Influence of Ground Water Level on the Growth of the Medicinal Plant Pinellia ternata Breit. in a Solid Substrate Culture System

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Influence of Ground Water Level on the Growth of the Medicinal Plant *Pinellia ternata* Breit. in a Solid Substrate Culture System

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Abstract: Many crude drugs used in Kampo medicines in Japan represent untapped medicinal resources. Domestication of the wild plants from which these drugs are derived and preservation of their natural habitats are necessary for establishing a stable supply of Kampo medicines. Here, we investigated the influence of the moisture condition of root medium on the growth of *Pinellia ternata* Breit. by using a sub-irrigation solid substrate culture system. The moisture condition was controlled by maintaining a constant ground water level (GWL). Plants were grown for 15 weeks under a GWL of either 4 cm (GWL-4) or 8 cm (GWL-8) below the corm base in a phytotron glass room at 25°C and 70% relative humidity. The water content of the root media around the corm in GWL-4 was only 1% higher than that in GWL-8. However, water content apparently affected corm enlargement and the development of new bulbils. Relative yield of the corm per plant, compared with the initial weight, was ~400% in GWL-4 and ~200% in GWL-8. The number of bulbils produced in a plant was 13.5 in GWL-4 and 4.4 in GWL-8. The effective ingredient, a kind of water-soluble polysaccharide consisting mainly of arabinose, was about 10% higher in GWL-8 corms than in GWL-4 corms. Moreover, when comparing the two conditions, the relative difference in corm yield was remarkably larger than that observed for the effective ingredient content. Overall, productivity was considered to be higher in GWL-4 than in GWL-8.

Keywords: Pinellia ternata, root water condition, corm yield, arabinan

1. INTRODUCTION

Crude drugs have been effectively included as part of Japanese medicine since ancient times. However, modern Kampo medicine in Japan mostly depends on the import of drugs from China (http://www.nikkankyo.org/aboutus/investigation/investigation03.html, Japanese text). Such crude drugs are principally made from wild plants, for which Chinese yields are typically decreasing and resulting in an increase in export prices. Many of the medicinal resources in herb medicine remain largely untapped, and the domestication of medicinal wild plants and preservation of their ecological systems are required to achieve a stable supply of herbal crude drugs 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 moisture condition of root media suitable for plant growth.

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2. MATERIALS AND METHODS

Plant material

Corms of *P. ternata* sold on the market (Nikkoseed Corp., Utsunomiya) were used for this experiment. The place of origin was unidentifiable.

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 the center of a phytotron glass room with an air temperature of 25±1°C and relative humidity of 70±5%. A commercial nutrient solution (Otsuka Chemical Co. Ltd., Osaka) adjusted to pH 4.0 was used. The root media were moistened well and 5 corms were transplanted into each box with the corm base located 3 cm below the medium surface. The ground water level in the root media was adjusted by controlling the level of nutrient solution within a tank with the drainage tube. Ground water levels were maintained at approximately 4 cm (GWL-4) or 8 cm (GWL-8) below the corm base. Plants were grown for 15 weeks from June 5th to September 18th, 2013. Corms and bulbils were harvested after cultivation and weighed. The harvested corms were stored at -30℃. Water contents of the root media at 2-3cm depth, where the corms were planted, were evaluated.

Content of the effective ingredient

Stored corms were lyophilized, and used to determinate the effective ingredient content by using ELISA. Each sample (10-50 mg) was pulverized and extracted with H_2O (1 ml per 100 mg powder) three times. The sample solutions were diluted 10 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 h 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 h. 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 min. Absorbance was measured with a micro plate reader at 405 nm.

3. RESULTS AND DISCUSSION

Neither of GWL-4 nor GWL-8 showed symptoms of water deficiency (e.g. leaf wilting) during the cultivation period. Water contents of root media around the corms were 26% and 25% in GWL-4 and GWL-8, respectively. In a previous study using a similar cultivation methiod for carrots, we confirmed that the water content at a certain depth remained almost constant during the cultivation period (Eguchi et al., 2009). The corms of the *P. ternata* might be also grown under the same stable water condition as those for carrots.

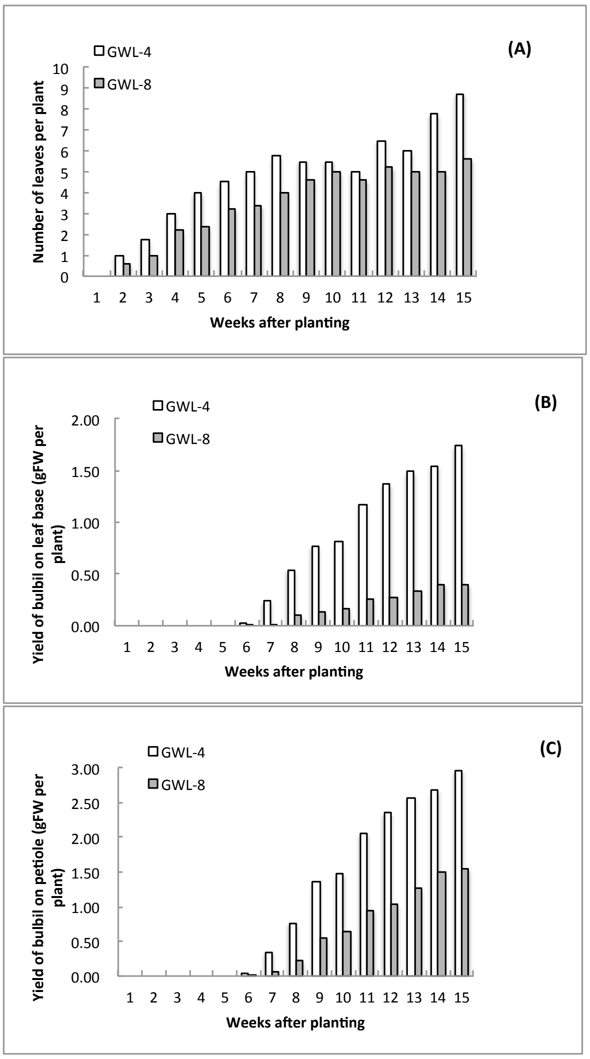

Fig. 1. Time-course changes of leaf number (A), cumulative fresh weight of bulbils on the leaf base (B), and cumulative fresh weight of bulbils on the petioles (C) in a *P. ternata* plant.

Figure 1 shows the time-course changes in leaf number (A), cumulative fresh weight of bulbils on the leaf base (B), and cumulative fresh weight of bulbils on the petioles (C) in a plant. GWL-4 was favorable for both of leaf development and bulbil production. Bulbil formation on the leaf base was particularly strong under GWL-4, where the bulbils were formed on the leaf base in almost all leaves, compared to 40% of leaves in GWL-8. Furthermore, the averaged fresh weight of one bulbil in GWL-4 was three times higher than that in GWL-8.

Table 1 shows the corm and bulbil yields. In GWL-4, the corms grew to about 4 times the fresh weight of that recorded at planting time, whereas this was only increased 2 times under GWL-8. Summing all of the bulbils obtained during the cultivation period (13.5 bulbils in GWL-4 and 4.4 bulbils in GWL-8) to the corm weight, the 8-fold yields were produced in GWL-4, compared to 4-fold yields in GWL-8. Statistically significant differences were not observed in the corm and bulbil yields between the two conditions. Distribution of the relative fresh weight of the corms (Fig. 2), however, suggests that the root water condition can affect *P. ternata* yields.

Table 1. Fresh weights of corms and bubils.

Ground water level	Corm fresh weight at planting (g/plant)	Corm fresh weight at harvest (g/plant)	Bulbils yield (g/plant)
GWL-4	1.29	4.76	6.22
GWL-8	1.29	2.86	2.76

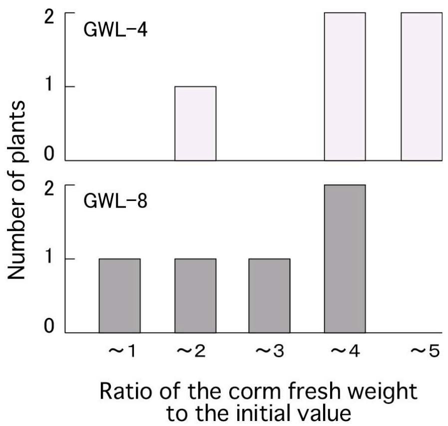

Fig. 2. Distribution of the relative fresh weight of the corms to the initial value.

Figure 3 shows the results of ELISA as the absorbance of the effective ingredient per 1 mg dry weight of the corms (A), dry weights of the corms per plant (B), and productivity of the effective ingredient per plant as the product of A and B (C). Concentration of the effective ingredient in the corm grown in GWL-4 was 10% lower than that in s GWL-8 (p>0.01, t-test). However, as the dry weight of the corm was 70 % higher in GWL-4, an approximately 50 % higher quantity of the effective ingredient was accumulated in one corm grown under GWL-4. Thus, when grown under a stable water condition, corms of homogenous quality can be obtained regardless of corm size. On the basis of these findings, we proposed that the root water condition may be more favorable in GWL-4 than in GWL-8 for the *P. ternata* cultivation.

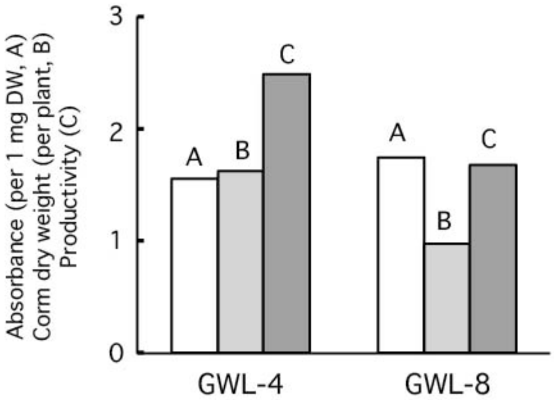

Fig. 3. Absorbance of the effective ingredient (A), dry weight of the corm (B), and the productivity of the effective ingredient per plant (C).

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4. REFERENCES

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