Effect of Root-zone Heating on Root Growth and Activity, Nutrient Uptake, and Fruit Yield of Tomato at Low Air Temperatures

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Low-cost heating is needed to reduce chilling injuries, heating costs, and CO2 emission during greenhouse tomato production. To acquire information about the physiological and morphological effects of root-zone heating, an economical option at low air temperatures, we grew tomato plants on a nutrient film technique hydroponic system in a heated nutrient solution. We investigated the effects of short-term root-zone heating after transplanting and long-term heating until harvest. We measured short-term plant growth, nutrient uptake, root activity (xylem exudation and root respiration rates), root indole-3-acetic acid (IAA) concentration, internal root structure, and long-term fruit weight and dry matter distribution. The minimum root-zone temperature was maintained at 16.6°C, while the minimum air temperature (5.9°C) and the minimum root-zone temperature in the control (5.8°C) were lower than optimal. After 7 days of root-zone heating, root dry weight and relative growth rate increased compared with those of the control, accompanied by increased mineral nutrient uptake and xylem exudation. These changes may explain the increased shoot growth after 21 days of heating. In roots, development of the epidermis and stele, including the xylem, was promoted by heating, in contrast to previous research on root-zone cooling at high air temperature, which promoted xylem-specific development. Although the proportion of dry matter distributed to the fruit was not changed by root-zone heating, individual fruit size and total yield were higher than in the control due to a higher total dry weight in the heating treatment. Our results suggest that root-zone heating is an effective low-cost heating technology at low air temperature because of its effects on root activity, growth, and fruit yield, but that the mechanisms may differ from those in root-zone cooling at high air temperature.

Key Words: chilling injury, greenhouse production, relative growth rate, root structure, xylem exudation rate.

Introduction

For greenhouse tomato production in winter, a heating system is used to avoid the growth inhibition (Criddel et al., 1997; Hoek et al., 1993; Smeets and Garretsen, 1986) and decreased yield (Adams et al., 2001) caused by low temperatures. However, heating cost has become expensive due to soaring fossil fuel prices in recent years. When this is combined with increasing public concern about CO2 emissions, the development of an ecological and economical heating technique is needed.

Root-zone heating has been proposed as a solution. This approach can reduce fuel consumption by heating only the root zone rather than the air (Jones et al., 1978). It makes it possible to obtain a yield equivalent to that achieved with conventional temperature management (Jones et al., 1978; Morgan and O’Haire, 1978). The effects of root-zone temperature on tomato growth have been investigated. For example, concentrations of internal plant growth regulators (Ali et al., 1996), carbon distribution to the roots (Shishido and Kumakura, 1994), root growth (Tindall et al., 1990), water and nutrient uptake (Gosselin and Trudel, 1983b), and the leaf area expansion and carbon dioxide exchange rate (Hurewitz and Janes, 1983) were decreased by low root-zone temperature. However, these results were obtained under optimal air temperatures, and few investigations have been conducted under low air temperatures (Jones et al., 1978; Orchard, 1980), especially in terms of the effects on yield. In addition, no studies have investigated changes in root morphology caused by root-zone heating.
In our previous study, we examined the effect of root-zone cooling at high air temperature (Kawasaki et al., 2013). We found that root growth and root activity were enhanced by root-zone cooling, leading to subsequent promotion of shoot growth. It is important to reveal the physiological and morphological mechanisms that determine the effects of temperature on roots, as this would provide fundamental knowledge to support the development of root-zone temperature-control techniques suitable for year-round greenhouse production and energy conservation. In the present study, we grew tomato plants in winter, with root-zone heating, and investigated short-term plant growth, root activity, and root morphological changes after transplanting and long-term fruit yield and characteristics.

Materials and Methods

Plant materials, growth conditions, and temperature management

Seeds of tomato (Solanum lycopersicum L.) ‘Momotaro-Yoku’ (Takii Seed Co., Ltd., Kyoto, Japan) were sown in 72-cell trays filled with vermiculite, and seedlings were grown for 3 weeks in a growth chamber with day/night air temperatures of 25/20°C. The seedlings were then transplanted into a nutrient-film-technique hydroponic system in a greenhouse on 7 December, 2012. Plants were pinched above the third truss, leaving two true leaves above the truss. To promote fruit set and growth, 80 μM 4-chlorophenoxyacetic acid was sprayed after three flowers had appeared on each truss.

Ventilation was used when the air temperature exceeded 25°C, and air temperature was maintained at 5°C or more using heating pipes. The nutrient solution, renewed every 2 weeks, contained 158 (µg·g⁻¹) NO₃-N, 4 NH₄-N, 31 P, 267 K, 110 Ca, 24 Mg, 2.1 Fe, 0.6 Mn, 0.4 B, 0.07 Zn, 0.02 Mo, and 0.02 Cu. The root-zone heating treatment began immediately after transplanting. Plants (n = 12) were cut off below the first true leaves, the cotyledons were removed, and we collected the xylem exudate for 10 min in pre-weighed absorbent cotton for later weighing (Yamaguchi et al., 1995). Some well-developed lateral roots were immersed from base to tip in 500 mL of stirred nutrient solution (the same solution used for cultivation), and the rate of oxygen consumption was measured for 10 min with an oxygen electrode (D-55; HORIBA, Ltd., Kyoto, Japan) at 25°C to calculate the root respiration rate. Xylem exudation and the root respiration rate were measured between 09:00 and 12:00. Shoots and roots were dried at 80°C for at least 48 h and then weighed to allow calculation of the relative growth rate (RGR), which was calculated using the following equation:

\[
RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}
\]

where \(W_2\) and \(W_1\) are the dry weights at times \(t_2\) and \(t_1\), respectively.

Ripened fruits (n = 24) from each treatment were harvested and weighed to determine the fruit yield, and the soluble solid content was measured using a digital refractometer (PR-101; ATAGO Co., Ltd., Tokyo, Japan). At the end of harvesting, dry weights of shoots and roots were measured as described above and the dry matter distribution among the plant parts was calculated.

Mineral nutrient analysis and quantification of auxin content

We pulverized 50 mg of dried samples of shoots and roots (n = 12) and ashed the samples in 10 mL of nitric acid and 2 mL of hydrogen peroxide at 180°C for 20 min in a microwave ashing system (START D; Milestone-General Co., Ltd., Kanagawa, Japan). P, K, Ca, and Mg were measured by inductively coupled plasma atomic-emission spectrometry (SPS7700; Hitachi High-Tech Science Corporation Co., Ltd., Tokyo, Japan). N was measured using a CN analyzer (JM1000CN; J-Science Lab Co., Ltd., Kyoto, Japan). For analysis of the indole-3-acetic acid (IAA) content, fresh roots (n = 4) were sampled using the same method as in our previous study (Kawasaki et al., 2013).

Root morphology

On day 21, lateral root samples (n = 3) near the root tip from each treatment were fixed in 40% formaldehyde/acetic acid/ethanol/water = 1:1:9:9 (volume basis). Then, samples were dehydrated in a graded alcohol series (70%, 80%, 90%, and 100%) and embedded in Technovit 7100 resin ( Heraeus Kulzer, Wehrheim, Germany). The embedded samples were sectioned on a microtome at a thickness of 2 μm and stained with toluidine blue. The cross sections were then observed and photographed under an optical microscope (CX41; Olympus Co., Ltd., Tokyo, Japan). The root photographs were then analyzed with an image processing program (Yamamoto, 2000) to allow measurement of the cross-sectional area of the epidermis and cortex and of the stele including xylem.
Results

Growth and root activity

Although the shoot dry weights did not differ significantly between the control and the root heating treatment on day 7, shoot growth was significantly greater in the heating treatment by day 21 (Fig. 1A). There was also no difference in shoot RGR from day 0 to day 7, but RGR was significantly greater in the heating treatment from day 7 to day 21 (Fig. 1B). Root dry weight in the heating treatment was significantly greater on days 7 and 21 (Fig. 1C). Root RGR was also significantly greater in the heating treatment from day 0 to day 7, but there was no difference from day 7 to day 21 (Fig. 1D).

The rate of xylem exudation did not change between day 0 and day 7 in the control, and only increased on day 21, but in the heating treatment, the increase was remarkable and the rate was significantly greater on days 7 and 21 than in the control (Fig. 2A). The root respiration rate decreased in both the control and the heat treatment groups from day 0 to day 7 and then increased slightly in both groups on day 21 (Fig. 2B). Root respiration on day 7 was significantly higher in the control than in the heating treatment, although the difference between the heating treatment and the control was not significant on day 21.

Determination of IAA and mineral concentrations

We determined the IAA concentration in the roots under root-zone heating in order to reveal its relationship with root growth. In both treatments, there was no significant change in the root IAA concentration between day 0 and day 7, and the concentration decreased significantly on day 21 (Fig. 3). We found no significant positive correlation between the root IAA concentration and root RGR, unlike in our previous study (Kawasaki et al., 2013).

We also investigated the effect of root-zone heating on the mineral concentrations in shoots and roots (Table 1). In the control, the concentration of every mineral that we investigated in the shoot decreased significantly from day 0 to days 7 and 21, except for the Ca concentration on day 21, which was not significantly different from the value on day 0. In contrast, only P and Mg concentrations on day 7 and K on day 21 decreased significantly in the heating treatment, and Ca increased significantly on day 21. Every mineral concentration was higher in the heating treatment than in the control on days 7 and 21, except for a nonsignificant increase in the K concentration on day 21. The root N concentration decreased significantly in the heating treatment by days 7 and 21, while it was unchanged in the control. Root P and K concentrations increased significantly in the heating treatment on days 7 and 21 compared with those on day 0 and were significantly higher than those on day 21.
in the control on day 7, but there was no significant difference between the treatments on day 21. The root Ca concentration was significantly lower on day 7 and significantly higher on day 21 in the heating treatment than in the control. The root Mg concentration decreased significantly in the control on days 7 and 21, but not in the heating treatment. The total nutrient uptake, which equaled the content per plant multiplied by the dry weight, increased for all minerals on day 21 in both treatments, but was much higher in the heating treatment.

**Internal root structures**

We also investigated root internal structures by microscopy (Fig. 4). The cross-sectional area of the epidermis and cortex was significantly larger in the heating treatment than in the control on day 21 (Table 2). The same trend occurred for the stele and total cross-sectional areas. However, there were no significant differences among the ratios of stele to total cross-sectional area and of xylem to stele.

<table>
<thead>
<tr>
<th>Days</th>
<th>Shoot conc. (mg·g⁻¹ DW)</th>
<th>Root conc. (mg·g⁻¹ DW)</th>
<th>Total uptake (mg per plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>K</td>
</tr>
<tr>
<td>0 Control</td>
<td>51.6 ab</td>
<td>7.6 a</td>
<td>70.0 a</td>
</tr>
<tr>
<td>7 Control</td>
<td>42.5 d</td>
<td>4.8 d</td>
<td>55.2 c</td>
</tr>
<tr>
<td>Heating</td>
<td>48.3 bc</td>
<td>6.7 b</td>
<td>63.3 ab</td>
</tr>
<tr>
<td>21 Control</td>
<td>46.5 c</td>
<td>6.0 c</td>
<td>54.3 c</td>
</tr>
<tr>
<td>Heating</td>
<td>52.8 a</td>
<td>8.0 a</td>
<td>59.6 bc</td>
</tr>
</tbody>
</table>

*Values within a column followed by the same letter are not significantly different (*P*<0.05, Tukey’s multiple-comparison test; *n* = 12).
Fruit yield, quality, and dry matter distribution

Fruit yield in the heating treatment was significantly higher than that in the control (Table 3). Although there was no difference in fruit number, individual fruit weight was significantly greater in the heating treatment. However, the soluble solid content was slightly but significantly lower in the heating treatment. At the end of the experiment, the dry weight of every plant part except the roots was significantly greater in the heating treatment than in the control (Table 4). The dry matter distributions and the T/R ratio did not differ between treatments.

Discussion

Under low air temperatures, reduced leaf area expansion (Hoek et al., 1993) and fruit set (Picken, 1984) have been reported in tomato plants. Fruit development is also inhibited below a daily mean air temperature of 14°C (Adams et al., 2001), and no vegetative growth is expected below 12°C (Criddle et al., 1997). Under low air temperatures at night, leaf area expansion and plant growth are also restricted (Smeets and Garretsen, 1986). In contrast, the root-zone temperature is optimal at 20 to 30°C (Gosselin and Trudel, 1984; Hurewitz and Janes, 1983; Shishido and Kumakura, 1994; Tindall et al., 1990), although the optimum varies slightly among air temperatures (Gosselin and Trudel, 1983a). Under low root-zone temperature, plant growth is inhibited. At optimal air temperatures, severe growth inhibition is shown at a root-zone temperature of 10°C or less (Hurewitz and Janes, 1983; Shishido and Kumakura, 1994). In the present study, the mean air temperature was 16.2°C and the minimum was 5.8°C, which was low enough to cause growth inhibition by chilling at night. The solution temperature in the control averaged 14.2°C and reached a minimum of 5.8°C, which was also low enough to cause damage. In contrast, the solution temperature in the heating treatment was a minimum of 16.6°C, which was sufficiently high to prevent growth inhibition.

Root-zone heating enhanced root growth. On day 7 of the heating treatment, root RGR and dry weight were

Table 2. Cross-sectional areas of root tissues with and without root-zone heating.

<table>
<thead>
<tr>
<th>Days</th>
<th>Cross-sectional area (10^3 μm^2)</th>
<th>Stele/Total (%)</th>
<th>Xylem/Stele (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Epidermis and cortex</td>
<td>Stele</td>
<td>Epidermis and cortex</td>
</tr>
<tr>
<td>0</td>
<td>Control</td>
<td>141.9 b</td>
<td>131.9 b</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>80.2 b</td>
<td>74.4 b</td>
</tr>
<tr>
<td></td>
<td>Heating</td>
<td>88.3 b</td>
<td>82.7 b</td>
</tr>
<tr>
<td>21</td>
<td>Control</td>
<td>174.1 b</td>
<td>162.5 b</td>
</tr>
<tr>
<td></td>
<td>Heating</td>
<td>411.1 a</td>
<td>372.6 a</td>
</tr>
</tbody>
</table>

* Values within a column followed by the same letter are not significantly different (P < 0.05, Tukey’s multiple-comparison test; n = 3).

Table 3. Fruit yield and soluble solid content of tomatoes with or without root-zone heating.

<table>
<thead>
<tr>
<th></th>
<th>Fruit yield (g per plant)</th>
<th>No. of fruits</th>
<th>Fruit weight (g per fruit)</th>
<th>Soluble solid content (Brix %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3444</td>
<td>14.8</td>
<td>230.4</td>
<td>5.9</td>
</tr>
<tr>
<td>Heating</td>
<td>4139</td>
<td>14.2</td>
<td>291.8</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Significance * NS * *

* significantly different (P < 0.05, t-test; n = 24).
NS: not significantly different (P < 0.05).

Table 4. Dry weights and dry weight distributions for the tomato plants at the end of the harvesting, with or without root-zone heating.

<table>
<thead>
<tr>
<th></th>
<th>Final dry weight (g)</th>
<th>Dry matter distribution (%)</th>
<th>T/R ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves and stem</td>
<td>Fruits</td>
<td>Roots</td>
</tr>
<tr>
<td>Control</td>
<td>171.0</td>
<td>167.4</td>
<td>15.1</td>
</tr>
<tr>
<td>Heating</td>
<td>223.2</td>
<td>214.8</td>
<td>18.6</td>
</tr>
</tbody>
</table>

Significance * * — y * NS NS — NS

* Including removed lateral shoots and shoot tips.
* No replication due to twined roots.
* significantly different (P < 0.05, t-test; n = 24).
NS: not significantly different (P < 0.05).
both higher than in the control (Fig. 1). Some previous research on tomato plants has also shown that root-zone heating enhances root growth under optimal air temperatures (Bugbee and White, 1984; Tindall et al., 1990), but because of the root heating, we observed the same phenomenon under suboptimal air temperatures. Shoot growth was also promoted by the root-zone heating, although it became significant later (by day 21) than the promotion of root growth. This suggests that the root-zone heating influences root growth first, and that this subsequently promotes shoot growth. Because shoot activity is promoted by root-zone heating, increased leaf area (Hurewitz and Janes, 1983) and transpiration rate (Shishido and Kumakura, 1994) have been reported, which supports our results.

Xylem exudation and root respiration rates have been used to represent root activity related to water and nutrient transport (Klock et al., 1997; Yamaguchi et al., 1995). We found that the xylem exudation rate was much higher in the heating treatment than in the control (Fig. 2). The root respiration rate at 25°C decreased on day 7 and then increased slightly by day 21. The decrease of root respiration may have been due to transplanting shock caused by low air temperature and the use of soilless culture. Root respiration was significantly higher in the control on day 7, but the difference was no longer significant on day 21. Previously, the root respiration rate at 25°C was shown to be related to the root-zone temperature, with a lower rate at low root-zone temperature (Shishido and Kumakura, 1994). Although the reason why root respiration was lower in the heating treatment is not clear, the decrease was transient and respiration had recovered by day 21. Root respiration generally approximately doubles when the temperature rises by 10°C (Atkin et al., 2000). Thus, any initial difference in respiration rates between treatments should quickly disappear as a result of the higher root-zone temperature; this agrees with our results (Fig. 2B). The observed changes in root activity suggest that the root-zone heating increases xylem exudation by day 7 and increases both xylem exudation and root respiration by day 21, which would improve nutrient transport to the shoot, thereby increasing shoot growth.

We measured the root IAA concentration because we found a correlation between this variable and root RGR with root-zone cooling under high air temperature in our previous study (Kawasaki et al., 2013). In Arabidopsis, IAA promotes root growth (Novickiè et al., 2010; Ohashi-Ito et al., 2013) and xylem development (Deng et al., 2012; Ohashi-Ito et al., 2013). In our study, root-zone heating did not affect the IAA concentration. Therefore, the effects of root-zone heating at low air temperature on auxin in root may differ from that of root-zone cooling at high air temperature. On anthers of Arabidopsis, the auxin level decreased under high temperature, while it did not change under low temperature (Sakata et al., 2010). This report supports our results that root IAA concentration increased by root-zone cooling under high-temperature conditions, while it was unchanged by root-zone heating under low-temperature conditions.

The root-zone heating promoted nutrient uptake and transport to the shoot (Table 1). These results may explain the increased shoot growth by day 21. The remarkable enhancement of xylem exudation as a result of root heating would improve nutrient uptake and transport. In contrast, inhibition of nutrient uptake and decreased shoot mineral concentrations have been reported under a low root-zone temperature (Gosselin and Trudel, 1983b; Kabu and Toop, 1970) and our results for the control support these previous findings. In addition, increased nutrient uptake under an optimal root-zone temperature was reported after root-zone cooling at high air temperature (Kawasaki et al., 2013).

Our analysis of internal root structures showed that the development of every tissue was promoted by root-zone heating (Table 2). The increased xylem development would increase xylem exudation and mineral nutrient uptake. In our previous study, xylem-specific development was observed in response to root-zone cooling at high air temperature, and this treatment also increased xylem exudation and nutrient uptake (Kawasaki et al., 2013). The difference in root structure development between plants with root-zone heating and those with root-zone cooling may be explained by the difference in the correlation between root IAA concentration and RGR: root heating did not lead to a significant correlation, whereas root cooling did. It is possible that the development of every root tissue by root-zone heating at low air temperature may occur not via auxin in root.

Fruit yield was higher in the heating treatment than in the control (Table 3). Because the number of fruits did not differ significantly, increased individual fruit dry weight was responsible for this difference. However, the soluble solids content decreased in the heating treatment. Previous research suggested the possibility of increasing fruit yield by root-zone heating at low air temperature (Jones et al., 1978), and our results support this finding. On the other hand, Gosselin and Trudel (1983a) reported only a small effect of root-zone temperature on fruit yield at a 12°C night air temperature. This suggests that root-zone heating would be more effective at low air temperature, particularly if the temperature is low enough to cause growth inhibition.

The dry matter distribution did not differ between the root-zone heating and the control, although the total dry weight of all plant parts was higher in the heating treatment (Table 4). This suggests that growth of every plant part increased by a roughly equal proportion under long-term root-zone heating. In previous research with an optimal air temperature, the effects of root-zone heating on the dry matter distribution in tomato seedlings differed among studies: for example, root dry mat-
ter decreased in one study (Bugbee and White, 1984) but increased in another (Shishido and Kumakura, 1994), and both studies contradict our results. In the early stages of our study, our results agree with the hypothesis of Shishido and Kumakura (1994) that root growth would be promoted earlier than shoot growth by root-zone heating. However, the dry matter distribution can be affected by both plant growth and the air temperature. Therefore, the difference in dry matter distribution between plants with and without root-zone heating at low air temperature may disappear by the harvesting stage.

In conclusion, we found that root-zone heating of tomato plants at low air temperatures affected root activity, plant growth, and nutrient uptake as a result of improved development of root tissues, and this subsequently improved shoot growth, leading to higher individual fruit dry weight and total fruit yield. This study therefore provides physiological and morphological evidence for the mechanisms of growth promotion by root-zone heating at low air temperature, and provides support for further investigations of the effectiveness of heating in tomato cultivation.

**Literature Cited**


