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<https://hdl.handle.net/2324/4774205>

出版情報 : Environmental Control in Biology. 54 (4), pp.183-185, 2016. 日本生物環境工学会
バージョン :
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Time-course Pattern of Electrolyte Leakage from Tuberous Roots of Sweetpotato (*Ipomoea batatas* (L.) Lam.) after Short-term High Temperature

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(Received May 11, 2016; Accepted July 7, 2016)

We investigated the time-course pattern of electrolyte leakage from the root flesh of growing tuberous roots of two sweetpotato cultivars, Koganesengan and Narutokintoki, after exposing them to high temperature for short duration. For both cultivars, the electrolyte leakage after 1 h of treatment was significantly higher than that at 24 h after treatment. This pattern was similar to the pattern observed following instantaneous flooding treatment previously reported by us. Electrolyte leakage from plant cells is an indicator of cellular responses to various stress factors. Similar stress, therefore, might be caused in heated and flooded tuberous roots.

Keywords : electrolyte leakage, environmental stress, sweetpotato, tuberous root

INTRODUCTION

Sweetpotato (*Ipomoea batatas* (L.) Lam.) plants which are surface-irrigated periodically, i.e., twice a week on root media, show greater α -tocopherol content in their tuberous roots with no apparent change in either tuberous root development or oxygen concentration around the roots as compared to sub-irrigated plants (Eguchi et al., 2012). 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 as a coping mechanism for the slight oxidative stress, i.e. hypoxia, occurring within the roots. Therefore, we performed instantaneous flooding, which completely covered the entire root surface of sweetpotato plants with water (Eguchi et al., 2015) and demonstrated that electrolyte leakage from the tuberous root flesh cells showed a temporal increase with flooding treatment and is an indicator of cellular responses to various stress factors (Demidchik et al., 2014).

Rise in temperature activates respiration within the heated plant part; in a similar way, high-temperature treatment applied to the tuberous root can also cause slight oxidative stress within the root similar to that caused by instantaneous flooding. We therefore investigated electrolyte leakage from tuberous roots subjected to high temperature for short durations.

MATERIALS AND METHODS

Plant materials

Sweetpotato cultivars Koganesengan and Narutokintoki were used in this study as the previous study (Eguchi et al., 2012). Plant materials were prepared as described

previously (Eguchi and Yoshida, 2007). The lowest node of a stem cutting with three leaves attached was rooted and grown in a phytotron at a temperature of $25^{\circ}\text{C} \pm 1$ and relative humidity (RH) of $70\% \pm 5\%$ for 10 d. Except for an approximately 25-cm-long single nodal root, all other roots were excised from the plant. Thus, plants with a single root and three leaves were used for all experiments.

Experimental conditions

Three plants were transferred to a hydroponic box as a foolproof method to induce tuberous root formation (Eguchi and Yoshida, 2004). The box consisted of a nutrient solution layer for water and nutrient uptake by roots and an aerial space to allow for the swelling of roots above the nutrient solution. Three cultivation systems were installed in a phytotron glass room (air temperature of 25°C and RH of 70%). The temperature of the nutrient solution was controlled at 25°C using a water temperature controller (Coolnics Circulator, CTA401, Yamato Scientific Co., Ltd., Tokyo, Japan). Half-strength of OAT Agrio A solution (OAT Agrio Co., Ltd., Tokyo, Japan) adjusted to pH 6.0 was used. Plants were subjected to 3 h high-temperature treatment, during which the root diameter swelled to about 10 mm. Air temperature within a steel case (150 mm W, 120 mm L, 200 mm H) surrounded with Styrofoam insulator was controlled at 65°C with a Peltier-effect applying heater (SL-3FF, Nippon Blower Co., Ltd., Tokyo, Japan) and a controller (SL-C7PE, Nippon Blower Co., Ltd., Tokyo, Japan), and the high-temperature air was circulated between the case and the aerial space of the hydroponic box using parallel arranged two air pumps (MAS-1, As One Corp., Osaka, Japan) during the high-temperature treatment. The cultivation systems were shaded for 18 h prior to the high-temperature treatment to avoid temperature disturbance by solar irradiation, and this shading continued 24 h after the treatment. Air temperature around the tuberous

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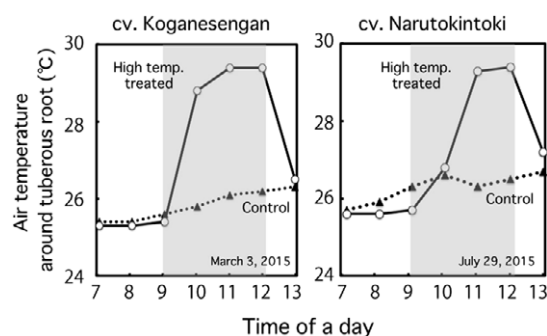


Fig. 1 Change in temperature around tuberous roots of sweetpotato during high-temperature treatment.

roots was measured with a thermo recorder (TR-71wf, T&D Corporation, Matsumoto, Japan). During the treatment, the temperature of the air-exposed part of the swollen root rose to about 29°C (Fig. 1). Koganesengan plants were cultivated between January 22 and March 4, 2015. Narutokintoki plants were cultivated between July 9 and July 30, 2015.

Evaluation of electrolyte leakage

Stress-induced electrolyte leakage was evaluated by measuring the time-course changes in electrolyte leakage from the tuberous root flesh, as described previously (Eguchi et al., 2015). Electrolyte leakage was measured using the method described by Campos et al. (2003). Three tuberous roots for each cultivar were sampled immediately before and 1 and 24 h after the high-temperature treatment. Three discs (8 mm diameter, 2 mm thickness) of tuberous root flesh were obtained from the thickest part of the root and were rinsed with deionized water three times. The discs were soaked in 6 ml of deionized water, and the electric conductivity of the solution was measured after 22 h using a conductivity meter (MM-60R, DKK-TOA Corp., Tokyo, Japan). Thereafter, the solution containing the root disc was kept at 90°C for 2 h, and the total conductivity of the solution was measured. Electrolyte leakage was evaluated as the percentage of electrical to total conductivity.

RESULTS AND DISCUSSION

Figure 2 shows changes in relative electric conductivity on exposure to high-temperature. In Koganesengan, the highest value, 19% of the relative electric conductivity was observed after 1 h of heat treatment, whereas the value decreased to about 16% after 24 h of treatment (Fig. 2A). A similar pattern was observed in Narutokintoki, for which the highest value, 21% of the relative electric conductivity was observed after 1 h of the heat treatment and decreased to about 18% after 24 h of treatment (Fig. 2B). For both cultivars, the value after 1 h of the treatment was significantly higher than that at 24 h after the treatment. Time-course patterns of electrolyte leakage after heat treatment were similar to the previously reported pattern observed after instantaneous flooding treatment (Fig. 2C, Eguchi et al., 2015). Demidchik et al. (2014) determined that electrolyte leakage, which is mainly related to K^+ efflux, is the hallmark of stress response in intact plant cells, and the

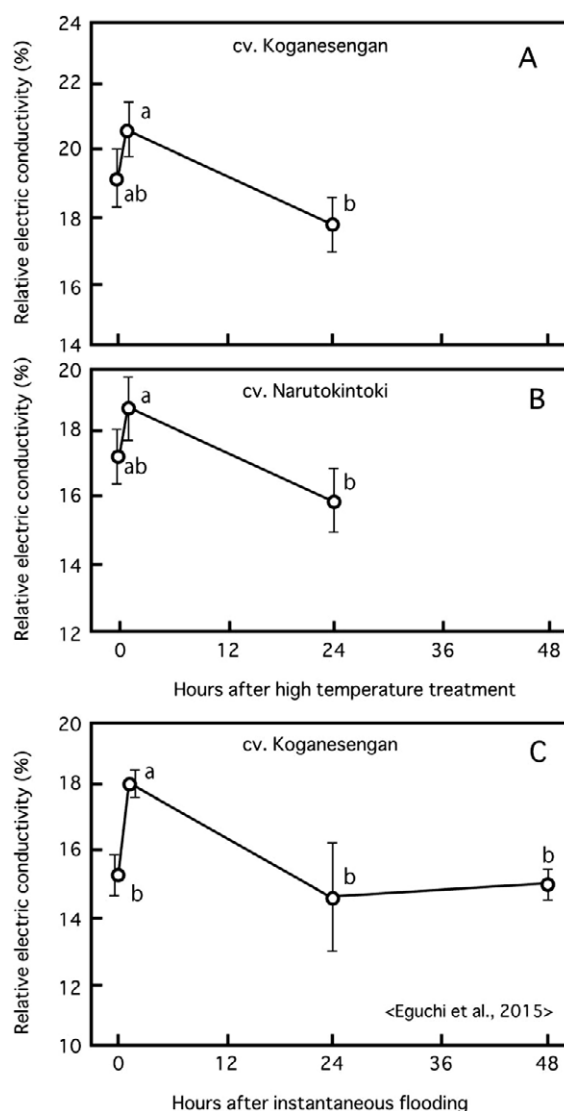


Fig. 2 Time-course changes in the relative electric conductivity of tuberous roots on application of high-temperature treatment in Koganesengan (A) and Narutokintoki (B). Different letters represent significant differences at $P < 0.05$ by Tukey's test ($n=3$), and bars represent \pm SE. A representative illustration (C) of the changes after the application of the instantaneous flooding is given (Eguchi et al., 2015).

reaction is generally activated after a few minutes and lasts for about 1 h. Our results showed that a similar reaction occurred within the tuberous root. According to Geigenberger (2003), bulky plant tissues with high metabolic activity, such as potato tubers, can become hypoxic because they lack large intercellular air spaces, contain poorly vacuolated cells and are located at sites remote from the sites of entry of O_2 . For example, Geigenberger et al. (2000) demonstrated that hypoxic conditions easily develop in the inner regions of growing potato tubers even when the tuber is under normoxic conditions. Because the anatomical features of sweetpotato tuberous roots are similar to those of potato tubers, the low O_2 stress may also easily develop within the growing tuberous root. In our experiments, short-term high temperature can also cause the low O_2 stress within the tuberous root.

This study was supported by JSPS KAKENHI Grant Number 24580371.

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