shown).

4. Discussion

The short-term dark treatment significantly reduced the endogenous carbohydrate concentration in different pelargonium cutting tissues (Fig. 3), especially starch in the leaf tissue. In source-leaves, starch mobilizes and supplements sucrose synthesis from photosynthesis during darkness and under low light conditions, and is assumed to involve a circadian component (Geiger et al., 2000). Sucrose, the main transportable form of carbohydrate, was consistently lower in all the tissues of the cuttings taken from preharvest dark treatment. This indicates that inhibition of photosynthesis under dark conditions consequently reduced the carbohydrate export capacity of source-leaves to various sinks. Surplus carbon assimilation can be transported to active sinks within the stock plant, thereby accumulating in young stem tissue (Rapaka et al., 2005). In leaves, the comparatively low glucose and fructose concentrations in the cuttings taken from the stock plants under light indicate that sucrose and starch are the major storage pools of newly assimilated carbon. These results are in agreement with the observations made by Jiao et al. (1999) in pelargonium plants using 14C.

1-MCP is a very effective, non-toxic gaseous antagonist of ethylene perception that competitively and irreversibly blocks ethylene receptors (Sisler and Serek, 1997). In addition, it is effective at low concentrations and a potent inhibitor of several different ethylene-dependent processes in various organs across a broad range of plant species (Blankenship and Dole, 2003). In experiment 1, postharvest blocking of ethylene receptors of cuttings by application of 1-MCP substantially increased ethylene production (Fig. 1). Similar responses to 1-MCP treatment have been observed in vegetative cuttings of the same species (Kadner and Druege, 2004) as well as Euphorbia pulcherrima (Faust and Lewis, 2005) and Lantana camara (Rapaka et al., 2007b). This response can be attributed to the interruption of the autoinhibition of ethylene biosynthesis from the blockage of ethylene receptors by 1-MCP (Ella et al., 2003) as already discussed by Kadner and Druege (2004). In the present study, blocking of ethylene receptors significantly reduced post-storage chlorophyll degradation and leaf decay due to senescence, whereas those responses were accelerated in ethylene-treated cuttings (Fig. 2A and B). These results strongly support the conclusion that ethylene action regulates postharvest leaf senescence of pelargonium cuttings.

In experiment 2, cuttings harvested from both preharvest dark and light treatments produced similar amounts of ethylene (Fig. 4). This suggests that the preharvest carbohydrate concentration of cuttings has no influence on subsequent ethylene production. Similar results were observed in studies with unrooted cuttings of Portulaca and Lantana (Rapaka et al., 2007a,b). In those studies, however, the preharvest cutting carbohydrate concentrations were a consequence of different harvest times during the photoperiod. In the present study, post-storage chlorophyll content of the oldest leaf after 4 days of propagation was highly correlated with preharvest carbohydrate status, irrespective of postharvest ethylene concentration (Fig. 6A–D). This indicates that endogenous carbohydrate status is the predominant regulator in the postharvest storage induced leaf senescence in pelargonium cuttings. Furthermore, exogenously applied ethylene elicited only a marginal breakdown in chlorophyll when cuttings were taken from preharvest light treatment which had relatively higher carbohydrate levels (Fig. 5B).
In contrast, when cuttings were taken from preharvest dark treatment, which had lower carbohydrate levels, chlorophyll degradation occurred even in untreated-control cuttings, and substantial senescence promoted leaf decay in ethylene-treated cuttings (Fig. 5A and B). These results indicate that postharvest ethylene action decreased as the preharvest carbohydrate concentration in cuttings increased. However, the highly significant correlations between leaf senescence process and carbohydrate concentrations in the whole cutting, as well as in individual cutting parts (Table 1), indicate that the interconnection between different parts of the cuttings (source/sink relationship) plays a major role in older leaf senescence of pelargonium cuttings. Therefore, it can be concluded that older leaf senescence to ethylene increases with the increase in carbohydrate export activity of that leaf. Considering the strong interactions between carbohydrates and the ethylene signal transduction pathway (Zhou et al., 1998; Leon and Sheen, 2003; Yanagisawa et al., 2003), the dependency of ethylene action on endogenous carbohydrate status further supports our hypothesis that preharvest endogenous carbohydrate status regulates postharvest ethylene sensitivity in vegetative leafy stem cuttings (Rapaka et al., 2007a,b).

Adventitious root formation relies on an adequate supply of carbohydrates to the region of root regeneration to promote root initiation and development (Haisig, 1986; Veierskov, 1988). In experiment 2, the lower preharvest individual and total carbohydrate concentrations in cuttings harvested following extended darkness did not decrease the subsequent rooting response in either nonstored or stored control cuttings (Fig. 5C). We postulate that the relatively high light (DLI: 7 mol m$^{-2}$) delivered during propagation provided the photosynthates needed for root regeneration to at least partially overcome the initial carbon deficiency of cuttings (Rapaka et al., 2005).

The role of ethylene sensitivity in adventitious root formation in leafy stem cuttings has been studied in ethylene-insensitive transgenic petunia (Clark et al., 1999). In the present study, postharvest transient blocking of ethylene sensitivity during storage by a receptor-directed inhibitor (1-MCP) decreased the number of adventitious roots (Fig. 2C). Furthermore, in cuttings with a high preharvest carbohydrate status, the exogenous application of ethylene increased the number of adventitious roots (Fig. 7C and D). It would be interesting to speculate from these observations that postharvest ethylene sensitivity promotes carbohydrate transport from source-leaves towards the basal stem, which becomes a sink immediately after harvest for root regeneration. This idea supports the hypothetical model proposed by Gapper et al. (2005) that ethylene triggers the expression of genes involved in carbohydrate transport and metabolism during harvest-induced senescence in broccoli florets.

In pelargonium cuttings, the basipetal transport of carbohydrates from source-leaves during the early rooting period is a major determinant of the intensity of adventitious root formation (Rapaka et al., 2005). In the present study, however, ethylene sensitivity was greater in ethylene-treated cuttings with a low preharvest carbohydrate concentration compared to those with higher concentrations, and the number of subsequently formed roots was lower (Fig. 5C). This decreased rooting response could at least partially be attributed to the lower initial carbohydrate export capacity of source-leaves and a lack of photosynthetic support during propagation due to rapid leaf decay.

These studies suggest that the postharvest ethylene sensitivity of pelargonium cuttings decreases with an increase in preharvest endogenous carbohydrate status and, as a result, leaf senescence decreases. Alternatively, when the endogenous carbohydrate status is greater, ethylene sensitivity promotes adventitious root formation without triggering rapid leaf senescence. Further studies are being conducted to clarify the role of postharvest ethylene sensitivity on carbohydrate transport and allocation towards the region of root regeneration during cutting propagation.

References


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