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Effect of Light and Scale Explant Conditions on Propagation Efficiency in *Lilium callosum* Scale Culture

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ABSTRACT Series of in vitro experiments in *Lilium callosum* were conducted to investigate efficient multiplication through finding the optimal cultural environment, and organogenic capability of cultural explants, and then to determine the progressive method for enhancing bulblet growth in *Lilium callosum* scale culture. Twenty-four hr photoperiod was most effective for the growth of bulblet and the formation of other organs. Optimum light intensity for bulblet growth was 2,500~5,000 Lux. When bulbets were subcultured, growth of bulblets were enhanced by removing excessive leaf blade. Number of bulblets per scale increased as mother scale size increased, whereas diameter of bulblet from the small size mother scale increased. Bulblet formation and development was induced when explants were placed above the medium to be exposed to more light.

Additional key words: bulblet development, bulblet formation, lily, photoperiod.

Introduction

There are 100 native *Lilium* species and 500~600 varieties, growing widely in the world. Korea is also well known as the important lily habitat, and total of 11 native *Lilium* species are distributed (Kim, 1996). Many species of Korean native lilies possess high ornamental values for their beautiful flower color, ranging from reddish orange to purplish pink, and unique flower form.

It was known that a few of them show disease resistance and cold hardiness characteristics which are attractive to lily breeders (Jeong and Kim, 1991). For these reasons, Korean native lilies have been used as important breeding materials in foreign countries such as the Netherlands, Japan, and the United States for many years.

Lilium callosum grows naturally in the southern region and Cheju Island, but is rare in the central area in Koera. This out-of-the-way plant is distinctive from other Korean native lilies in that *L. callosum* has nearly no color spot on the petal, whereas other species have dark purple spots. A flower of *L. callosum blooms* down-facing the earth in mid July, growing at the sunny side of the foot of mountains, and it can be used as a valuable ornamental plant at park and garden, or as a material for flower arrangement. Thus, it is necessary to conserve, exploit, and popularize this useful native genetic resource by a mass-propagation method (Huh, 1994; Kim et al., 1990; Jeong et al., 1991).

The objectives of this study were to determine the efficient multiplication method through cultural environment, and organogenic capability of cultural explants, and then to determine the effective method for enhancing bulblet growth in *Lilium callosum* scale culture.

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Materials and Methods

Plant material

Scales were received from Andong National Universtity where they were detached from *L. callosum* bulbs, washed by tap water to remove soil, and then surface sterilized by submerging scales for 2 min in 95% ethanol followed by 20 min in a 0.5% sodium hypochlorite solution and rinsed 3 times in sterile water. Culture room were maintained at 24±2℃, 16 hr photoperoid and 2,000 Lux, which was supplied by florescent lamps. The scales were cultured on MS medium containing 30g/L of sucrose for proliferation of bulblets. The average diameter of newly formed bulblets was 0.4~0.5 cm. The scales separated from these newly formed bulblets were used as experimental materials.

Light condition

Explants were cultured under various photoperiods of 24, 16, 8, or 0 hr. The MS basal medium was supplemented with 60 g/L of sucrose and 0.01 mg/L BA. Other environmental conditions were the same as previously described. Culture vessel was wrapped with aluminum foil for dark culture. Explants were cultured under various light intensity which were controlled by shading with 24 hr photoperiod in a growth chamber. Shading material was used on the iron frame which covered culture vessels to achieve 500, 2,500, 5,000, 10,000 and 20,000 Lux light intensity. Artificial light source was methyl halide lamp.

Scale explant condition

The shoot clumps were subjeted to four different methods of cutting. Senescing leaves and leaves over 10 cm in length were trimmed to a length of 1 cm, 2 cm, or 4 cm before transferring, respectively. For the 'severe cutting', all shoots were cut down to the basal plate region, removing all green tissues.

Different size of bulbscales (0.2, 0.3, 0.4 cm diameter) were inoculated. Explants were cultured with different inoculation methods (above medium, below medium, upright, upside down) in solid medium. In these experiments, all explants were cultured on MS media containing 30 g/L of sucrose, 8 g/L agar, under 16 hr photoperiod and 25℃ condition. pH was adjusted to 5.8,

Results and Discussion

Effect of light on propagation efficiency in scale culture

The influence of four photoperiods on bulblet formation and growth is shown in Table 1 and Fig. 1. Dark treatment for 24 hr had no effect on bulblet growth and callus formation. Only 24hr photoperiod was significantly effective in growth of

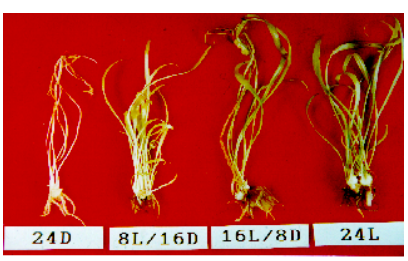

Fig. 1. Bulblet formation and their growth under different photoperiod.

Table 1. Effect of light on bulblet growth of *L. callosum* scale culture at 12 weeks after treatment.

Day /night (hr)	Bulblet			No. of scales /bulblet	Leaf				Root			Callus[z] formation (%)
	No. (ea)	Diam. (cm)	F.W. (g)		No. (ea)	Length (cm)	Width (cm)	F.W. (g)	No. (ea)	Length (cm)	F.W. (g)	
24/0	3.2a[y]	0.56a	0.20a	14.1a	11.7a	10.40 a	0.42a	0.36a	++[x]	1.42a	0.57b	60
16/8	2.1b	0.38b	0.12b	7.4b	9.8a	10.20 a	0.41a	0.31a	+++	1.24a	1.39a	33
8/16	2.4b	0.27b	0.09bc	4.7b	10.2a	9.96a	0.22b	0.30a	+	1.35a	0.49bc	73
0/24	1.6b	0.33b	0.05c	4.9b	5.6b	10.20 a	0.10c	0.14b	+	1.79a	0.09c	0

[z]Callus formation (%) = $\frac{\text{No. of scales with callus}}{\text{No. of scales inoculated}} \times 100$

[y]Mean separation within columns by Duncan's multiple range test at 5% level.

[x]+ : 1~10; ++ : 11~25; +++ : more than 25 roots.

Table 2. Effect of light intensity on bulblet formation and growth of *L. callosum* in scale culture at 8weeks after treatment.

Light Intensity (Lux)	No. of bulblets/ scale	Bulblet diameter (cm)	No. of leaves	Leaf length (cm)	No. of roots	Root length (cm)	Bulblet formation[z] (%)
500	1.7 bc[y]	0.27 ab	4.7 bc	6.24 b	3.1 c	0.78 b	75
2,500	2.1 ab	0.30 ab	7.7 ab	8.06 ab	6.0 b	1.50 a	100
5,000	3.0 a	0.31 ab	9.7 a	9.51 a	7.5 b	2.04 a	93.7
10,000	3.0 a	0.39 a	7.3 ab	2.85 c	11.0 a	0.83 b	75
20,000	0.9 c	0.18 b	3.0 c	1.56 c	1.1 c	0.19 b	25

[z]Bulblet formation(%) = $\frac{\text{No. of scales with formed newly bulblet}}{\text{No. of scales inoculated}} \times 100$

[y]Mean separation within columns by Duncan's multiple range test at 5% level

Table 3. Effect of remaining shoot length on bulblet growth of *L. callosum* in scale subculture at 4 and 12 weeks after treatment.

Remaining shoot length(cm)	Weeks after treatment	No. of bulblets/ scale	Bulblet diameter (cm)	No. of leaves	Leaf length (cm)	No. of roots	Root length (cm)
0	4	1.53 a[z]	0.15 a	4.9 a	3.18 a	1.3 c	2.33 a
1		0.70 ab	0.08 b	1.8 b	1.55 b	1.0 c	4.00 a
2		0.43 b	0.04 b	0.8 b	0.36 d	3.3 b	2.33 a
4		0.70 ab	0.07 b	1.4 b	1.25 bc	4.0 b	4.00 a
no cut		0.50 b	0.08 b	0.5 b	0.70 cd	5.0 a	3.00 a
0	12	4.60 a	0.50 a	13.5 a	8.76 a	15.4 a	2.59 a
1		1.78 b	0.26 bc	6.7 b	3.84 b	9.7 b	3.39 a
2		1.72 b	0.21 c	5.3 b	4.23 b	5.8 b	2.13 a
4		2.12 b	0.30 bc	7.7 b	5.14 b	7.9 b	1.69 a
no cut		2.94 b	0.42 ab	10.4 ab	6.83 ab	10.5 b	1.81 a

[z]Mean separation within columns in each month by Duncan's multiple range test at 5% level.

bulblet and other organs. Stimart and Asher (1978) reported that darkness increased the number of bulblets and size, apparently at the expense of leaf number and size. Conversely, the lighted environment supressed bulblet formation and increased leaf formation, root weight and callus fresh weight. Therefore, scale of *L. callosum* seemed to be sensitive to light in bulblet formation and development. Taeb (1990) reported that long day condition (16 or 22 hr) enhanced the regeneration of bulbing shoots in tulip scale culture. This result supports the finding of Sobeih and wright (1987) that bulb formtion in onion is promoted by long-day in vitro onion culture.

Effect of light intensity on bulblet formation and growth is shown in Table 2. Light intensity of 2,500~5,000 Lux was effective in bulblet formation and growth. When they were grown under 10,000 Lux, total bublet formation rate was low, but the number of bulblets per scale and bulblet size (diameter) tended to be similar to those grown under 2,500~5,000 Lux. This result is corresponding to the report of Mondal et al. (1986) where increasing light intensity promoted the bulbing ratio of five onion cultivars. They explained that high light intensity appeared to increase the number of bulbing shoot by either (a) reducing dominance of the older shoots and then allowing the young bulbing shoots to grow, or (b) increasing the amount of available assimilate by active photosynthesis of the older shoots. So, it was assumed that when scales of *L. callosum* are cultured under high light intensity, bulblet growth are promoted by active photosynthesis of small shoots as well as nutrient absorption from medium through short and multiple roots. However, too high light intensity such as 20,000 Lux inhibited bulblet formation and growth by poor shoot and root growth.

Effect of explant condition on propagation efficiency in scale culture

Effect of new shoot formation by removing long shoots on bulblet growth is shown in Table 3. Removing whole shoots was most effective in bulblet and shoot formation at both 4 and 12 weeks after treatment. But, the number of roots was increased as remaining shoot length was longer. At 12 weeks after treatment, there was no significant difference between no cut and complete shoot removal in both bulblet diameter and shoot growth. However removing off entire shoots was very effective in the number of bulblets. Paek et al. (1987) reported the same result that in *L. lancifolium*, narcissus, hyacinth, the number of bulblets were increased by removing leaves. Moreover, in case of narcissus and hyacinth, after bulblets were divided, removing shoots produced more multiple shoots and bulblet formation than simple removal of shoots from distal part of bulblet did.

Especially, explants of hyacinth showed strong apical dominance in vitro scale culture, which resulted in poor propagation efficiency (Paek et al., 1987). Hussey (1982) and Seabook et al. (1976) reported that apical dominance is released in vitro by removing disital part of bulblet or splitting large shoots. As the basal plate of bulbs was suggested to be the site of exertion of apical dominance in *Narcissus*, cutting down toward the basal plate region of shoot clump could stimulate shoot production and prevent or delay the loss of vigor by breaking apical dominance (Chow, 1992).

The influence of three scale sizes such as 0.2, 0.3, and 0.4 cm in diameter is

Table 4. Effect of scale size on bulblet formation in *L. callosum* scale culture at 12 weeks after treament.

Scale diameter (cm)	No. of bulblets/scale	Bulblet diameter (cm)
0.2	2.0 b	0.53 a
0.3	4.0 ab	0.48 ab
0.4	6.0 a	0.43 ab

[z]Mean separation within columns by Duncan's multiple range test at 5% level.

Table 5. Effect of inoculation method on bulblet growth of L. callosum in scale culture at 12 weeks after treament.

Treamentz	No. of bulblets/scale	Bulblet diameter (cm)
Above	3.9 ab[y]	0.50 a
Below	4.8 a	0.33 b
Upside down	2.8 b	0.38 ab
Upright	2.5 b	0.45 a

[z]media surface (above, below)
basal part upward (upside down)
basal part downward (upright)

[y]Mean separation within columns by Duncan's multiple range test at 5% level.

shown in Table 4. Number of bulblets per scale were increased as mother scale size increased, whereas bulblet diameter was rather slightly longer when mother scale was small. During the early growth of the daughter shoot, stored reserves in the mother scale tissue are hydrolyzed to soluble carbohydrate (primarily sucrose) and used to support growth of the new shoots (Miller and Langhans, 1989). Thus, this result suggested that produced bulblets compete with each other for absorption of nutrient from a mother scale and medium. Bulblet weight and diameter were promoted by using whole scaling or bisectional scaling method than when it was planted by bisectional or trisectional scaling method. However, it was thought that bisectional scaling method was more effective in the number of bulblets per scale (Jeong, 1994). Pierik and Post (1975) found the shape and size of bulb scale explants from hyacinth to be critical factors in maximizing bulb production in vitro.

When the scales were dissected transversally, a total of 15.6 bulblets were formed on one healthy scale cut into 4 segments. When the bulbscale was cut longitudinally, a total of 32.2 bulblets was generated on one scale splited into 5 segments compared to 2 bulblets on non-divided scale (Lee et al., 1994).

Bulblet induction was found on the basal part of the scale, but it was retarded on the apical part irrespective of inoculation methods (data not shown). Table 5 showed that bulblet formation and development was induced best from explants placed above the medium. Effect of light was very important in *L. callosum* scale culture. Twenty-four hr photoperiod promoted the number of bulblets and their promoted their development (Table 1). In order to enhance bulblet growth, optimum inoculation method placing explants above the surface of the media was recommended because whole part of explants can be irradiated.

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땅나리 기내 인편 배양시 광환경과 배양 절편체의 조건이 증식 효율성에 미치는 영향

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초 록

본 실험은 자생나리인 땅나리의 기내 대량생산 체계를 확립하기 위한 기초 연구로서 배양환경중 광환경 및 배양 절편체의 분화 능력 조건을 유도하여 자구의 생장을 촉진시킬 수 있는 방법을 모색하기 위하여 실시하였다. 광조건은 24시간 지속적인 일장처리와 2,500~5,000 Lux 정도의 광도가 자구의 발달에 효과적이었다. 계대배양시 번무한 지상부를 자구의 연접부위까지 완전히 절단해 줌으로써 전반적인 식물체 생육을 촉진시킬 수 있었고, 절편체의 크기가 클수록 자구수가 많았으나, 자구의 비대는 모인편의 크기가 작은 처리구에서 약간 우수하였다. 광을 많이 필요로 하는 땅나리는 기내 인편 배양시 치상방법을 배지면 위에 노출시켜 치상하는 것이 자구생산면에 있어서 효율적이었다.

추가주요어 : 광주기, 자구 형성, 자구 발달