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Temperature Effects on the Yield and Quality of the Medicinal Plant *Pinellia ternata* Breit.

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The medicinal plant *Pinellia ternata* Breit., which is a non-domesticated plant, is widely distributed in Japan. However, the crude drug made from the plant corm is not currently produced in Japan. We investigated the influences of air temperature on the growth of *P. ternata* collected from 2 regions, the Kyoto and Nagasaki prefectures, for both of summer and winter seasons. The temperature effects on the effective ingredient contents in the corms were also investigated. At Kyushu University, plants were grown for 15 weeks in phytotron glass rooms controlled at air temperatures of 20, 25, or 30°C. The corm yields and effective ingredient content in the winter cultivation were poorer than those in the summer, because the cumulative solar radiation during the winter cultivation period was almost half of that in the summer. In the Kyoto lines, the highest corm yield was obtained at 25°C in the summer cultivation, while the Nagasaki lines did not show significant differences with respect to yield among the three growth temperature conditions. The effective ingredient contents in the corm did not differ significantly among the three temperature conditions for both lines, although the amount of effective ingredient in the Kyoto lines were significantly higher than those of Nagasaki in summer cultivation.

Keywords : arabinan, corm yield, growth temperature, *Pinellia ternata*

INTRODUCTION

Crude drugs have been effectively included as part of Japanese medicine since ancient times. However, modern Kampo medicine in Japan mostly depends upon the import of drugs from China. In recent years, Japanese Kampo manufacturers have suffered from the rapid increase in export prices of Chinese drugs (Yamaoka et al., 2017). Many of the medicinal resources in herb medicine remain largely untapped. The domestication of medicinal wild plants and preservation of their ecological systems are required to achieve a stable supply of herb medicines in Japan. The medicinal plant *Pinellia ternata* Breit., which is a non-domesticated plant, is widely distributed in Japan (from Hokkaido to the Ryukyu islands). However, the drug made from *P. ternata* is not currently produced in Japan. The *P. ternata* drug is made from the corm and has an antiemetic effect: the major effective ingredient is a water-soluble polysaccharide mainly consisting of arabinose (Maki et al., 1987). Therefore, we aimed to domesticate *P. ternata* and select favorable lines as medicinal material. Here, we investigated the air temperature effects on the plant growth and the effective ingredient content.

MATERIALS AND METHODS

Plant material

Bulbils of *P. ternata* collected from two regions, the

Kyoto and Nagasaki prefectures, were used for this experiment.

Cultural conditions

The porous solid material PUMICE® (grain size, 0.5–2.4 mm; porosity, 0.58; OhE Chemicals Inc., Osaka) was used as root media. A square cultivation box (inner size: width, 300 mm; length, 150 mm; height, 210 mm) was filled with the root media at 180 mm thickness. A drainage tube was connected to the bottom of the box. Boxes were installed in three phytotron glass rooms of Kyushu University controlled at air temperatures of 20, 25, and 30±1°C and a relative humidity of 70±5%. A commercial nutrient solution (OAT Agrio Co., Ltd., Osaka) adjusted to pH 4.0 was used. The root media were moistened well and 10 bulbils were transplanted into each box. In each box, the bulbils were equally placed at intervals of about 6 cm in 2 lines, one line was for the Kyoto region and another was for the Nagasaki region, with the bulbil base located 3 cm below the surface of the medium. The ground water level in the root media was adjusted by controlling the level of nutrient solution within a tank with the drainage tube. The ground water levels were maintained at approximately 4 cm below the bulbil base, which is favorable for the plant growth (Eguchi et al., 2014). During the cultivation using this box, the water content of the root media around the bulbils is maintained almost constant, regardless of the irradiation to the box (Eguchi et al., 2009). Plants were grown for 15 weeks in summer (from May 14 to August 27, 2014) and winter (from November 19, 2014 to March 4, 2015).

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Corms grown from bulbils were harvested after cultivation and weighed. The harvested corms were peeled, then soaked in 1% acetic acid solution for 4 hours. Thereafter, the corms were washed well with distilled water. Washed corms were dried at room temperature for 2 weeks, and the dry weights were measured.

Content of the effective ingredient

Dried corms were used to determinate the effective ingredient content by using the enzyme-linked immunosorbent assay (ELISA). Each sample (50 mg) was pulverized and extracted with H₂O (1 ml per 100 mg powder) three times, then heated at 95°C for 10 minutes. The sample solutions were diluted 4,000 or 20,000 times with 50 mM carbonate buffer (pH 9.6) absorbed on the wells of a 96-well immunoplate (100 µl), and then treated with 300 µl of 5% skimmed milk in PBS for 1 hour to reduce non-specific adsorption. The plate was washed with a washing buffer and then treated with a monoclonal antibody (MAb) against the polysaccharide fraction of *P. ternata*. The MAb was combined with 100 µl of a 1:1000 dilution of peroxidase-labeled anti-mouse IgG for 1 hour. After washing the plate, 100 µl of the substrate solution [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) solution] was added to each well and incubated for 10 minutes. The absorbance was measured with a micro plate reader at 405 nm. The effective ingredient content was also measured for the medicine on the market, produced in China, that was purchased in 2014.

Statistical analysis

Data for corm yield, and effective ingredient content in the corm were analyzed by analysis of variance ($n = 5$). The significant differences between regions or seasons were tested by Tukey test at $P < 0.05$.

RESULTS AND DISCUSSION

Figure 1 shows the *P. ternata* yield, dry weight of the corm, affected by the growth temperature. In the Kyoto lines, the highest corm yield was obtained at 25°C in summer cultivation. While the Nagasaki lines did not show a significant difference between the yields among the three growth temperature conditions for both seasons. In *Colocasia esculenta*, that belongs to the same genus as *P. ternata*, tuber growth was also promoted under soil temperature of around 25°C (Sugimoto et al., 2001). The corm yield appeared to be higher in the summer cultivation than that in the winter cultivation for both of the two lines.

Figure 2 shows the contents of the effective ingredient in the corm of *P. ternata* grown under the air temperature of 20, 25, and 30°C for the summer and winter seasons. The dotted line shows the effective ingredient content of the Chinese medicine, 21.4 mg g⁻¹, which was obtained from the market. These two lines of Japanese *P. ternata* also contained a high level of the effective ingredient in the corm. The effective ingredient contents in the corm did not differ significantly among the three temperature conditions for both lines, although the values in the Kyoto lines were significantly higher than those of the Nagasaki lines in the summer cultivation. The highest value of the effective

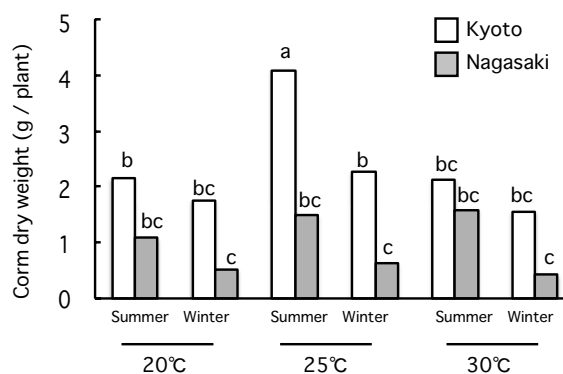


Fig. 1 Effect of growth temperature on the corm yields of *P. ternata* originated from two regions, the Kyoto and Nagasaki Prefectures, in the summer winter cultivation. Mean values are indicated. Different letters represent significant differences at $P < 0.05$ by Tukey test ($n = 5$).

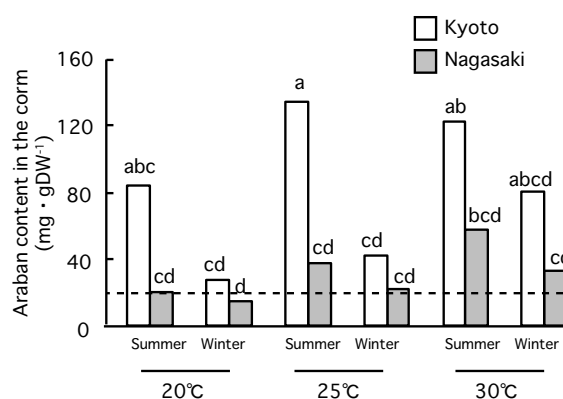


Fig. 2 Effects of growth temperature on the contents of effective ingredient, araban, in the corm of *P. ternata*. The dotted line shows the effective ingredient content of the Chinese medicine obtained from the market. Different letters represent significant differences at $P < 0.05$ by Tukey test ($n = 5$).

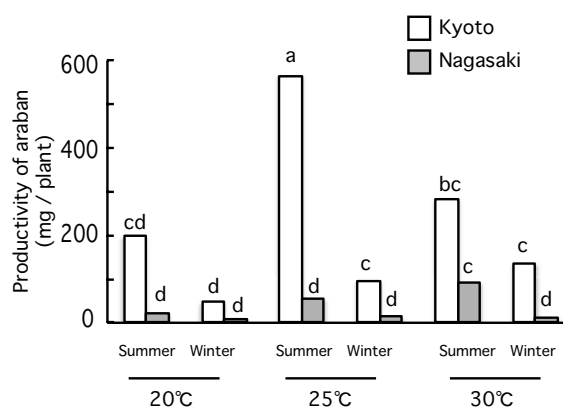


Fig. 3 Effects of growth temperature on the productivities of effective ingredient by *P. ternata*. Different letters represent significant differences at $P < 0.05$ by Tukey test ($n = 5$).

ingredient content was observed in the Kyoto lines grown at 25°C in the summer cultivation, where the highest corm yield was observed.

Figure 3 shows the productivity of the effective ingre-

dient per plant as the product of the corm dry weight and the content of the effective ingredient under the respective growth temperatures. The significantly highest value of the productivity was observed in the Kyoto lines grown at 25 °C in the summer cultivation.

Cumulative solar radiation at Fukuoka city was 1,571 MJ m⁻² during the summer cultivation period, whereas that decreased to 829 MJ m⁻², which was almost half of the summer radiation, during the winter cultivation period. Flowering was observed in both seasons, and new leaf emergence occurred through cultivation periods in both seasons. It was considered that plants were not introduced to dormancy phase in summer or winter seasons. Thus, the solar irradiance might cause the difference of the corm yields from the summer and winter cultivation.

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