In Vitro Propagation and Post-in Vitro Establishment of Bulblets of *Lilium japonicum* Thunb.

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Summary

Lilium japonicum growing in the experimental field were collected in early May and segments of their scales, leaves and stems of the plants were cultured in vitro on a basal medium consisting of Murashige and Skoog's inorganic salts (1962) supplemented with several organic nutrients. Young leaf-segments were a suitable source for propagation, because they were least contaminated and almost had the same capacity as scale segments for bulblet regeneration. Bulblets were proliferated by culturing whole scales or segments on the basal medium supplemented with $0.1 \text{ mg} \cdot \text{liter}^{-1}$ NAA and $0.01 \text{ mg} \cdot \text{liter}^{-1}$ BA. A cold treatment at 4°C for more than 12 weeks broke the dormancy of in vitro cultured bulblets. In vitro light conditoin affected both post-in vitro bulblet-rot infection and plant growth in soil. The bulblets incubated in the dark were more susceptible to soil-born organisms than were those incubated in the light. Nearly all the bulblets of more than 400 mg in weight grew into plants with elongated axes (epigeous type plant; ETP). The ETP plantlets required at least 2 years' cultivation before they flowered.

Introduction

Lilium japonicum Thunb., 'Sasa-yuri' is indigenous to the hills and low mountains of the central and western districts of Honshu, Shikoku, and Kyushu, Japan (Shimizu, 1987). Reductions in population and limited distribution have led *L. japonicum* close to extinction. In addition, it has considerable potential as an ornamental crop because of its trumpet-shaped beautiful flowers endowed with good pink color and pleasant fragrance. Some growers have attempted to produce bulbs commercially through seed and scale propagation, but an appropriate commercial method of proliferating the bulbs has not been established. This paper describes the aseptic cultures, in vitro proliferation, and post-in vitro establishment of *L.*

japonicum bulblets.

Materials and Methods

Experiment 1. Establishment of aseptic cultures

A basal medium consisted of Murashige and Skoog's inorganic salts (1962), $2 \text{ mg} \cdot \text{liter}^{-1}$ glycine, $100 \text{ mg} \cdot \text{liter}^{-1}$ myoinositol, $0.1 \text{ mg} \cdot \text{liter}^{-1}$ thiamine hydrochloride, $0.5 \text{ mg} \cdot \text{liter}^{-1}$ nicotinic acid, $0.5 \text{ mg} \cdot \text{liter}^{-1}$ pyridoxine hydrochloride, 5% sucrose, 0.7% Difco Bacto agar, and, except where otherwise stated, $1 \text{ mg} \cdot \text{liter}^{-1}$ NAA and $0.1 \text{ mg} \cdot \text{liter}^{-1}$ BA (Niimi and Onozawa, 1979).

Three plants, having stems about 15 cm in length, were harvested from the experimental field in early May. Young growing leaves, terminal portion of stem (about 10 cm long), and a bulb were separated from each plant, washed with soap, and then rinsed thoroughly with tap water.

1) Sterilization

Leaves and stems were sterilized with 70% ethanol, rinsed with tap water, swirled for 5 minutes in 0.6% sodium hypochlorite. Bulbs were im-

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mersed in aqueous 0.1% Benlate for 30 minutes after removal of damaged outer-scales.

Healthy scales separated from the bulbs were sterilized with the same methods as used for leaves and stems, except that the scales were swirled for 20 minutes in 2% sodium hypochlorite.

Finally, each organ was rinsed three times in sterile water.

2) Preparation of explants and inoculation

(1) *Scale explants*: A scale segment, about 1 cm², was excised from the lower portion of each scale and placed with the abaxial side down on the 20 ml basal medium in a test tube (18×180 mm).

(2) *Leaf explants*: A 3~5 mm long proximal regions including petiole was cut away from a young leaf, and then three leaf-strips, 5 mm in length, were excised transversely from the basal portion. They were designated as L-I, L-II, and L-III and placed, abaxial side down, on the 20 ml basal medium in a 50-ml Erlenmeyer flask.

In an additional experiment, five 3-mm leaf-strips from the proximal portion of leaves (designated as GL-I) and five other strips from the mid-portion of leaves (designated as GL-II) were excised and cultured on the basal medium containing BA at concentrations of 0.005, 0.01 and 0.05 mg·liter⁻¹ in combination with 0.5 mg·liter⁻¹ NAA (Table 2).

(3) *Stem explants*: Ten stem-segments, 5 mm long, were prepared by transversely cutting the terminal portion of a stem; each segment was placed with the basal cut end, surface down, on the basal medium in a test-tube (18×180 mm).

3) Culture conditions

All explants were kept in the dark at 24±1 °C for 90 days at the end of which the number of bulblets per explant, fresh weight of bulblets excluding roots, and the percentage of contamination were determined.

Experiment 2. Proliferation of bulblets by culturing whole scales and scale segments

Scales, excluding a few inner small scales, were excised aseptically from bulblets that had been cultured on the basal medium containing 0.05 mg·liter⁻¹ NAA for more than 12 weeks at 24±1 °C in the dark.

1) *Whole scale culture*: Five randomly selected

scales were placed, abaxial side down, in a 50-ml Erlenmeyer flask containing 20 ml of the basal medium supplemented with different concentrations of NAA and BA. Eight flasks per treatment were kept for 12 weeks under continuous white fluorescent lamps giving about 1000 to 1200 lx at the culture level (Table 3).

2) *Scale segment culture*: Scales were cut transversely into two (basal and terminal portions) or three (basal, middle and terminal portions). Five comparable segments were placed, abaxial side down, in a 50-ml Erlenmeyer flask containing 20 ml of the basal medium supplemented with 0.1 mg·liter⁻¹ NAA and 0.01 mg·liter⁻¹ BA. The 13 to 15 flasks per treatment were kept in the dark for 12 weeks (Table 4).

In both experiments, the number of bulblets per explant and weight of fresh bulblets excluding roots were determined after 12 weeks of culture.

Experiment 3. Establishment of bulblets in soil

1) Low temperature treatment and transplanting to soil

Bulblets that had been cultured for more than 3 months on the basal medium containing 0.1 mg·liter⁻¹ NAA and 0.01 mg·liter⁻¹ BA at 24±1 °C in the dark were washed with tap water. After cutting the roots at the base, bulblets weighing 100 to 200 mg, were mixed with moist vermiculite, placed in polyethylene bags (0.03 mm in thickness), and stored at 4 °C for 0, 2, 4, 6, 8, 10, 12, and 14 weeks. After the cold treatment, 10 bulblets, with 5 replicates for each treatment, were transplanted, 1 cm deep, in a plastic tray (50 × 35 × 7.5 cm) containing sandy-loam and leaf mold mix (1:1 v/v). The trays were transferred to a glasshouse, where the night (10~20 °C) and day (15~30 °C) temperatures fluctuated depending on the weather. The data of leaf emergence was recorded every 3 day for 45 days. All bulblets were gathered 13 weeks after transplanting to determine the number of surviving bulblets, their fresh weight and that of roots (Fig. 1, Table 5).

2) Post-in vitro growth of bulblets in soil

It was tested whether in vitro dark- and light-conditions affect the post-in vitro growth of bulblets in soil. Bulblets that had been cultured on the basal medium containing 0.1 mg·liter⁻¹ NAA and

0.01 mg·liter⁻¹ BA in the dark or light (about 150~300 lx) were treated as in *Experiment 3-1*. After being stored at 4 °C for 12 weeks, each was classified according to its weight and planted 1 cm deep in a plastic tray (Table 6). The bulblets were grown for 8 weeks under the same condition as in *Experiment 3-1*. Then, the numbers of rotten bulblets, surviving bulblets with growing leaves, and plant growth types (bolted plantlets without scaly leaves; epigeous type plant, ETP or unbolted plantlets only with scaly leaves; hypogeous type plant, HTP) (Matsuo et al., 1982) were counted.

Results

Experiment 1. Establishment of aseptic cultures and determination of initial propagation-materials

Scale-segments formed 3.1 bulblets per explant.

Leaf segments, of L-I and L-II, and stem-segments developed 2 to 2.4 bulblets per explant.

The average fresh weight of bulblets differed considerably; those developed on the leaf-segments were heavier and those from scale-segments were lightest.

No leaf-segments became contaminated, whereas 3 and 18% of the stem- and scale-segments, respectively, were contaminated (Table 1).

In an additional experiment (Table 2), leaf-segments or GL-I formed as many bulblets as those in scale-segments. However, GL-II formed no bulblets on the medium without BA, and 52% of the explants formed bulblets when they were cultured on the medium with 0.05 mg·liter⁻¹ BA and 0.5 mg·liter⁻¹ NAA.

Thus, it was found that GL-I and -II were the most suitable initial materials to establish aseptic tissue cultures of *L. japonicum*.

Table 1. In vitro bulblet-productivity of explants excised from different organs of mother bulbs of *L. japonicum*. ± indicates standard error of the mean.

Explants	Number of explants cultured	Number of explants forming bulblets ^z	Number of bulblets per regenerated explant	Mean fresh weight of bulblets (mg)	Number of explants contaminated ^y
Scale segments	33	25 (76)	3.1	39±6	6 (18)
Leaf strips					
L- I	18	15 (83)	2.2	99±16	0
L- II	18	13 (72)	2.0	104±15	0
L- III	18	9 (50)	1.1	78±17	0
Stem segments	33	27 (82)	2.4	55±7	1 (3)

^z Figures in parenthesis represent % of explants with bulblets.

^y Figures in parenthesis represent % of explants contaminated.

Table 2. Bulblet formation in leaf-segments of *L. japonicum* cultured in a basal medium containing NAA and BA at different concentrations. ± indicates standard error of the mean.

NAA (mg · liter ⁻¹)	BA	Leaf strips	Number of strips cultured	% of strips forming bulblets	Number of bulblets per cultured strip	Mean fresh weight of bulblets (mg)
0.5	0	GL- I	25	40±10	1.0±0.3	61±11
		GL- II	25	0	0	0
0.5	0.005	GL- I	25	80±11	3.2±0.5	60±6
		GL- II	25	16±7	0.5±0.2	16±2
0.5	0.01	GL- I	25	60±10	2.1±0.4	78±12
		GL- II	25	8±7	0.3±0.2	37±13
0.5	0.05	GL- I	25	84±10	2.6±0.5	100±12
		GL- II	25	52±15	1.6±0.4	60±7

Experiment 2. Proliferation of bulblets through bulb-scale cultures

Of excised scales incubated on the basal medium without growth regulators, 98% formed 1.8 bulblets per explant. The addition of NAA and BA at different concentrations to the basal medium had little effect on increasing the number of bulblets. The fresh weight of bulblets increased with the increasing concentration of NAA, whereas BA had little effect on increasing the weight (Table 3).

Culturing bi- or tri-sectioned scale-segments on the basal medium containing $0.1 \text{ mg} \cdot \text{liter}^{-1}$ NAA and $0.01 \text{ mg} \cdot \text{liter}^{-1}$ BA, the basal segments produced more than 2.2 bulblets, whereas the middle and terminal segments scarcely formed bulblets

(Table 4). Thus, the method of culturing bi- and tri-scale-sections had little effect on increasing the bulblet numbers.

Experiment 3. Establishment of bulblets in soil

Leaf emergence from chilled bulblets depended on the duration of the cold treatment (Fig. 1). None of the control bulblets kept at 25°C sprouted during the 45-day experimental period, whereas bulblets chilled for more than 12 weeks sprouted in 15 days after transplanting. Bulblets chilled for less than 8 weeks sprouted later and produced fewer new leaves.

The duration of the cold treatment affected the rate of surviving bulblets and plant growth in the soil (Table 5). All bulblets chilled for more than

Table 3. Effect of NAA and BA concentrations on bulblet formation in the cultures of bulb-scales isolated from in vitro a-xenic developing bulbs. $\pm$ indicates standard error of the mean.

NAA ($\text{mg} \cdot \text{liter}^{-1}$)	BA	% of scales with bulblets	Number of bulblets per regenerating scale	Mean fresh weight of bulblets (mg)	Weight classes of bulblets (%)				
					10~ 49mg	50~ 99mg	100~ 149mg	150~ 199mg	Above 199mg
0	0	98 $\pm$ 2	1.9 $\pm$ 0.2	47 $\pm$ 5	66	23	7	1	3
0.01	0	98 $\pm$ 2	2.3 $\pm$ 0.2	47 $\pm$ 4	67	23	9	0	0
0.01	0.005	100	2.5 $\pm$ 0.2	41 $\pm$ 3	72	25	3	0	0
0.05	0	100	2.8 $\pm$ 0.2	55 $\pm$ 4	53	32	11	4	0
0.05	0.01	95 $\pm$ 3	2.3 $\pm$ 0.2	60 $\pm$ 6	58	28	6	6	2
0.1	0	98 $\pm$ 2	2.4 $\pm$ 0.2	61 $\pm$ 4	49	38	9	3	1
0.1	0.01	100	2.5 $\pm$ 0.1	69 $\pm$ 6	52	31	7	5	5
0.1	0.05	90 $\pm$ 4	1.9 $\pm$ 0.2	58 $\pm$ 8	66	23	4	3	4
0.5	0	75 $\pm$ 7	2.9 $\pm$ 0.4	69 $\pm$ 5	40	39	19	1	1
0.5	0.01	85 $\pm$ 5	2.7 $\pm$ 0.3	64 $\pm$ 6	48	31	16	3	2
0.5	0.05	93 $\pm$ 3	2.4 $\pm$ 0.2	58 $\pm$ 5	66	20	9	2	3
0.5	0.1	90 $\pm$ 5	2.5 $\pm$ 0.2	66 $\pm$ 6	49	30	9	10	2

Table 4. Bulblet productivity in explants of non-divided scales, two- and three-divided scale-segments. $\pm$ indicates standard error of the mean.

Explants	Number of explants cultured	% of explants forming bulblets	Number of bulblets per explant cultured	Number of bulblets per explant with bulblets	Mean fresh weight of bulblets (mg)
Non-divided scales	70	93 $\pm$ 3	2.7 $\pm$ 0.2	3.0 $\pm$ 0.2	81 $\pm$ 4
Bi-sectioned segments					
basal portion	75	69 $\pm$ 6	2.3 $\pm$ 0.2	3.4 $\pm$ 0.3	87 $\pm$ 6
terminal portion	75	39 $\pm$ 7	1.0 $\pm$ 0.2	2.6 $\pm$ 0.3	41 $\pm$ 5
Tri-sectioned segments					
basal portion	65	71 $\pm$ 8	2.2 $\pm$ 0.2	3.1 $\pm$ 0.2	71 $\pm$ 6
middle portion	65	34 $\pm$ 7	1.0 $\pm$ 0.2	2.8 $\pm$ 0.3	57 $\pm$ 7
terminal portion	65	20 $\pm$ 6	0.3 $\pm$ 0.1	1.7 $\pm$ 0.3	58 $\pm$ 5

Fig. 1. Leaf emergence from bulblets of *L. japonicum* after transplanting in soil. The bulblets were stored at 4 °C for different duration, 0 (□), 2 (○), 4 (△), 6 (○), 8 (▲), 10 (●), 12 (□), and 14 (■) weeks, prior to the transplanting.

Table 5. Effect of different duration of cold treatment at 4°C on growth of bulbs after transplanting in the field. Ten bulblets, with 5 replicates for each treatment, were used. $\pm$ indicates standard error of the mean.

Duration of treatment at 4°C (weeks)	Mean fresh weight of bulbs at transplanting (mg)	Bulbs harvested 13 weeks after transplanting		
		Number of bulbs	Mean fresh weight of bulbs (mg) ^z	Mean fresh weight of roots (mg)
0	217 $\pm$ 13	8.8 $\pm$ 0.5	190 $\pm$ 9 (88)	16 $\pm$ 1
2	156 $\pm$ 8	9.4 $\pm$ 0.6	146 $\pm$ 6 (94)	12 $\pm$ 1
4	231 $\pm$ 9	9.4 $\pm$ 0.2	226 $\pm$ 10 (98)	33 $\pm$ 3
6	249 $\pm$ 20	8.6 $\pm$ 0.7	229 $\pm$ 10 (92)	91 $\pm$ 4
8	186 $\pm$ 5	9.8 $\pm$ 0.3	230 $\pm$ 10 (124)	123 $\pm$ 14
10	191 $\pm$ 12	9.4 $\pm$ 0.4	227 $\pm$ 12 (119)	146 $\pm$ 8
12	166 $\pm$ 7	10.0 $\pm$ 0	273 $\pm$ 24 (164)	213 $\pm$ 25
14	213 $\pm$ 16	10.0 $\pm$ 0	350 $\pm$ 33 (164)	271 $\pm$ 30

^z Figures in parenthesis represent growth increment calculated by the difference between before and after transplanting.

12 weeks survived. Bulblets chilled for more than 8 weeks grew, whereas those exposed to less than 8 weeks failed to grow. The prolonged chilling duration also stimulated growth of roots.

Bulblets weighing less than 100 mg were more perishable than the heavier ones; about 50% of bulblets cultured in the light and 35% of those in

the dark rotted. Among bulblets weighing more than 100 mg, fewer cultured in the dark survived, compared with those cultured in the light (Table 6).

In vitro light- and dark-conditions also affected some morphogenetic features of bulblets weighing more than 400 mg at transplanting (Table 6, Fig.

Table 6. Number of surviving bulbs and plant growth types (HTP and ETP) 8 weeks after transplanting in soil.

Weight classes of bulbs at transplanting (mg)	Bulblets cultured in vitro in			
	light condition		dark condition	
	Number of bulblets transplanted	Number of surviving bulblets ^z	Number of bulblets transplanted	Number of surviving bulblets
below 100	89	43	46	30
100–199	32	32	22	14
200–299	17	15	11	3
300–399	15	15	5	2 (1)
400–499	7	7	6	4 (3)
500–599	2	2	6	4 (3)
above 599	2	2 (1) ^y	12	12 (12)

^z After 8 week-cultivation, bulblets with growing leaves were regarded as surviving bulblets and those without growing leaves as rotten bulblets.

^y Figures in parenthesis represent the number of bulblets with an elongated axis, ETP.

2). Nearly all bulblets that had been cultured in the dark developed into epigeous type plants with elongated axes (ETP), while the bulblets that had been incubated in the light grew only scaly leaves, or had hypogeous growth habit (HTP), except one bulblet that developed an elongated axis. The plantlets with elongated axes required at least 2 years' cultivation in soil before they flowered (Fig. 3).

Discussion

1. Choosing initial propagation-materials

Using higher regenerative and pathogen-free tissue materials is essential in in vitro vegetative propagation. Robb (1957) reported that in vitro scale-segment culture is useful for the proliferation of bulblets of *L. speciosum*. Many experiments have used bulb-scales as very old common practice in the in vitro vegetative propagation of *Lilium* species. My data confirm that scale-segments possess the highest regeneration capacity among explants, but the highest incidence of contamination occurs in spite of careful sterilization (Table 1). This phenomenon was also observed in *L. rubellum* (Niimi and Onozawa, 1977) and *Ornithogalum* (Nel, 1981). Young leaf-explants of *L. japonicum* are easily sterilized and the explants form as many bulblets as bulb-scale segments. This is consistent with the previous results that young leaf-strips of

several *Lilium* species have high regeneration potential and are among the most reliable sources for in vitro propagation of *Lilium* (Niimi, 1986). Thus, young leaf-strips were chosen as initial propagation-materials to establish a system of aseptic tissue culture of *L. japonicum*.

There were clear differences in the regeneration potential of the leaf-segments (Table 2) and scale-segments (Table 4) of *L. japonicum*. Strips excised from the basal portion of each organ had higher regenerative capacity than did the others. The low regeneration capacity of GL-II can be improved to a certain extent by modifying the concentration of NAA and BA (Table 2) which is consistent with previous results (Niimi, 1986). Van Aartrijk and Blom-Barnhoorn (1981) showed that BA does not increase the number of bulblets in the bulb-scale cultures of *L. speciosum*. The effect of BA on the formation of plantlets may be partially due to the differences in the physiological or ontogenetic ages of leaves and scales.

2. Proliferation of bulblets

The addition of NAA, or BA, or both at different concentrations to the medium has insignificant effects on increasing the number of bulblets per scale or on improving the growth of generated bulblets (Table 3). Similar results were obtained with *L. rubellum* (Niimi, 1985) and *L. longiflorum* (Stimart and Asher, 1978). In these lilies auxins

Fig. 2. Post-in vitro development of *L. japonicum* bulblets. (A) unbolted bulblets (hypogeous type plant; HTP) cultured in the light. (B) bolted bulblets (epigeous type growth; ETP) cultured in the dark.

The weight of bulblets at transplanting were as follows; 1, less than 100 mg; 2, 100~199 mg; 3, 200~299 mg; 4, 300~399 mg; 5, 400~499 mg; 6, 500~599 mg; 7, above 599 mg.

at low concentration are suitable for the regeneration and growth of bulblets, whereas cytokinins are ineffective. The genus *Lilium* is known to have a high capacity to generate bulblets through in vivo scale propagation, or scaling. Based on these results it was decided that the addition of 0.05 and 0.1 mg·liter⁻¹ NAA only or 0.1 mg·liter⁻¹

NAA in combination with 0.01 mg·liter⁻¹ BA to the basal medium was the optimum condition for the regeneration of bulblets in the scale cultures.

A method of culturing bi- or tri-sectioned scale-segments had little effect on increasing the number of bulblets (Table 4), because segments excised from terminal- and middle-portions of a scale had

Fig. 3. *L. japonicum* flowering in the third spring after transplanting.

low regeneration potential, compared with the basal segment and a whole scale. In the in vitro proliferation of *L. japonicum*, Asao et al. (1992) reported that, like a whole scale, each of the four individual segments excised from a whole scale forms nearly the same number of bulblets. This discrepancy probably results from the differences in the physiological conditions of cultured explants.

3. Establishment in soil

A cold treatment of more than 12 weeks broke the dormancy of bulblets of *L. japonicum* (Fig. 1) and accelerated the growth of each organ (Table 5). This response is comparable to those of *L. rubellum* (Niimi, 1988) and *L. speciosum* (De Klerk et al., 1992), which demonstrated that bulblets require a cold treatment at 4 °C for at least 8 weeks before the bulblets transplanted into soil. Therefore, the cold treatment of bulblets is one of the essential treatments for post-in vitro establishment of most *Lilium* species.

Table 6 shows that there may be two factors responsible for bulblet-rot infection in soil: 1) small bulblets of *L. japonicum*, weighing less than 100

mg, are more perishable in soil than are larger ones, implying that smaller bulblets have a lower degree of resistance to pathogen attack as compared with larger ones; and 2) light probably makes the outer scales of bulblets physically tough, resulting in imparting some tolerance to moisture stress and a resistance to certain pathogens.

Bulblets cultured in the dark, weighing more than 400 mg at the time of transplanting developed elongated axes (Table 6), which indicates that not only the sizes of bulblets but the light also modified the developmental morphology of the plants. Thus, dark condition which favors ETP instead of HTP may be more suitable for commercial bulb production because ETP plants seem to be more photosynthetically efficient than HTP ones. Therefore, it is recommended that bulblets are cultured in the dark until they weigh more than 400 mg and subsequently cultured in the light for a few weeks before transplanting into soil.

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ササユリの試験管内増殖と圃場への移植

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摘 要

萌芽間もないササユリの母球を掘り上げ、組織培養による増殖と形成された子球の移植条件について調べた。

1. 母植物を実験圃場より採集し、りん茎、未展開葉、茎に分離した。りん片および葉ではその基部付近、茎では先端部分から外植体を調整し、それらを Murashige・Skoog (1962) の無機塩培地と数種類の有機物、しょ糖を含む基本培地に $\text{NAA } 1.0 \text{ mg} \cdot \text{liter}^{-1}$, $\text{BA } 0.1 \text{ mg} \cdot \text{liter}^{-1}$ 添加した培地で培養した。

各外植体の子球形成数、子球重および汚染率の結果から、初代培養の外植体として葉切片がよいことがわかった。また、葉切片の子球形成は $\text{NAA } 0.5 \text{ mg} \cdot \text{liter}^{-1}$, $\text{BA } 0.05 \text{ mg} \cdot \text{liter}^{-1}$ の添加で促進されることもわかった。

2. 無菌培養中の子球から分離したりん片を培養した結果、これらのりん片からの子球の形成および成長には $\text{NAA } 0.1 \text{ mg} \cdot \text{liter}^{-1}$, $\text{BA } 0.01 \text{ mg} \cdot \text{liter}^{-1}$ が適していた。またりん片当たりの子球形成数を増加させる

ためにりん片を分割して培養したが、りん片当たりの子球数の増加はわずかであった。

3. 出葉開始時期、最終出葉率、子球と根の生長および子球の腐敗率の結果から、試験管内で培養した子球を圃場に移植する前に12週間の 4°C での低温処理が必要であることがわかった。

4. 圃場への移植時に100 mg未満の子球は培養期間中の明・暗条件に関係なく腐敗しやすく、100 mg以上の子球でも暗条件下で培養した子球は明条件下で培養したものと比べ腐敗する子球が多かった。

5. 移植時に300 mg未満の子球は培養中の光環境条件で関係なくすべて地中型植物 (HTP) となった。試験管内で400 mg以上に生長した子球では、明条件下で培養した子球はほとんど地中型植物であったが、暗条件のものはほとんどすべて地上型植物 (ETP) となった。これら地上型植物が開花するまでには少なくとも2年間の圃場での栽培を必要とした。