Growth Characteristics, Yield and Fruit Soluble Carbohydrate Content of Hydroponically Grown Strawberry with Carbon Dioxide Fertilization

Hye-Rran Ryu¹, Eun-Young Choi†, and Ki-Young Choi²*

¹Department of Agricultural Science, Korea National Open University, 86 Daehak-ro, Jongro-gu, Seoul 03087, Korea
²Department of Controlled Agriculture, Kangwon National University, 1 Kangwondae-gil, Chuncheon, Gangwon-do 24341, Korea

*Corresponding author: choiky@kangwon.ac.kr
†This author contributed equally to this study.

Abstract

This study aimed to investigate the effects of CO₂ fertilization on vegetative and reproductive growth and fruit yield of plants grown in a commercial strawberry farm. Leaf length and width were 14.3 and 12.2% lower in the CO₂ fertilization than in the control (without CO₂ fertilization), respectively. Photosynthesis rate, stomatal conductivity, and transpiration rate were significantly lower in the CO₂ fertilization than that in the control treatment. The fresh and dry weights of fruit grown in the CO₂ fertilization were 19.9% and 27.3% higher, respectively, in January. Total yield was higher at 6 to 8 weeks when the CO₂ was applied, but it was not significant. When the fruit carbohydrate concentration was measured in triplicate at a 3-day intervals from the 6th to 13th of January, the sucrose concentration was initially lower in the CO₂ fertilization than that in the control. However, at the second and last time points, it was higher, about 41 µg·g⁻¹ (30 µg / fruit) and 21 µg·g⁻¹ (89 µg / fruit), respectively, than the control. Both glucose and fructose were also about 14 µg·g⁻¹ (68-73 µg / fruit) higher than the control treatment groups at the last time point. Vertical and horizontal hardness, soluble sugar content, and acid content of fruit grown with CO₂ fertilization were slightly higher than the control, although there was no statistical significance. Together, these data suggest that a long-term CO₂ fertilization for a protected cultivation of strawberry crop can reduces vegetative growth, but improves reproductive growth and fruit quality.

Additional key words: fructose, glucose, photosynthesis, stomatal conductivity, sucrose, transpiration rate

Introduction

Strawberry (Fragaria × ananassa Duch.) is one of the most popular fruits in the world and is a good source of vitamins, minerals, and anthocyanin (Cordenunsi et al., 2002). The approximate area and production yield of strawberries cultured in protected facilities in Korea is 6,403 ha and 195,964 tons (MAFRA, 2016), with and about 2,647 tons exported in 2015 (aT, 2016).
Proper light intensity, temperature, humidity, and carbon dioxide (CO\textsubscript{2}) are important components of growth environments that can affect fruit quality and yield. CO\textsubscript{2} fertilization is commonly used on commercial farms because the CO\textsubscript{2} saturation point of most horticultural crops is higher than the atmospheric CO\textsubscript{2} concentration. The application of CO\textsubscript{2} to strawberry crops was found to increase fruit sucrose content and yield up to 30-40\% (Jeong et al., 1996). Previous studies provided proper ranges of CO\textsubscript{2} fertilization in strawberry cultivation; Mortensen (1987) suggested that elevated CO\textsubscript{2} levels of up to 700-900 mg·L\textsuperscript{-1} can be applied in winter and early spring for commercial strawberry cultivation, and Jeong et al. (1996) suggested that CO\textsubscript{2} fertilization with 900 mg·L\textsuperscript{-1} at 20°C was an optimal condition for strawberry cultivation. Conversely, some studies found that high CO\textsubscript{2} concentration reduced yield and fruit quality (Koch and Mooney, 1996; Lee and Lee, 1994; Yu et al., 2015). When the two strawberry cultivars ‘Benihoppe’ and ‘Frandy’ were cultivated under high CO\textsubscript{2} concentrations in a closed ecosystem, 2,000 mg·L\textsuperscript{-1} CO\textsubscript{2} (over saturation point, 1,500 mg·L\textsuperscript{-1}) inhibited the net photosynthetic rate of ‘Benihoppe’ cultivar by 12.4\% than that of ‘Frandy’ (2.3\%) (Yu et al., 2015).

In the long-term (over 50 d), CO\textsubscript{2} fertilization with 800 mg·L\textsuperscript{-1} in tomato cultivation increased the photosynthetic rate until 20 d but it had no effect ever since 30 d after the treatment and decrease more than untreated plant (Lee and Lee, 1994). The biochemical basis for the decline of photosynthesis with long-term elevated CO\textsubscript{2} treatments is not clearly understood, although sink imbalance, carbohydrate accumulation, and negative feedback mechanisms have been proposed as models (Bowes, 1991; Stitt, 1991). An increase of 600 mg·L\textsuperscript{-1} in the CO\textsubscript{2} level promoted leaf net photosynthesis of strawberry, but a further increase to 750 and 900 mg·L\textsuperscript{-1} decreased net CO\textsubscript{2} assimilation, which was accompanied by a distinct decrease of optimal quantum yield and macronutrient deficiency (Keutgen et al., 1997).

Although there are numerous studies of effects elevated CO\textsubscript{2} has on photosynthesis in plants grown in a large range of experimental conditions, little information is available on the effects of elevated CO\textsubscript{2} concentrations have on strawberry productivity, which would benefit the strawberry industry. The aims of this study were to provide information on the effects of CO\textsubscript{2} fertilization on the vegetative and reproductive growth and yield in commercial strawberry cultivation.

Materials and Methods

Plant Growth and Environments

The experiment was carried out in two double plastic houses [100 m (L) × 10 m (W) × 3.3 m (H)] located in Kyoungnam Sacheon-si starting on the 5th of September 2016. Nine hundred strawberry plants ‘Maehyang’ were planted in two rows with an interval of 15-16 cm on a cocopeat substrate [dust:chip = 7:3 (v/v), Kims Trade Ltd., India] placed in beds [0.3 m (W) × 80 m (L) × 0.2 m (H)] 1.0 m from the ground. The UOS nutrient solution developed for strawberry cultivation at the University of Seoul (UOS; Lee et al., 2017) was supplied 3-4 times in 2-3 min duration each day at the EC 1.0-1.2 dS·m\textsuperscript{-1} level and at pH 5.8.

Carbon Dioxide Treatments

Each plastic house had air circulating and CO\textsubscript{2} sensor systems (Mirae sensor, Seoul, Korea) to monitor the CO\textsubscript{2} concentration. The CO\textsubscript{2} gas was applied from the 12th of December 2016 to the 12th of March 2017. The CO\textsubscript{2} concentration was controlled every 15 min from 7 a.m. to 9 a.m. and then every 30 min from 9 a.m. to 3 p.m. with a
maintained set value of 600-800 mg·L\(^{-1}\) during the day (Fig. 1). The CO\(_2\) gas was automatically injected into the house from a LPG gas cylinder (2.8 ± 0.5 kPa) that was used for a gas heater system (KCH-20S, Katsura, Kyoto, Japan) and could produce 5.0 kg·h\(^{-1}\) CO\(_2\) gas. For the control treatment, the CO\(_2\) was applied to the house through natural ventilation when the temperature was higher than 23°C.

**Measurements**

Ten plants in each treatment were labeled in developing leaves and used for the measurement of leaf length and width without destruction of the plant. The photosynthetic rate was measured in four fully expanded leaves from plants in each treatment using a CO\(_2\) gas analyzer (LI-6400XRT, LI-COR Biosciences, Lincoln, NE, USA). Block temperature and standard CO\(_2\) concentration were kept at 25°C and 400 mg·L\(^{-1}\), respectively. The artificial photosynthetically active radiation (PAR) was gradually increased from 100 to 2,000 µmol·m\(^{-2}\)·s\(^{-1}\) during all measurements.

Strawberry fruits were harvested at 2-day intervals when 60-70% of the fruits had turned red. At each harvest, yield data (fruit fresh weight) were determined. Acid and soluble sugar content was measured in 15 fruits for each treatment, which were harvested from the first, second, and third clusters using a picket brix-acidity meter (PAL-BX/ACID, Atago, Tokyo, Japan). The vertical and horizontal hardness of fruit were measured in the outer layer, about 5-10 mm apart from the fruit center using a meter equipped with a 3 mm probe (FR-5105, Rheo Meter Compac-100 II, Sun Scientific Co., Tokyo, Japan) after cutting 0.5 cm off the top and bottom of the fruit.

Fifteen fruits from the first and second clusters from fifteen plants in each treatment were labeled for the measurement of soluble carbohydrates (sucrose, glucose, and fructose). Samples were taken three times at 3-day intervals on the 6th and 13th of January 2017. The selected fruits were of similar size (average fruit length: 5-7 mm, average fruit diameter: 11-12 mm). As soon as the fruits were harvested, they were kept on dry ice prior to being freeze-dried. Then, the freeze-dried fruit was analyzed for soluble carbohydrates using HPLC (Ultimate 3,000, Dionex, Sunnyvale, CA, USA), a detector (Shodex RI-101, Showa Denko, Tokyo, Japan), sugar-pak column (300 × 6.5 mm, Waters, Milford, MA, USA), and analysis software (Chromeleon 6.0 Chromatography Data System Software, Dionex, Sunnyvale, CA, USA).

The data were analyzed for statistical differences using the SAS package (statistical analysis system, version 9.3, SAS)
Growth Characteristics, Yield and Fruit Soluble Carbohydrate Content of Hydroponically Grown Strawberry with Carbon Dioxide Fertilization

Institute Inc.). The data were analyzed using ANOVA (analysis of variance) and t-test at 5%, *p < 0.05.

Results and Discussion

After the 30th day of CO$_2$ fertilization, leaf lengths were 13.21% and 15.45% lower on the 10th of Jan. and 4th of Feb., respectively, with CO$_2$ fertilization than in the control conditions (Fig. 2A). Leaf width was also significantly lower in the plants that received CO$_2$ fertilization than that in the control. Leaf width was 12.70% and 11.81% lower on the 10th of Jan. and 4th of Feb., respectively, compared to plants that received the control treatment (Fig. 2B). This period was done at the same time vegetative and reproductive growth in strawberry.

The photosynthetic rate in strawberry plants was measured by increasing photosynthetically active radiation (PAR) gradually from 100 to 2,000 µmol·m$^{-2}$·s$^{-1}$. We observed significantly lower rates in plants treated with CO$_2$; rates of 30.9, 29.3, 31.1, 30.6, 29.9, 26.6, and 31.0% were measured at the PAR levels 100, 250, 500, 750, 1,000, 1,500, and 2,000 µmol·m$^{-2}$·s$^{-1}$, respectively (Fig. 3A). Stomatal conductivity was 22.8, 21.8, and 22.0% at PAR 1,000, 1,500, and 2,000 µmol·m$^{-2}$·s$^{-1}$, respectively, with CO$_2$ fertilization, which was significantly lower than that measured in the control plants (Fig. 3B). Transpiration rates were slightly lower in the CO$_2$ fertilization group at PAR between 100-1,500 µmol·m$^{-2}$·s$^{-1}$; specifically, the rate was 13.24% lower at PAR 2,000 µmol·m$^{-2}$·s$^{-1}$ (Fig. 3C). The leaf to air vapor pressure deficit (VpdL) and the intercellular CO$_2$ concentration were higher with CO$_2$ fertilization compared to the control treatment (Fig. 4).

Fig. 2. Changes in leaf length and width in plants grown in CO$_2$ fertilization or control conditions. Vertical bars indicate standard error of the mean (n = 6).
These results support previous results that were reviewed by Pospisilova and Catsky (1999), in which long-term elevated CO$_2$ led to a decrease in stomatal conductance and transpiration rate, although the decline can be affected by environmental factors such as irradiance, temperature, and vapor pressure deficit. Lee and Lee (1994) also postulated that a decrease in photosynthesis under high CO$_2$ (800 mg·L$^{-1}$) conditions was associated with an increase of stomata resistance and decrease of transpiration rate.

The fresh and dry weights of fruits with CO$_2$ fertilization were both significantly higher (19.9% and 27.3%) than the untreated controls in January, but not in February (Table 1). There was also no significant difference in fruit ripening between the treatment and control groups (Table 1). Total yield was higher at 6 to 8 weeks when the CO$_2$ was applied (Fig. 5), but it was not significant.

When the fruit carbohydrate concentration was measured, the initial sucrose concentration was lower in the plants treated with CO$_2$ than in the control plants. However, at the second and last time points, this concentration was higher, about 41 µg·g$^{-1}$ (30 µg/fruit) and 21 µg·g$^{-1}$ (89 µg/fruit), respectively, than the control. Both glucose and fructose were also higher, measuring 14 µg·g$^{-1}$ (68-73 µg/fruit) at the final time point (Table 2). Vertical and horizontal fruit hardness

---

**Fig. 3.** Photosynthesis rate (Ps, A), stomatal conductivity (SC, B), and transpiration rate (TR, C) in leaves from plants grown in CO$_2$ fertilization or control conditions. Vertical bars indicate standard error of the means from four replicates measured in January.
Growth Characteristics, Yield and Fruit Soluble Carbohydrate Content of Hydroponically Grown Strawberry with Carbon Dioxide Fertilization

The acid content of fruit grown in the CO$_2$ fertilization was higher than the control when measured in January (Table 3). These data support a previous study in which CO$_2$ fertilization induced reproductive growth and improved fruit quality and yield (Nilsen et al., 1983). Our results show that the CO$_2$ fertilization during hydroponic strawberry cultivation decreased leaf growth, which is consistent with the lower photosynthesis, stomatal conductivity, and transpiration rate; and the higher fruit carbohydrate content and fruit yield. In contrast, photosynthesis was more active in the control plants, which also had higher stomatal conductivity and transpiration, consistent with the greater leaf growth and lower fruit yield.

Soluble sugar is the most critical factor for fruit quality, 99% of which in strawberry fruits is sucrose, glucose and fructose (Montero et al., 1996). Reduced leaf growth accompanied by lower photosynthesis, transpiration, and stomatal conductivity with CO$_2$ fertilization may have improved fruit growth by increasing photosynthates and water translocation.

**Table 1.** Fresh and dry weights and ripening of fruits grown in CO$_2$ fertilization or control conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Ripening (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO$_2$</td>
<td>Control</td>
<td>CO$_2$</td>
</tr>
<tr>
<td>January</td>
<td>18.73*</td>
<td>15.01*</td>
<td>2.13*</td>
</tr>
<tr>
<td>February</td>
<td>14.72</td>
<td>13.45</td>
<td>1.84</td>
</tr>
</tbody>
</table>

* Asterisks indicate significant differences (t-test, *p* < 0.05).
* Each value is the mean from 10 replicates.

Fig. 4. VpdL (leaf to air vapor pressure difference, A) and intercellular CO$_2$ concentration (IC, B) of leaves from plants grown in CO$_2$ fertilization or control conditions. Vertical bars indicate standard error of the means from four replicates measured in January.

![Graph A: VpdL vs. CO$_2$ concentration](image)

![Graph B: IC vs. PAR](image)
Growth Characteristics, Yield and Fruit Soluble Carbohydrate Content of Hydroponically Grown Strawberry with Carbon Dioxide Fertilization

CO₂ fertilization in strawberry increased sugar content but decreased organic acid content (Jeong et al., 1996; Wang et al., 2003). These results indicate that CO₂ has an effect on carbohydrate and organic acid metabolism. On the other hand, ‘Maehyang’ strawberry increased not only fruit hardness and sugar content but also acidity in CO₂ fertilization compared to those in the non-CO₂ control treatment after harvest (Choi et al., 2017).

Some studies suggest that feedback inhibition of photosynthesis with the long-term CO₂ fertilization can restrict root growth and lead to nutrient insufficiency, carbohydrate accumulation and, subsequently, diminished photosynthetic capacity, which usually results from a decreased Rubisco activity (Bowes, 1991; Sicher et al., 1995).

**Fig. 5.** Changes in fruit dry weight of 1st fruits from second fruit clusters, and total fruit yield in CO₂ fertilization or control conditions.

**Table 2.** Concentration and content of sucrose, glucose, and fructose of fruit grown with CO₂ fertilization or control treatments. Measurements were conducted three times at 3-day intervals from 6th to 13th of January on the 15 first fruits from the second cluster. Fifteen plants were sampled from each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CO₂</th>
<th>Control</th>
<th>CO₂</th>
<th>Control</th>
<th>CO₂</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg·g⁻¹ fruit DW</td>
<td>µg/fruit</td>
<td>µg·g⁻¹ fruit DW</td>
<td>µg/fruit</td>
<td>µg·g⁻¹ fruit DW</td>
<td>µg/fruit</td>
</tr>
<tr>
<td>6-Jan</td>
<td>82⁺</td>
<td>115</td>
<td>131</td>
<td>136</td>
<td>163</td>
<td>167</td>
</tr>
<tr>
<td>10-Jan</td>
<td>179</td>
<td>138</td>
<td>156</td>
<td>194⁺ yan</td>
<td>178</td>
<td>213⁺ y</td>
</tr>
<tr>
<td>13-Jan</td>
<td>190</td>
<td>169</td>
<td>196⁺ y</td>
<td>182</td>
<td>212⁺ y</td>
<td>198</td>
</tr>
<tr>
<td>6-Jan</td>
<td>46</td>
<td>57</td>
<td>71</td>
<td>66</td>
<td>88</td>
<td>80</td>
</tr>
<tr>
<td>10-Jan</td>
<td>166</td>
<td>136</td>
<td>142</td>
<td>186</td>
<td>161</td>
<td>203</td>
</tr>
<tr>
<td>13-Jan</td>
<td>254⁺ yan</td>
<td>165</td>
<td>261⁺ y</td>
<td>193</td>
<td>283⁺ y</td>
<td>210</td>
</tr>
</tbody>
</table>

*Each value is the mean of five replicates.
⁺Asterisks indicate significant differences (t-test, *p < 0.05).
Although long-term CO\(_2\) fertilization in the present study affected photosynthetic activity and transpiration rate, the CO\(_2\) concentration we applied was not as high as that used in previous studies. Thus, we speculate that the long-term CO\(_2\) fertilization of a protected strawberry crop can reduce vegetative growth and improve reproductive growth and fruit quality.

### Literature Cited

**aT (Korea Agro-Fisheries & Food Trade Corp)** (2016) Import and export statistics. aT, Seoul, Korea


MAFRA (Ministry of Agriculture, Food and Rural Affairs) (2016) Status of vegetable production statistics. MAFRA, Sejong, Korea


---

**Table 3.** Vertical and horizontal hardness, sugar content, and acid content of fruit grown in CO\(_2\) fertilization or control conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vertical hardness (N)</th>
<th>Horizontal hardness (N)</th>
<th>Sugar content (°Brix.)</th>
<th>Acid content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO(_2)</td>
<td>Control</td>
<td>CO(_2)</td>
<td>Control</td>
</tr>
<tr>
<td>Jan.</td>
<td>2.95(^z)</td>
<td>1.92</td>
<td>1.93</td>
<td>1.45</td>
</tr>
<tr>
<td>Feb.</td>
<td>2.95</td>
<td>2.31</td>
<td>1.93</td>
<td>1.43</td>
</tr>
</tbody>
</table>

\(^z\)Each value is the mean of five replicates.

\(^*\)Asterisks indicate significant differences (t-test, *p* < 0.05).