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Effect of Day Length and Tuber Storage Temperature and Duration on Sprouting, Enlargement and Flowering of Tubers in *Pinellia ternata* (Thunb.) Makino

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Summary

Crowdipper (*Pinellia ternata* (Thunb.) Makino) is a perennial plant in the aroid family (Araceae), which grows naturally in various parts of East Asia. It can be propagated by dividing tubers. Large tubers that have been peeled and dried have long been used as a material in Kampo medicine called "Hange". We investigated the effects of temperature and light on sprouting, growth, and flowering of tubers. Tubers sprouted 100% in 3.2 weeks under long-day conditions (L). On the other hand, under the short-day condition (S), no sprouting occurred even after 7 weeks. From these results, it was expected that the photoperiod was the main factor for sprouting. The tubers cultivated for 20 weeks after planting swelled about 5 times under the L-L condition and S-L conditions. Flowering was only observed where the storage temperature was 4℃ for 6 weeks. On the other hand, in the treatment plots stored at 16℃, the tuber enlargement rate tended to be higher in the 3-week storage than in the 6-week storage.

INTRODUCTION

Crowdipper (*Pinellia tern*ata (Thunb.) Makino) is a perennial plant in the aroid family (Araceae), which grows naturally in various parts of East Asia. One leaf is usually developed from one tuber from spring to summer, and one spadix (inflorescence) peculiar to Araceae is formed at the top of a large tuber. It can be propagated by seeds (Nagao 1980), but the tuber forms a small daughter tuber on the surface, and in addition, it forms a propagule at the base of the petiole of the leaf and the base of the leaflet. Large size of tubers that have been peeled and dried have long been used as a material in Kampo medicine called "Hange". The main ingredient is homogentisic acid, which is also an ingredient in many traditional Kampo medicine (Harashima 2012). At present, materials imported from China are mainly used for the manufacture of Japanese Kampo medicines, but it is expected that these imports will become unstable due to the recent international situation (Tohoku Revitalization Research Center 2013).

Depending on the temperature of the cultivation area, sprouting and growth from tubers occurs once or twice a year in the Kanto region in Japan (Nagao 1979), and no method has yet been established for stably cultivating tubers suitable for Kampo medicine raw material. The authors have cultivated *Pinellia ternata* in a plant factory with a stable environment, and confirmed that under certain environmental conditions, the dormancy period is very short, and the growth and multiplication rate can be increased up to about 4 growth cycles per year (Amaki *et al.*, 2015). However, although there are some studies that have examined the effects of temperature on growth and tuber enlargement (Eguchi 2019; 2020), there are not many studies on the effects of tuber storage temperature and light during cultivation (Nagao 1977; 1978). Therefore, we investigated the effects of temperature and light on sprouting, growth, flowering, and enlargement of tubers.

MATERIALS AND METHODS

Tubers growing wild in the sandy soil of Niigata City, Niigata Prefecture were collected and cultivated and multiplied in a greenhouse with a minimum temperature of 16℃ at the Atsugi Campus of Tokyo University of Agriculture, Kanagawa Prefecture. Harvested tubers were cleaned, lightly dried, placed in plant boxes (Magenta Box G7, Magenta Co., Ltd), and stored at room temperature (20 - 24℃) until used for following experiments. In the daylength experiment, tubers were planted in 6 cm plastic pots with a mixture of Metro Mix 360 JPN (Sungro Horticulture, Canada): akadama clay soil (S-size) = 1:1. In the storage study, tubers were planted at a depth of 1 cm in 6 cm pots filled with Metro Mix 360. Cultivation was performed without fertilization, irrigation was performed with tap water, and temperature was 23 ± 1℃.

Effect of day length on sprouting and subsequent growth of tubers

Tubers stored at room temperature for about 6 months were planted, and 10 tubers for each treatment were cultivated under two conditions: long day (16h-light / 8h-dark: L) and short day (8h-light / 16h-dark: S). After 7 weeks, 5 unsprouted tubers from the short-day conditions were moved to long day conditions (S-L) or 12h-light/12h-dark conditions (S-M), and then cultivation was continued for 20 weeks. The light source used for cultivation was a white fluorescent lamp (FLR40S-EX-N/M-H,

Toshiba Lighting & Technology Co., Ltd.), and the irradiance (photosynthetic photon flux density: PPFD) was 100 µmol·m^{-2}·s^{-1}.

Effect of tuber storage temperature, duration, and cultivation light on sprouting and subsequent growth and flowering of tubers

Room temperature-stored tubers were moved to 4℃ or 16℃ for 3 months (3 plants per plot) or 6 months (2 plants per plot). After planting, tubers were grown under different LED light environments of mixed white (blue + green + red: W), blue (peak wavelength 470 nm: B), green (525 nm: G), or red (660 nm : R) with a PPFD set to 100 µmol·m^{-2}·s^{-1}.

RESULTS AND DISCUSSION

Effect of day length on sprouting and subsequent growth of tubers

Tubers sprouted 100% in 3.2 weeks under long-day conditions (**Table 1**). On the other hand, under the short-day condition (S), no sprouting occurred even after 7 weeks. From these results, it was expected that the photoperiod was the main factor for sprouting of *Pinellia ternata* tuber. The tubers cultivated for 20 weeks after planting swelled about 5 times under the L-L condition and S-L conditions. In the S-M condition with delayed sprouting, the average fresh weight of tuber was about 3 times larger than that of L-L and S-M (Table 1).

Table 1. Effects of day length on sprouting rate, tuber growth rate and sprouting time of *Pinellia ternata*

Daylength[z]			Sprouting (%)			
0 to 7 weeks	7 to 14 weeks		After 7 weeks	After 14 weeks	Sprouting time (weeks)	Tuber enlargement (%)[y]
16-L/8-D	16-L/8-D	L-L	100	-	3.2	467.0
8-L/16-D	16-L/8-D	S-L	0	100	9.8	488.4
8-L/16-D	12-L/12-D	S-M	0	100	14.2	296.6

[z]16-L/8-D means 16 hours of lighting and 8 hours of darkness (Same for others).
[y]Enlargement rate calculated by the fresh weight at the end of treatment divided by the weight at the start of treatment as 100.

Effect of tuber storage temperature, duration, and cultivation light on sprouting and subsequent growth and flowering of tubers

Flowering was observed only in tubers stored at 4℃ for 6 weeks (**Table 2**). For tubers stored at 16℃, the tuber enlargement rate tended to be higher after 3-weeks of storage compared to 6-weeks storage. No significant difference was observed in the influence of light quality of cultivation light. Tuber enlargement after cold storage was generally smaller compared to tubers held at room temperature, but this is because the storage period at room temperature after

digging is long and the cultivation period after planting is short (20 weeks vs. 12 weeks). In addition, almost no flowering was observed in the greenhouse at 16℃ clarifing that 4℃ for 6 months is a factor that promotes flowering.

Table 2. Effects of storage temperature, period of tubers and light quality during cultivation on tuber sprouting, flowering, and enlargement of *Pinellia ternata*.

Storage temperature (℃)	Storage period (month)	Light quality for cultivation	Sprouting time (weeks)	Tuber enlargement (%)[z]	Flowering (%)
4	3	Mixed White	1	77	0
		Blue	1	62	0
		Green	1	66	0
		Red	1	97	0
	6	Mixed White	1<	98	50
		Blue	1<	96	50
		Green	1<	107	50
		Red	1<	94	100
16	3	Mixed White	3	112	0
		Blue	3	103	0
		Green	3	76	0
		Red	3	82	0
	6	Mixed White	3	64	0
		Blue	1	68	0
		Green	2	75	0
		Red	3	82	0

[z] Enlargement rate calculated by the fresh weight at the end of treatment divided by the weight before storage treatment as 100.

Based on these results, if only tuber enlargement is to be promoted, white light LEDs or white fluorescent lamps should be used as the light source under long-day conditions. However, if this condition is continued, the shape of the tubers becomes unsuitable for materials for “Hange”, as shown in **Figure 1**. Therefore, the tubers are divided into large and small size in a cycle of about half a year, and the large size tubers are continued to be cultivated. In order to increase the size of the tubers, it is thought that low-temperature treatment of the small tubers and the obtained propagules to promote sprouting and growth will lead to efficient tuber production.

Figure 1 Morphology of *Pinellia terunata* tubers cultivated for 18 months in a plant factory environment (24°C, white fluorescent lamps as light source, 150 μmol$m^{-2}s^{-1}$, 16 hours illumination per day) (Amaki *et al*., 2015). The planted parent tuber is in the center. The lateral buds were enlarged as secondary tubers one after another, making it an unsuitable form for processing as "Hange".

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