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Instantaneous Flooding and α-Tocopherol Content in Tuberous Roots of Sweetpotato (*Ipomoea batatas* (L.) Lam.)

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Compared to sub-irrigated sweetpotatoes (*Ipomoea batatas* (L.) Lam.), periodic surface-irrigated plants, i.e., twice a week on root media, showed increased α-tocopherol content in their tuberous roots with no apparent changes in both of tuberous root development and oxygen concentration around the roots. We speculated that surface irrigation might temporarily cover the tuberous root surface with water and inhibit oxygen movement into the roots, thereby increasing the antioxidant α-tocopherol content, for coping with the slight oxidative stress occurring within the roots. Therefore, we performed 1–3 times instantaneous flooding, with different intervals, which perfectly covered the whole root surface with water, of sweetpotato plants grown in a phytotron glass room (25°C, 70%RH). Electrolyte leakage from the tuberous root flesh cells showed a temporal increase for the flooding treatment, while it immediately recovered within 24 h. Instantaneous flooding did not affect the storage root development in any of the experiments. Apparent increases in the α-tocopherol content were observed during the 3-time flooding at 3-day intervals. Our results suggest that more frequent root surface wetting is necessary for increasing the α-tocopherol content, which is released because of the oxidative stress that occurs within the roots.

Keywords : antioxidants, flooding, oxidative stress, root growth, storage organ

INTRODUCTION

In a previous study (Eguchi et al., 2012), tuberous root growth and antioxidant contents of two sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars were examined using two different irrigation schemes: periodic surface-irrigation and continuous sub-irrigation. Although no apparent differences in tuberous root development were observed between the two irrigation methods, the content of α-tocopherol in the surface-irrigated tuberous root was significantly higher for both the cultivars. We speculated that the periodical wetting of the tuberous root surface might increase the content of the antioxidant, α-tocopherol. The inner portion of bulky plant tissues such as tuberous roots can become hypoxic because they are located at sites remote from the sites of oxygen entry (Geigenberger, 2003). Furthermore, hypoxia causes oxidative stress in plant tissue (Blokhina et al., 2003). A thin water film that covers the root surface may inhibit oxygen movement into the roots, and cause an increase in the content of the antioxidant α-tocopherol, for coping with the slight oxidative stress occurring within the roots. However, there was doubt as to whether the surface-irrigated water completely coated the tuberous roots.

Small container cultivation as we previously used for sweetpotatoes (Eguchi et al., 2012) can readily apply instantaneous flooding, which perfectly covers the whole surface of the tuberous root. In the previous study, the O_2 concentration around the tuberous root was maintained at approximately 21% during the cultivation period and was unaffected by irrigation because of the good gas permeability and water drainage of the root media. In that case, instantaneous flooding may not greatly disturb O_2 concentrations when we use the same root media. Therefore, in this study, we performed instantaneous flooding of sweetpotato plants grown in a small container. The effects of the flooding treatments with different times and different intervals were investigated with regards to the α-tocopherol contents in the tuberous roots. Electrolyte leakage from the root flesh was also measured for examination of the occurrence of physiological stress within the root.

MATERIALS AND METHODS

Plant materials

A sweetpotato cultivar "Koganesengan" was used in this study. Plant materials were prepared as shown in our previous study (Eguchi et al., 2012). In brief, the lowest node of a stem-cutting with three leaves was rooted in a phytotron (air temperature of 25°C and relative humidity of 70%) and grown for one week. Except for the longest nodal root (approximately 15 cm in length), all other roots were excised from the plant, and only such plants, with a single root and three leaves, were used for this study (refer Fig. 1A). The material plants ensure to form one tuberous root per one plant that simplifies the carbon translocation flow (Eguchi et al., 1994) and also makes analyses of envi-

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ronmental effects on the plant growth easy, although the plant life is shorten by the limitation of leaf number. Such plants grown in the same cultural condition represent uniformity in the initiation time of tuberous root formation and in the degree of tuberous root thickening.

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 Kosan, Fukuoka, Japan) 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. O_2 concentration was monitored using a fluorescent O_2 analyzer (FO-960; accuracy ±0.5%; Automatic System Research Co., Tokyo, Japan) at a depth of −70 mm from the root media surface. This depth was considered as the optimum for root thickening (Eguchi et al., 1994). 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%. Half-strength of OAT Agrio A solution (OAT Agrio Co., Ltd., Tokyo, Japan) adjusted to pH6.0 was used, and the electric conductivity of the nutrient solution was about 1.2 dS m^{-1}. The root media were moistened well, and then four plants with a single root were transplanted into each box. The 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 (Fig. 1A). Instantaneous flooding was applied to the plants either once, twice, or three times with different intervals during the cultivation period as shown in Fig. 1B. At the flooding treatment (Fig. 1A), water was added until the water level rose above the root media surface, and this situation was maintained for one minute, whereupon the water was immediately drained. The plants were grown for six weeks. Growth periods of the respective flooding regimes were as follows: 1-a, December 1, 2011 to January 12, 2012; 1-b, July 12 to August 23, 2012; 2-a, 2-b, and 2-c, February 14 to March 28, 2013; 3-a, 3-b, and 3-c, May 16 to June 27, 2013; and 3-d, July 18 to August 29, 2013. As the control experiment for each growth period, one box was not exposed to the flooding treatment during cultivation. Adventitious buds emerged from nodes were all removed anytime whenever they were found.

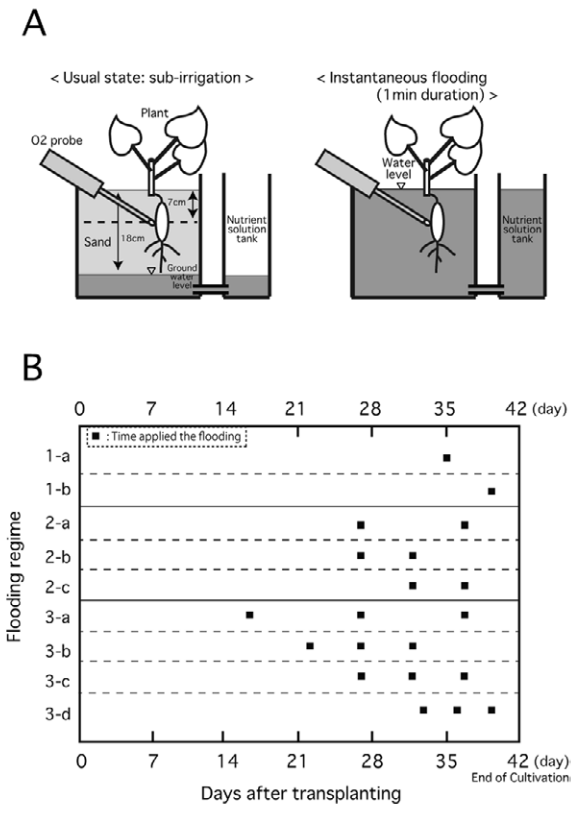

Fig. 1 Schematic diagrams of the cultivation system and instantaneous flooding treatment using the system (A), and the date (■) of the instantaneous flooding application during the cultivation period in nine flooding regimes that differed from one another in frequency and interval of the flooding treatment (B).

Growth measurement and quantification of α-tocopherol

Length and width of the tuberous root were measured after the 6-wk cultivation. The tuberous root was weighed and then lyophilized, and the dry matter ratio (%) was determined. The lyophilized tuberous root was analyzed for determination of its α-tocopherol content. 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; spectrofluorometric detector, RF-10A, Shimadzu Corp., Kyoto, Japan). α-Tocopherol (GR 34114-54) purchased from Nacalai Tesque (Kyoto) was used as a standard reagent.

Evaluation of electrolyte leakage

Stress-induced electrolyte leakage, an indicator of cellular response to various stress factors (Demidchik et al., 2014), was evaluated by measuring the time-course changes in the electrolyte leakage from the tuberous root flesh. The electrolyte leakage was measured applying the method described by Campos et al. (2003). Material plants were cultivated on the same flooding regime as 3-d during November 7 to December 18, 2013. The third flooding was applied to the plants on December 16, and four tuberous roots were sampled immediately before, and 1, 24, and 48 h after the flooding. Three discs (10-mm diameter, 2-mm thickness) of tuberous root flesh were obtained from the most thickened part of the root, and discs were rinsed with deionized water three times. The discs were soaked in six ml of deionized water, and the electric conductivity of the solution was measured using a conductivity meter (MM-60R, DKK-TOA Corp., Tokyo, Japan) after 22 h soaking at room temperature. Thereafter, the solution containing the root disc was kept at 90°C for two hours, and the total conductivity of the solution was measured. The electrolyte leakage was evaluated as the percentage of the electrical to total conductivity. For comparison, the electrolyte leakage was also evaluated for the plants that did not experience the instantaneous flooding during the 6-wk cultivation period during July 18 to August 29, 2013.

Statisitical analysis

Data for tuberous root size, yield, antioxidant content, and relative electric conductivity were analyzed by analysis of variance ($n=4$). In the individual flooding regime, the significant differences between flooded roots and non-flooded roots were tested by *t*-test at $P<0.01$. The relative electric conductivity data were tested by Tukey test at $P<0.05$.

RESULTS AND DISCUSSION

In all experiments, O_2 concentration at 7-cm depth in the root media was kept over 20% during the cultivation period. This concentration may not cause the oxidative stress in the root because a symptom of oxygen deprivation such as the decrease in respiration rate occurs when the oxygen concentration below 8% (Geigenberger et al., 2000). Figure 2 shows an example of the O_2 concentration changes by the depth in the root media on the day that the flooding treatment was applied. O_2 concentration appeared slightly decreased after the instantaneous flooding while the lowest value was 20.4%. Figure 3 shows changes in the relative electric conductivity as affected by the instantaneous flooding. The values were obtained from tuberous roots for which the third flooding treatment was applied (refer Fig. 1, 3-d). The dotted line is the mean value obtained from the plants that were not subjected to the flooding during the cultivation period of 6-wk. The relative electric conductivity rapidly rose to 18% at one hour after the flooding, whereas the value decreased to about 15% at 24h after the flooding and was maintained thereafter. The value at one hour after the flooding was significantly higher than that at the other measurement times, which was almost the same as that of the roots that did not experience flooding. Demidchik et al. (2014) reviewed the literature and determined that electrolyte leakage, which is mainly related to K^+ efflux, is the hallmark of stress response in intact plant cells and that the reaction is generally activated after a few minutes and lasts for about one hour. Our results showed that a similar reaction occurred within the tuberous root. Thus, although the instantaneous flooding may have caused the oxidative stress within the tuberous root, the effect is just temporary. The thickening growth of the tuberous root is rapidly affected by hypoxia, i.e., it ceases within one hour when the root is treated with a hypoxic condition (Eguchi and Yoshida, 2011). Thus, instantaneous flooding can temporally disturb the tuberous root thickening.

In all the flooding regimes, significant differences in the length, width, fresh weight, and dry matter ratio of tuberous roots were not observed in comparison with the control plants that were not applied the flooding treatment (data not shown). Thus, in the long-term, the tuberous root development was not affected by the instantaneous flooding applied in this study. Table 1 shows the α-tocopherol

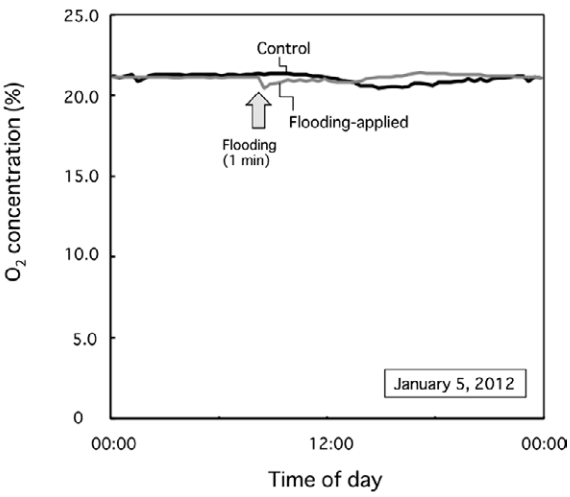

Fig. 2 O_2 concentration at the 7 cm depth of the root media during the day of flooding application.

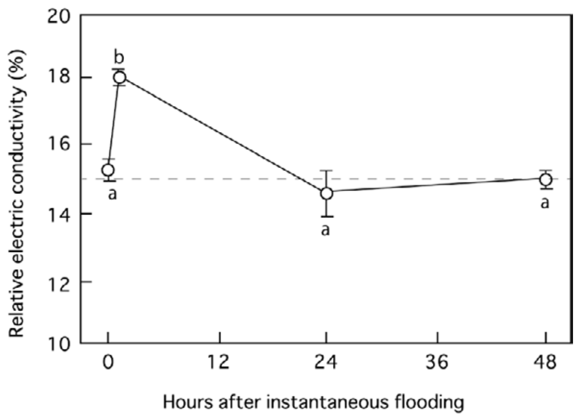

Fig. 3 Time-course changes in the relative electric conductivity of the tuberous root on application of the flooding treatment. Mean values are indicated with 95% confidence intervals. Different letters represent significantly different at $P<0.05$ by Tukey test ($n=4$). Dotted line is the mean value (15.05 ± 1.21) obtained from the plants that were not flooded during the cultivation period of 6-wk.

Table 1 Contents of α-tocopherol ($\mu g \cdot gDW^{-1}$) in sweetpotato tuberous roots grown under different flooding regimes. Mean values with 95% confidence intervals are indicated.

	Flooding regimes								
	1-a	1-b	2-a	2-b	2-c	3-a	3-b	3-c	3-d
α-tocopherol, $\mu g \cdot gDW^{-1}$	81.8±14.8	95.5±5.3	109.3±10.0	116.3±8.2	111.0±12.1	88.4±3.7	89.3±6.0	96.1±4.3	105.0±11.4
(Control: no flooding treatment was applied)	(79.7±11.3)	(93.5±8.7)	(104.8±5.4)	(104.8±5.4)	(104.8±5.4)	(86.4±1.8)	(86.4±1.8)	(86.4±1.8)	(84.6±10.8)
Significance[x]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	**

[x] $**P<0.01$, n.s.. not significant, by *t*-test ($n=4$).

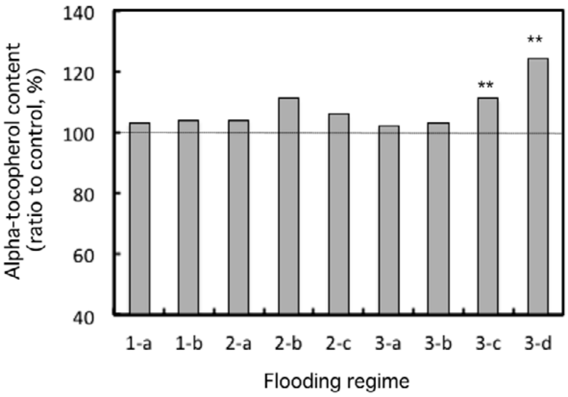

Fig. 4 Relative contents of α-tocopherol in the different flooding regimes. Ratios to the control are indicated. Asterisks show the significant differences between the contents in the regime and the control as shown in Table 1 ($P<0.01$, *t*-test).

content in the tuberous roots grown under different flooding regimes. In comparison with the control plants, significantly higher content was observed in regimes 3-c and 3-d. Figure 4 shows the relative α-tocopherol content against the control in different flooding regimes. The content was the highest in 3-d, but the value (124%) was lower than that (140%) of roots periodically wetted by surface irrigation twice a week (Eguchi et al., 2012). L-ascorbic acid content in the storage organ was unaffected by the instantaneous flooding (data not shown). Munné-Bosch (2005) suggested that α-tocopherol plays a role in enhancing plant stress tolerance, the stress that is related to reactive oxygen species formation, and that the decreased level favors oxidative damage. α-tocopherol acts with other antioxidants, rather than with only a single antioxidant, such as ascorbate, glutathione, carotenoids, and terpenoids, in the whole set of antioxidant defenses, because recent studies have shown the existence of a compensatory mechanism in the absence of some antioxidant (Havaux et al., 2000; Munné-Bosch and Alegre, 2002; Munné-Bosch and Alegre, 2003; Kanwischer et al., 2005; Llusià et al., 2005; Pẽnuelas et al., 2005). In this study, the instantaneous flooding caused an increase in the content of α-tocopherol in the root flesh, but not the L-ascorbic acid content. The instantaneous flooding may increase the α-tocopherol content when the treatment is applied more than three times with short intervals of less than five days, and the treatment should be applied much closer to harvest time.

The instantaneous flooding disturbed the membrane cation conductances of the tuberous root flesh, which could be because of the oxidative stress. The periodical wetting of the root, as we performed previously (Eguchi et al., 2012), may cause the slight oxidative stress within the sweetpotato roots, but the significant increase of α-tocopherol content requires frequent and short-interval application of water.

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