Periodical Wetting Increases $\alpha$-Tocopherol Content in the Tuberous Roots of Sweetpotato 
(*Ipomoea batatas* (L.) Lam.)

Toshihiko Eguchi$^1$, Yuji Ito$^2$ and Satoshi Yoshida$^1$

$^1$ Biotron Application Center, Kyushu University, Fukuoka 812–8581, Japan
$^2$ Institute of Lowland and Marine Research, Saga University, Saga 840–8502, Japan

(Received March 22, 2012; Accepted June 27, 2012)

Tuberous root growth and antioxidant contents of 2 sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars were examined using 2 different irrigation schemes: surface-irrigation and sub-irrigation. Coarse silica sand was used for root media, which maintained well the gas permeability and water drainage around the roots. The root surface was periodically wetted for watering in the surface-irrigated roots, while the sub-irrigated roots were not. The irrigation methods did not affect the oxygen concentration around the roots. No differences in plant growth were observed between the 2 irrigation methods. However, the content of $\alpha$-tocopherol in the tuberous root was significantly higher in the ordinary-irrigated roots for both of two cultivars. Thus, the periodical wetting increased the $\alpha$-tocopherol content in the tuberous root of sweetpotato cultivars without any apparent changes in tuberous root development.

Keywords: antioxidants, growth, sweetpotato, tuberous root, wetting

INTRODUCTION

Growth of tuberous roots of sweetpotato (*Ipomoea batatas* (L.) Lam.) is directly influenced by reduction of oxygen (O$_2$) concentrations around the root, and its thickening ceases under hypoxic conditions (Eguchi and Yoshida, 2011). Bulky plant tissues such as banana fruits and potato tubers which poses high metabolic activity can become hypoxic because they lack large intercellular air spaces, contain poorly vaculated cells, or are located at sites remote from the sites of entry of O$_2$ (Geigenberger, 2003). In growing potato tubers, for instance, hypoxic conditions easily develop in the inner region even when the tuber is surrounded by normoxic conditions (Geigenberger et al., 2000). The anatomical features of sweetpotato tuberous roots are similar to those of bulky organs, with low O$_2$ stress easily developing within the growing tuberous root. Water is considered as a barrier to O$_2$ movement because it impedes the rate of O$_2$ diffusion ($10^{-4}$ times the rate in air). Therefore, watering or rainfall may often cause hypoxia in the inner parts of the tuberous roots that are grown in the field. Influences of the slight low O$_2$ stress on the tuberous root development are required to be elucidated if hypoxic conditions are readily occurred within the roots.

The review by Blokhina et al. (2003) suggests that hypoxia and reoxygenation lead to the generation of reactive oxygen species (ROS) in plants cells. O$_2$ deprivation, which results in ROS gen-
eration, activates the expression of antioxidant biosynthesis genes (Geigenberger, 2003). Hypoxia occurring within cells of the sweetpotato tuberous roots thus facilitates accumulation of antioxidants. Temporary hypoxic conditions do not necessarily result in termination of thickening and formation of hard fibers within the fleshy part of the tuberous root (Eguchi and Yoshida, 2007). In such case, occurrence of temporary hypoxia may be indirectly estimated by changes in antioxidant content.

This study examined the growth and antioxidant content of roots grown using 2 different irrigation methods: surface-irrigation which involves periodic wetting of the root surface by watering on the root media similar to rainfall, and sub-irrigation wherein the tuberous root surface is not covered with irrigation water.

MATERIALS AND METHODS

Plant materials
Two sweetpotato cultivars “Koganesengan” and “Narutokintoki”, both the major cultivars in

![Sub-irrigation system](image1)

**Fig. 1** Schematic diagram of the 2 different irrigation methods. In the sub-irrigation system, groundwater level in the root media was adjusted by controlling the level of nutrient solution. In the surface-irrigation system, the root media surface was irrigated with 2 L of the nutrient solution twice a week using a watering can, and the excess solution was drained out through the connecting tube. Oxygen concentration and volumetric water content of the root media were measured on-line for both irrigation systems.
Japan, were employed in this study. The cream-skinned cultivar “Koganesengan” is mainly used as an industrial raw material for the production of starch and alcohol, whereas the red-skinned cultivar “Narutokintoki” is mainly consumed as a fresh vegetable. Plant materials were prepared as follows: the lowest node of a stem-cutting with 3 leaves was rooted and grown in a phytotron at an air temperature of 25°C±1°C and relative humidity (RH) of 70%±5% for 1 week. Except

**Fig. 2** Changes in the volumetric water content (VWC) and oxygen concentration of the root media in the different irrigation methods for 2 sweetpotato cultivars. Lines show the time-course changes of VWC and oxygen concentration, and circles and triangles show the weekly mean VWC in the sub-irrigation system and surface-irrigation system, respectively.
for the longest nodal root (approximately 15 cm in length), all other roots were excised from the plant. Such plants with a single root and 3 leaves were used for this study.

Experimental conditions

A square cultivation box (inner size: width, 300 mm; length, 250 mm; height, 210 mm) was filled with a layer of silica sand (grain size, 0.2–1 mm; porosity, 0.46; Saitozaki Kosen, Fukuoka) of 180-mm thickness over a 20-mm thick bottom layer of glass beads (diameter, 5 mm). A drainage tube was connected at the bottom of the box. Moisture content and O₂ concentration were monitored at a depth of ~70 mm from the root media surface: this depth was considered as optimum for root thickening (Eguchi et al., 1994). Volumetric water content of the root media was measured using ECH2O probes (EC-5; Decagon Devices, Inc. Pullman) that were previously calibrated for the root media (Miyamoto et al., 2009). O₂ concentration was measured using a fluorescent O₂ analyzer (FO-960; Automatic System Research Co., Tokyo). Two boxes were installed in the center of the phytotron glass room controlled at an air temperature of 25±1°C and RH of 70±5%, and were arranged in the east-west direction leaving 25 cm. Around the boxes, there was no other objects as plants or pots shading the sunlight. CO₂ concentration in the glass room could be maintained at atmosphere level since the air in the room was well ventilated at the rate of 10 times h⁻¹. Half-strength of a commercial nutrient solution (Otsuka Chemical Co., Ltd., Osaka) adjusted to pH6.0 was used. The root media were moistened well, and then 5 plants with a single root were transplanted into each box. Two boxes were subjected to 2 different methods of irrigation (Fig. 1). In 1 box, groundwater level in the root media was adjusted by controlling the level of nutrient solution in a tank connected to the drain tube, and the groundwater level was maintained at approximately ~180 mm from the root media surface. In this sub-irrigation system, the nutrient solution reached the roots through capillary motion, and the thickened part of the nodal root was never covered by water during the process. In the other box, the root media surface was irrigated with 2 L of nutrient solution twice a week (on Mondays and Thursdays) using a watering can, with any excess solution drained out through the connecting tube. In this surface-irrigation system, the surface of the nodal root was supposed to be periodically wetted for watering. The plants were grown for 6 weeks (“Narutokintoki” growth period: August 19 to September 30, 2010; “Koganesengan” growth period: December 30, 2010 to February 10, 2011). For “Koganesengan”, the preliminary 5-weeks experimental cultivation was carried out from July 1 to August 5, 2010 for investigation of antioxidant content. Adventitious buds emerged from nodes were all removed anytime when those were found.

Growth measurement

Plants were divided into 4 parts namely, leaves, stem with petioles, tuberous root, and fibrous roots. Leaf area, tuberous root size, and fresh and dry weights of each plant part were measured. For the tuberous root, the fresh weight was measured, and a 2-mm-thick cross-section from the thickest region was cut out to observe the lignified region after staining with 2% phloroglucinol and HCl (Eguchi and Yoshida, 2007). The rest of the tuberous root was weighed and then lyophilized, and the dry matter ratio (%) was determined and used to calculate the dry weight of the whole tuberous root. The lyophilized tuberous root was analyzed for determination of antioxidant content. Other plant parts were oven-dried at 70°C for 24 h.

Quantification of antioxidants

L-ascorbic acid, α-tocopherol, and polyphenol composition of the roots were investigated. The α-tocopherol content in the tuberous root was quantified by the method described by Okuno et al. (1998), using a high-performance liquid chromatography (HPLC) system (pump, LC-10AD; column oven, CTO-10A; UV-VIS detector, SPD-10AV; RF-10A, spectrophotometric detector, Shimadzu Corp, Kyoto). The L-ascorbic acid content was quantified by the method reported by Hanada et al. (2011), using an HPLC system (pump, LC-20AD; column oven, CTO-10A; UV-VIS detector, SPD-10AV, Shimadzu Corp, Kyoto). Total polyphenol content was determined by using
the Folin-Ciocalteu method as previously described (Eguchi et al., 2007).

**RESULTS AND DISCUSSION**

Figure 2 shows changes in volumetric water content (VWC) of the root media and O₂ concentration at −70 mm depth during 6 weeks of cultivation of 2 sweetpotato cultivars. The O₂ concentration was maintained at approximately 21% in all experiments and was shown to be unaffected by irrigation possibly because of the good gas permeability and water drainage of the root media. VWC in the surface-irrigation system highly fluctuated during periodic watering: the VWC instantly increased to approximately 30% after watering, after which it immediately dropped to 20%, and gradually decreased thereafter until the next watering. In contrast, the sub-irrigation system showed less fluctuations and a gradual decrease in the VWC. The weekly mean VWC in the ordinary irrigation system was almost the same as that in the sub-irrigation system for the “Koganesengan”, but it was slightly lower than that in the sub-irrigation system for the “Narotokintoki”.

As shown in Table 1, total leaf area, tuberous root size, and tuberous root yield did not significantly differ between the 2 irrigation methods in both sweetpotato cultivars. The dry matter content of the tuberous root of “Koganesengan” was significantly high when grown using the sub-irrigation system. Dry matter partitioning in the plant was also the same for the 2 irrigation methods (Table 2). The lignified tissue observed in the cross-section of thickest part was mainly comprised of xylem tubes, and no differences were found between in the cultivars grown using the 2 different irrigation methods. Thus, the irrigation methods appeared to little affect the plant growth.

Table 3 shows the antioxidant content in the tuberous roots. For the 6 weeks cultivated plants, the α-tocopherol content in the surface-irrigated root was significantly higher than that in the sub-irrigated root for both cultivars. The L-ascorbic acid content was also relatively higher in the surface-irrigated roots, whereas the total polyphenol content was relatively higher in the sub-irrigated

**Table 1** Total leaf area, tuberous root size and yield, and dry matter content of tuberous root in sweetpotato plants grown with different irrigation methods.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Irrigation</th>
<th>Total leaf area (cm²)</th>
<th>Length of tuberous root (mm)</th>
<th>Maximum width of tuberous root (mm)</th>
<th>Fresh weight of tuberous root (g)</th>
<th>Dry weight of tuberous root (g)</th>
<th>Dry matter content of tuberous root (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koganesengan</td>
<td>Surface-irrigated</td>
<td>693 ± 21</td>
<td>78 ± 5</td>
<td>22.8 ± 0.6</td>
<td>17.3 ± 0.4</td>
<td>4.22 ± 0.12</td>
<td>24.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Sub-irrigated</td>
<td>697 ± 21</td>
<td>85 ± 2</td>
<td>22.2 ± 0.9</td>
<td>17.8 ± 1.8</td>
<td>4.56 ± 0.43</td>
<td>25.7 ± 0.3</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>*</td>
</tr>
<tr>
<td>Narotokintoki</td>
<td>Surface-irrigated</td>
<td>259 ± 15</td>
<td>87 ± 3</td>
<td>23.5 ± 1.1</td>
<td>20.6 ± 1.1</td>
<td>4.76 ± 0.31</td>
<td>23.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Sub-irrigated</td>
<td>286 ± 12</td>
<td>83 ± 5</td>
<td>25.0 ± 0.8</td>
<td>21.9 ± 1.6</td>
<td>5.25 ± 0.43</td>
<td>23.9 ± 0.3</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>*</td>
</tr>
</tbody>
</table>

* *P* < 0.05, n.s. — not significant, by *t*-test (*n* = 10).

**Table 2** Dry matter partitioning in sweetpotato plants grown with different irrigation methods.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Irrigation</th>
<th>Leaf partitioning (%)</th>
<th>Stem with petioles</th>
<th>Tuberous root</th>
<th>Fibrous root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koganesengan</td>
<td>Surface-irrigated</td>
<td>20.9 ± 0.4</td>
<td>16.8 ± 1.1</td>
<td>55.3 ± 1.5</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Sub-irrigated</td>
<td>20.5 ± 0.6</td>
<td>16.9 ± 1.2</td>
<td>56.4 ± 2.3</td>
<td>6.2 ± 0.7</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Narotokintoki</td>
<td>Surface-irrigated</td>
<td>16.5 ± 0.3</td>
<td>14.9 ± 0.8</td>
<td>65.3 ± 1.4</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Sub-irrigated</td>
<td>16.5 ± 0.7</td>
<td>15.3 ± 0.6</td>
<td>64.9 ± 1.0</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* n.s. — not significant, by *t*-test (*n* = 10).
roots. For the 5 weeks cultivated ‘Koganeseengan’, the α-tocopherol content in the surface-irrigated root was also significantly higher than that in the sub-irrigated root. The antioxidant content in the surface-irrigated roots relative to that of the sub-irrigated roots was also shown in Table 3. Thus, periodic watering on the root media surface increased the α-tocopherol content in the tuberous root even though gas permeability and water drainage of the root media were maintained as observed in time-course changes of O2 concentration around the roots (refer Fig. 2).

The 2 different irrigation methods used in this study showed significant differences in the α-tocopherol content in the tuberous roots, although the irrigation methods scarcely affected the plant growth. About 80% of the phenolic compounds were concentrated in the outer 5–6 mm of tissue of the tuberous root cross-section (Walter and Schadel, 1981). Padda and Picha (2007) also reported that the highest phenolic content was found in the periderm tissue. On the other hand, L-ascorbic acid and α-tocopherol are supposed to be entirely distributed in the fleshy part of the root (Naka and Tamaki, 1967; Woolfe, 1992). In this study, the surface-irrigation would temporarily wet the whole or a part of the surface of the tuberous root, while the sub-irrigation would not wet the surface of the tuberous root. Antioxidants distributed in the fleshy part of the tuberous root, such as L-ascorbic acid and α-tocopherol, showed relatively high contents in the surface-irrigated roots. Because periodic wetting of the root surface is thought to cause hypoxia within the root, and therefore, it would accelerate antioxidant accumulation within the root flesh, distal to the sites of O2 entry (refer Geigenberger, 2003). Accordingly, the significantly high content of α-tocopherol in the surface-irrigated roots was attributable to periodic and slight ROS generation due to hypoxia. Our results suggest that environment control techniques that induce temporary hypoxia within the tuberous root increase the level of antioxidants such as α-tocopherol in the root flesh. The major causes of temporary hypoxia need to be examined, and the appropriate strength, duration, and timing of the stress application should also be examined for practical applications.

REFERENCES


Table 3  L-Ascorbic acid, α-tocopherol, and total polyphenol contents in tuberous root of sweetpotato plants grown with different irrigation methods.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Irrigation</th>
<th>L-Ascorbic acid (mg g DW)</th>
<th>α-Tocopherol (μg g DW)</th>
<th>Total polyphenol (mg g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koganeseengan</td>
<td>Surface-irrigated</td>
<td>1.57±0.10</td>
<td>61±6</td>
<td>3.52±0.15</td>
</tr>
<tr>
<td>&lt;6 weeks cultivated&gt;</td>
<td>Sub-irrigated</td>
<td>1.38±0.03</td>
<td>44±2</td>
<td>3.78±0.29</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td>n.s.</td>
<td>114%</td>
<td>(97%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(139%)</td>
<td>(82%)</td>
</tr>
<tr>
<td>Koganeseengan</td>
<td>Surface-irrigated</td>
<td>2.08±0.11</td>
<td>56±3</td>
<td>4.21±0.52</td>
</tr>
<tr>
<td>&lt;5 weeks cultivated&gt;</td>
<td>Sub-irrigated</td>
<td>1.83±0.06</td>
<td>44±2</td>
<td>5.13±0.63</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td>n.s.</td>
<td>**</td>
<td>n.s.</td>
</tr>
<tr>
<td>Narutokintoki</td>
<td>Surface-irrigated</td>
<td>2.07±0.01</td>
<td>49±2</td>
<td>2.39±0.67</td>
</tr>
<tr>
<td>&lt;6 weeks cultivated&gt;</td>
<td>Sub-irrigated</td>
<td>1.99±0.04</td>
<td>41±1</td>
<td>2.78±0.29</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td>n.s.</td>
<td>**</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* * P < 0.05, ** P < 0.01, n.s. — not significant, by t-test (n = 10).
† Relative values of antioxidant content to the values of the sub-irrigated roots are shown in the parenthesis.

tops production of a sweetpotato cultivar ‘Suish’. (in Japanese text with English abstract) J. SHITA 19:


247–256.


Hanada, Y., Yasunaga, E., Uchino, T., Tanaka, F., Nakano, K., Chikushi, J.  2011.  A model to predict the ef-
fector of postharvest environment on quality deterioration of common bean (*Phaseolus vulgaris* L.). (in

Miyamoto, H., Cho, H., Ito, Y., Chikushi, J., Eguchi, T.  2009.  General calibration of capacitance soil mois-
ture sensor for various electrical conductivity conditions. (in Japanese text with English abstract) J. SHITA


Okuno, S., Yoshimoto, M., Kumagai, T., Yamakawa, O.  1998.  Contents of β-carotene and α-tocopherol of
sweetpotato cultivars newly developed for processing purposes. Tropic. Agric. (Trinidad) 75: 174–176.

Padda, M. S., Picha, D. H.  2007.  Effect of low temperature storage on phenolic composition and antioxidan-

Walter, W. M., Schadel, W. E.  1981.  Distribution of phenols in “Jewel” sweet potato (*Ipomoea batatas* (L.)

643.