Acclimatization of ex Vitro Strawberry Plantlets in CO2-enriched Environments and Supplementary Lighting

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Additional index words. tissue culture, Fragaria × ananassa, relative growth rate, net assimilation rate

Abstract. The effect of CO2 enrichment (CE) and supplemental lighting (SL) on the growth of ex vitro strawberry (Fragaria × ananassa Duch.) plantlets was studied during acclimatization. Three different concentrations of CO2 [330, 900, and 1500 ppm (v/v)] and two SL treatments (0 and 150 μmol·s−1·m−2) were applied. There was no significant interaction between light and CO2 for root and leaf dry weight and leaf area. CE had no effect on these parameters in the early period following transfer but resulted in significant increases at days 20 and 30. CE had no significant influence on leaf and root relative growth rate (RGR) over the three sampling periods, but had a significant effect on net assimilation rate at a 20- to 30-day period. At the end of the experiment, 900- and 1500-ppm treatments had a significantly higher root and shoot dry weight than the 300-ppm treatment. SL resulted in increased dry weight at 10 days and even greater increases at days 20 and 30. CE was more effective than SL in stimulating root growth, whereas SL increased shoot growth significantly. There was a synergistic effect between CE and SL. The period needed to obtain plants of a similar size to an acclimatized plantlet was shortened by 15 days with 900 ppm CO2 and SL. At the end of the experiment, SL and CE at 1500 and 900 ppm increased leaf and root dry weight by a factor of 3 and 5 for ‘Honeyoye’ and ‘Kent’, respectively. These increases were less important for SL or CE used alone.

One of the most critical steps in the micropropagation of strawberry and most other plants is the transition from an heterotrophic to a phototrophic mode of nutrition. During ex vitro acclimatization, plantlets are transferred from an in vitro-controlled environment to an external uncontrolled environment, where they have to establish normal photosynthetic activity and water relations. This transition usually is achieved by providing transplants with conditions of high relative humidity and is essential to minimize transplants loss (10, 24, 27). Cauliflower plantlets transferred to the acclimatization stage have been shown to possess insufficient photosynthetic activity to achieve a positive carbon balance (11). Carbon fixation rates of in vitro-produced strawberry leaves were low and insufficient to sustain autotrophic growth but increased significantly in subsequently formed leaves (12). It has been suggested that leaves produced in vitro are used as a carbon source for growth and development immediately following transplant, prior to the emergence of new leaves (12, 26). Under this premise, two possible alternatives are available to improve acclimatization success of micropropagated strawberry: a) increasing the amount of stored products in vitro leaf, and/or b) increasing the rate of new leaf production ex vitro. The first alternative, larger leaf area, could lead to increased evapotranspiration during the acclimatization stage, which is conducive to the development of water stress. The second alternative is therefore the more promising. Supplemental lighting (SL) and CO2 enrichment (CE) represent two methods to hasten the development of autotrophy. A number of researchers have tried to improve the survival and growth of tissue-cultured plants during acclimatization by avoiding water deficit and/or improving water regulation (3, 24, 27). Instead, we attempted to hasten the development of autotrophy as Grout and Millam (12) had suggested by SL and/or CE. SL plays an important role in the morphological and physiological adaptation of leaves (1, 18). Lichtenthaler (18) demonstrated that leaves of seedlings grown under high irradiance were thicker and had higher rates of dry matter accumulation than those grown under low irradiance. He also showed that root development was improved by high irradiance (18). In the greenhouse industry, high-pressure sodium (HPS) SL is used to increase yield and quality of crops (5). However, there is no study on the effects of HPS SL on the acclimatization of ex vitro plantlets.

There are numerous reports of the beneficial effects of CE on plant growth (2, 13, 14). However, there are few results published on the effect of CE on ex vitro plants. Lakso et al. (17) have shown that CE at a level of 1200 ppm improved the growth and development of grapevine plantlets during the acclimatization period. However, their experiment was done in controlled humidity chambers at only two concentrations of CO2. They did not determine the optimum CO2 level, nor could they measure the effects of CE under stress-hardening regime.

The objectives of this research were a) to measure the effect of CE and determine an optimum level of enrichment, b) to study the effects of HPS SL, and c) to quantify the interactions between CE and SL of ex vitro strawberry plantlets during the acclimatization stage.

Materials and Methods

Apical meristems of strawberry crowns (‘Kent’ and ‘Honeyoye’) were excised according to Boxus (2). After an initiation period on Boxus medium (2), the plantlets were transferred to a proliferation medium (16) and subcultured five to six times at regular 4-week intervals. Axillary branches located at the base of cultures then were excised and transferred onto rooting medium. The rooting medium was one-half strength MS salts (19) with 0.12 μM thiamine-HCl, 0.56 μM myo-inositol, 0.40 μM nicotinic acid, 2.4 μM pyridoxine-HCl, 3% sucrose, 0.7% Difco Bacto-agar, 0.5 g-liter−1 activated charcoal (Darco-90), without growth regulators. The pH of the media was adjusted to 5.8 and media were dispensed in 20-ml aliquots to 25 × 100 mm test
tubes during initiation and in 40-ml aliquots to Magenta GA-7 containers during multiplication and rooting prior to autoclaving at 1.4 kg·cm⁻² and 121°C for 15 min. The cultures were maintained in a growth room at a temperature of 24° ± 2° under cool-white fluorescent light that supplied a photosynthetic photon flux (PPF) of 48 μmol·s⁻¹·m⁻² for a 16-hr photoperiod.

After 4 weeks in rooting medium, plantlets with two to five well-formed, 3-cm-long roots were removed from culture and the agar washed off. Plantlets were transferred to a Pro-mix bx artificial medium (Premier peatmoss, Rivière-du-Loup, Quebec, Canada) in Sutton plastic trays with fifty 2 × 2 cm cells. Plantlets were placed in the glasshouse under high humidity tents for 1 week. Humidity was maintained by misting so the interior of the tent was always wet. After this period, humidity tents were removed and trays were distributed to three identical glasshouse compartments where CE and SL treatments were applied. The treatments began during the first week of March and ended in mid-Apr. 1985. The experiment was repeated in 1986.

The CO₂ concentration within each 24-m² glasshouse compartment was maintained at 330 (control, atmospheric concentration), 900, or 1500 ppm. Within each compartment, there were two repetitions of two light treatments [natural or supplemental light at 150 μmol·s⁻¹·m⁻² (PAR)]. Experimental units were randomly distributed in each compartment and contained two flats of 50 transplants.

CO₂ concentration was monitored (± 50 ppm) in each compartment with an infrared gas analyzer (Priva Computers, APBA 250 E CO₂). Carbon dioxide was added during the photoperiod from a compressed supply of pure gas (Liquid Air Canada). Natural radiant energy was measured with a LI-COR photometer (LI-185) with a quantum sensor. When natural light was <250 μmol·s⁻¹·m⁻², lamps were turned on. Supplemental lighting, supplied by 400-W HPS lamps, provided 150 μmol·s⁻¹·m⁻² at plant level for a photoperiod of 16 hr. Over the period of the experiment, SL contributed 20% of the light energy received by the transplants. Ambient temperature was maintained at 24°/ 18° ± 1°C day/night; when air temperatures were higher than 25°, CO₂ enrichment was stopped and forced-air ventilation started.

After 0, 10, 20, and 30 days of treatment, plants were harvested and washed to remove soil particles. Leaf and root fresh and dry weights, leaf area, and total number of roots and new leaves longer than 3 cm and characteristically with green petiole (reddish color when developed in vitro) were obtained on 25 plants randomly sampled at each of four dates from each experimental unit. Leaves and roots were dried for 48 hr at 70°C for measurement of dry weights. Leaf area was measured with a LI-COR portable area meter (Li-3000).

This experiment was done with the cultivar Kent during Spring 1985 and with ‘Kent’ and ‘Honeyoye’ in 1986. The experimental design was a split-plot with CO₂ concentration as main plots replicated over 2 successive years and supplemental light as subplots replicated two times. An analysis of variance (ANOVA) was performed using a general linear model (20) on the means of each sample. Orthogonal 1 degree of freedom contrasts (linear and quadratic) were performed to determine the response of growth parameters to CO₂ levels. An F test was used to compare effect of light levels.

Results and Discussion

Since strawberry plantlets were placed in a humidity tent for 1 week, there were minimal losses during CE and SL treatments. The survival was almost 100% with all CO₂ concentrations in the absence of SL. However, the survival under SL was 85% with ‘Kent’ and 65% with ‘Honeyoye’ over the three sampling dates. Plant loss caused by SL occurred mainly in the first few days of treatment and was characterized by a rapid drying of in vitro-produced leaves and ultimately by the destruction of the growing point.

When transplants were removed from the humidity tents, they did not all have a first new leaf formed and were therefore very susceptible to wilting when subjected to water stress. Sutter and Langhans (25) and Grout and Aston (11) showed that in vitro-produced leaves of carnation and cauliflower, respectively, had no structured epicuticular wax and were very susceptible to dessication. These authors (11) also showed that these leaves failed to become photosynthetically active and degenerated. New raspberry leaves, produced in vivo, have a transitional anatomy that allows for improved photosynthetic capacity and water regulation (8). Upon removal from humidity tents, ‘Kent’ had 0.81 ± 0.07 new leaves per plant formed, whereas ‘Honeyoye’ had only 0.49 ± 0.07 new leaves per plant formed. It appears that if a plant is producing its transitional leaves later, after transfer to normal conditions, it cannot regulate its water loss and degenerates before new leaves become functional and support growth. This condition was specifically apparent with ‘Honeyoye’. Presumably, ‘Honeyoye’ would require a prolonged period under humidity tents or a delay between removal from high humidity environment and placement under high light.

Effect of light. There was no interaction between SL of 150 μmol·s⁻¹·m⁻² and CE of 300, 900, and 1500 ppm on each sampling date for all growth parameters. Consequently, data from CO₂ treatments were combined for comparing the effect of SL. Supplemental light had a significant effect on the dry matter accumulation of strawberry plantlets at days 20 and 30 (Table 1). The SL treatment produced higher dry weights than no SL by 125% and 139%, respectively, for leaves and by 100% and 200%, respectively, for roots. Light also resulted in significant 38%, 45%, and 49% increases in total leaf area after 10, 20, and 30 days of treatment, respectively. Similar results had been found by Donnelly and Vidaver (9), where high light intensities increased leaf area of ex vitro raspberry plantlets during acclimatization. The root : shoot ratio was reduced with SL after 10 and 20 days of treatment (Table 2). In other words, the relative growth rate (RGR) was higher for leaves as compared to roots for the first two time intervals (0–10 and 10–20 days). Not only was more dry weight accumulated, but also the whole plant grew faster. At the end of the experiment, SL maintained a high growth rate in leaves and roots with 0.101 and 0.069 g/g per day, respectively, whereas it was lower under natural light with 0.066 and 0.038 g/g per day, respectively. Supplementary lighting has been found to increase root to shoot ratio in a number of species [tomato (13) and cucumber (7)]. In our experiment, root : shoot ratio decreased. This decline is particularly evident over the second sampling period (days 10–20). Similar results have been obtained with several species of bedding plants receiving SL (4).
Table 1. Growth of ex vitro ‘Kent’ strawberry plants maintained under three concentrations of atmospheric CO$_2$ (330, 900, and 1500 ppm) and two levels of light (ambient, supplemented) during acclimatization.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry weight</th>
<th>Root growth</th>
<th>Leaf area (cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$ (ppm)</td>
<td>Days of treatment</td>
<td>Days of treatment</td>
<td>Days of treatment</td>
</tr>
<tr>
<td>330</td>
<td>0</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>900</td>
<td>0</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>1500</td>
<td>0</td>
<td>0.03</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Significance:
- Linear
- Quadratic

Light ($\mu$mol·s$^{-1}·m^{-2}$):
- Ambient
- 150 SL

Table 2. Effect of three concentrations of atmospheric CO$_2$ and two levels of light on growth analysis of in vitro cultured ‘Kent’ strawberry during acclimatization.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative growth rate (g/g per day)</th>
<th>Net assimilation rate (g/LA per m$^2$ per day)</th>
<th>Leaf area ratio (dm$^2$/LA per g dry wt)</th>
<th>Root : shoot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$ (ppm)</td>
<td>Leaf (g)</td>
<td>Root (g)</td>
<td>Leaf (g)</td>
<td>Root (g)</td>
</tr>
<tr>
<td>330</td>
<td>0-10</td>
<td>10-20</td>
<td>20-30</td>
<td>0-10</td>
</tr>
<tr>
<td>900</td>
<td>0.04</td>
<td>0.11</td>
<td>0.05</td>
<td>0.039</td>
</tr>
<tr>
<td>1500</td>
<td>0.06</td>
<td>0.13</td>
<td>0.08</td>
<td>0.052</td>
</tr>
</tbody>
</table>

Significance:
- Linear
- Quadratic

Light ($\mu$mol·s$^{-1}·m^{-2}$):
- Ambient
- 150 SL

been produced to create a positive carbon balance. Root growth was minimal in the early period following transfer to treatment (Table 1). Root relative growth rate (RGR) with supplemental light was similar to root RGR without SL over the first 10 days, whereas the leaf RGR was higher with SL than without (Table 2). Our results show a significant effect of SL at 150 $\mu$mol·s$^{-1}·m^{-2}$ on the accumulation of leaf dry weight from days 10 to 20. This accumulation resulted in an increase of 200% in root dry weight by day 30 for the SL treatment. Over the 30 days of treatment, similar rates of root growth did not appear in unsupplemented plants. Consequently, 20 days after the beginning of the treatment, plantlets grown under SL had a leaf and root dry weight of 0.27 and 0.08 g/plant, respectively, which was higher than the final leaf (0.23 g/plant) and root (0.05 g/plant) dry weight of nonlighted plants. The period needed to obtain a plant of similar size to a fully acclimatized strawberry plantlet was reduced by more than 10 days in the lighted treatment (Table 1). Plants were considered fully acclimatized when
they had reached at least a leaf area of 30 cm² and a dry weight of 0.3 g. Plants having such characteristics were shown to survive well after transfer to soil (Y. Desjardins, A. Gosselin, and M.-J. Trudel, unpublished results). Light intensity influences pigment content and dry weight accumulation of raspberry (8). Moreover, Chabot et al. (6) showed that total light energy received during the day had a greater influence on leaf adaptation of strawberry than peak photosynthetic photon flux. According to these authors (15), the physiological and anatomical characteristics of the leaves were determined by the conditions prevailing for the longest period during their development. Under our conditions, SL favored dry weight accumulation over leaf expansion, since leaf area ratio decreased significantly (Table 2). It is possible that these leaves had an increased photosynthetic capacity and were probably better adapted to the field conditions and higher light levels normally encountered in late spring. It remains to be seen, however, if productivity would be improved under field conditions.

**Effects of CO₂.** Carbon dioxide enrichment did not have a significant effect on dry matter accumulation after 10 days of treatment (Table 1). After 20 days, there was a significant quadratic trend in the increase in leaf area, with the 900-ppm treatment superior to 330- and 1500-ppm treatments. At that time, the 900-ppm treatment was producing the highest leaf and root dry weight, but this difference was not significant. RGR of leaf and roots did not differ significantly among CE treatments. After
Growth was small in the early stages of treatment and that the to synthesis increased rapidly and did not reach such a plateau.

It has been reported (17) that CO₂ had a greater effect on root growth than on shoot growth. We did not find this response in our experiment. The root:shoot ratio declined rapidly from day 10 to days 20 and 30 for the 330- and 1500-ppm treatments, whereas it remained constant on day 20 for the 900-ppm concentration (Table 2).

The 900-ppm CO₂ treatment appeared as the optimum level of enrichment early in the development of the first leaf produced ex vitro, both for the dry weight accumulation and the leaf and root RGR, even if differences were not significant. The 900-ppm treatment was also preferable to the 1500-ppm treatment with greenhouse tomatoes (28). This study showed negative effect of 1500 ppm of CO₂ on growth and yield of greenhouse tomatoes. The comparable results obtained with 900- and 1500-ppm treatments supplemented with light gave comparable increases in growth parameters, with 1500 ppm offering a larger increase in leaf and root dry weight with 'Kent' and 900-ppm CO₂ offering a larger increase with 'Honeyoye' (Fig. 1).

When the data were analyzed with 'Kent' during 1986 only, a synergistic effect between SL and CE on growth of strawberry transplants appeared. A significant interaction between CE and SL was found for root dry weight (P = 0.04). Both the 900- and 1500-ppm treatments supplemented by light increased the leaf and root dry weight by a much larger extent than CE or SL separately (Fig. 1 A and C). For instance, root dry weight of 'Kent' was increased by 400% and 360% at 1500 and 900 ppm CO₂, respectively, in presence of SL, whereas without light, the resulting increase was of 40% and 80%, respectively. Furthermore, SL induced a 140% increase in root dry weight. A similar synergistic effect between CO₂ and light was observed for leaf dry weight. Supplementary lighting at the 300-ppm level caused a larger increase in dry weight than CE at 900- or 1500-ppm without light (Figs. 1 A–C and 2). The effect of supplemental light on growth was more pronounced than the effect of CE. Hurd (13) showed a similar phenomenon, with young tomato growth being much larger with SL as compared to CE. As demonstrated previously, CE and SL contributed to a reduction of the time required to obtain a fully grown transplant. A definite synergistic effect occurred between CO₂ and light. A plant of similar size to those under 300-ppm CO₂ and no SL was produced 15 days earlier with 900-ppm CO₂ and light and 13 days earlier with 1500-ppm CO₂ and light (Fig. 2).

Lakso et al. (17) found that CE increases shoot and root growth and reduces the time to acclimatize grapevine plantlets. However, this experiment was at relatively low light intensity in controlled humidity chambers with nonrooted plantlets. Our results, which were obtained under normal greenhouse conditions, agree with those of Lakso et al. (17). More specifically, they indicate that increased growth can be obtained under stress-hardening regimes used by commercial growers.

Our results demonstrated the benefit in enriching the atmospheric CO₂ to the levels of 900 ppm or higher, since these treatments produced growth responses superior to those at 330 ppm. These results also clearly demonstrated that HPS SL increased growth of in vitro strawberry plantlets during acclimatization. Moreover, SL was very important for the CO₂ treatment to be fully effective. Proportionally, light was more effective than CO₂ to increase growth of strawberry plantlets (Fig. 2). Under our natural growing conditions in early spring, low light was probably a limiting factor reducing the rate of growth during acclimatization. Both 'Kent' and 'Honeyoye' responded similarly to CE but differently to supplemental light. Care should

Fig. 2. Effect of three concentrations of atmospheric CO₂ and two levels of supplemental light (CO₂, ppm; light, µmol·s⁻¹·m⁻²) on plant dry weight of 'Kent' strawberry in 1986. Bars represent ±s.e.
be taken to maintain plantlets under humidity tents until the first transitional leaf is formed. It is possible that supplemental light supplied excessive PPF for 'Honeyoye' and reduced growth.

Changes in anatomy and physiology brought about by CE and SL should improve adaptation of plants to field conditions. Hence, it would be of great interest to determine how these treatments alter the anatomy and physiology and to what extent these changes influence growth and survival in the field. We showed that growth was hastened by SL and CE. However, we did not determine if this increase in growth was a result of an advance in the autotrophic status of the plant or of an increase in growth once autotrophic status was obtained by the plantlets. To answer this question, gas exchanges of developing leaves under treatments will have to be measured. The effect of CE and SL should be investigated with other species, such as micropropagated foliage plants and woody plants, where the acclimatization period could be reduced and transfer to field conditions improved.

**Literature Cited**


