

Effect of Sucrose and Supplementary Substances on the Germination Ecology and the Seedling Growth of Native *Bletilla striata*¹

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자생 자란의 발아생태와 유식물 생육에 미치는 당과 첨가물의 영향¹

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ABSTRACT

The study was carried out to elucidate the effect of various medium, sucrose, peptone and tryptone on the seed germination and seedling growth of *Bletilla striata* under in vitro culture. The seedling growth after subculture were surveyed too. Sucrose increased the germination rate of *B. striata* seeds by the concentration 10g/L onto the ½MS medium. Its rate was 5.45% and about 2 times higher than that of control. The supplement of tryptone or peptone didn't affect the seed germination. The dark conditions was superior to light onto Hyponex media in germination rate of *B. striata*, but the seedling growth without light was poor. The more sucrose contents, the more root length and fresh weight of *B. striata* seedlings. The root length and fresh weight were 3.0mm and 4.7mg under the 30g/L of sugar concentration. The roots were not developed in control. It seemed that the Hyponex media including tryptone 2g/L was very proper media for seed germination of *Bletilla striata* because of its relatively high germination rate and excellent early growth of seedlings, especially its shoot length(3.1mm) and fresh weight (2.6mg) for 6 weeks. After subculture, the growth of *B. striata* showed much differences by the kinds of supplementary substances. Peptone increased the leaf and root growth for the most part in comparison to any other substances. The fresh weight was 180mg and about 3 times higher than that of control.

KEY WORDS : *Bletilla striata*, IN VITRO SEED GERMINATION, PEPTONE, TRYPTONE

요약

본 연구는 조경소재로 이용가능성이 크지만 현재 자생지가 파괴되어 복원이 요구되고 있는 야생자란의 대량 번식을 위해 무균배양시 배지 내 당함량의 변화와 펩톤, 트립톤 등의 첨가가 종자발아와 계대배양 후 유식물의

1 접수 9월 30일 Received on Sep. 30, 2000

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생육에 미치는 영향을 알아보려고 실시하였다. 배지 내 펩톤과 트립톤의 첨가는 발아에 영향을 주지는 않았지만, 당의 함량은 그 농도가 10g/L까지 증가함에 따라 발아율을 높였다. 또한 발아 후 유식물의 생육시 당의 첨가는 뿌리의 생육을 두드러지게 향상시켰으며, 생체중도 거의 2~3배 정도 많았다. 하이포넥스 배지(대조구)에서는 높은 발아율을 보였지만 유식물의 생육은 트립톤 첨가배지(2g/L)에서 많았는데 엽수, 뿌리수, 엽장 근장, 생체중 등이 모두 다른 처리구의 2~3배에 이르는 초기생육을 보였다. 계대배양 이후의 생육상은 펩톤 첨가배지에서 가장 많은 생육량을 보였는데 특히 엽장과 엽폭 그리고 근장이 다른 처리구보다 월등히 높은 경향을 나타냈다. 생체중도 한 개체당 0.18g으로 가장 높게 나타나 펩톤의 첨가가 계대배양 이후의 생육을 크게 촉진시키는 것으로 나타났다. 결론적으로 하이포넥스 배지에 트립톤 2g/L를 첨가하였을 때 발아율과 유식물의 생육이 다른 배지에 비해 매우 양호한 것으로 나타나 자생 자란의 종자발아용 배지로 가장 적당한 것으로 사료된다. 그리고 이후 계대배양시에는 펩톤의 첨가(3g/L)가 유식물의 생육을 가장 촉진시키는 것으로 나타났다.

주요어 : 자란, 무균발아, 펩톤, 트립톤

Introduction

Bletilla striata(Orchidaceae) is indigenous in the south regions of Korean peninsula and its ornamental and economic values may be increased in terms of landscaping plants. Especially its flowers are very beautiful in early spring and it is the only plant of Orchidaceae to be possible in outdoor planting(Ahn and Lee, 1997). Now its natural habitat were endangered to extinct by man and it is necessary to develop the method of multiple propagation. In general, it is propagated by division and limited in multiplication and popularization to the common peoples, who have difficulty to propagations by means of aseptic germination for without proper equipments and laboratory.

The germination of orchid seeds is affected by many factors, i.e. nutrient composition, its supplementary materials, the kinds of medium, its concentration, culture period and cultural conditions, etc. Orchid seeds are non-embryonic and its germination and seedling are heterotrophic, and do not grow beyond an undifferentiated protocorm stage without infection of an appropriate mycorrhiza in nature (Ichihashi and Yamashita, 1977). Thus these cultural conditions are the most significant factors and always have to be considered. In previous papers, the authors

reported on the proportion of ions in medium and the relationship between ionic treatments and culture condition, i.e. sucrose and charcoal concentrations, plant growth regulators, etc.

The research was experimented to elucidate the effect of various medium, sucrose, peptone and tryptone on the seed germination and seedling growth of *Bletilla striata* under in vitro cultural environment. And the seedling growth after subculture were surveyed too.

Materials and Methods

Seeds of *Bletilla striata* Reichb. fil. were used as a experimental materials. Seeds were collected at Hantaik Botanical Garden, located in Yongin, Kyungkido, Korea, in November 1996. Preserving in refrigerator at 5°C, they were transferred into a small glass bottle of 10×2cm in November 1997 and sterilized for about 5min., agitating with 2 percent sodium-hypochlorite solution and then washed repeatedly with sterile distilled water. The seeds were sown into sterilized petridish containing about 10ml of agar medium composed of various nutrient solution. The petridish were wrapped and transferred and incubated into a growth chamber, of which cultural conditions were 25°C tem-

perature, 60% relative humidity, and 16 hours photoperiods with fluorescent lamps (maximum 2,000 Lux).

The basic medium used in these experiments were $\frac{1}{2}$ MS (Murashige and Skoog basal salt mixture, SIGMA) and Hyponex. To elucidate the effect of sucrose on the germination of seeds, 0, 5, 10, 20g/L of sucrose respectively were contained into $\frac{1}{2}$ MS. Before being added agar 7g/L, pH was adjusted to 5.7. Peptone or tryptone as hydrolysate protein supplemented to Hyponex medium to survey their effect on the germination of seeds. The supplementary concentrations were 0, 2, 5g/L peptone or tryptone respectively to Hyponex 3g/L, sucrose 30g/L and agar 10g/L. Aluminum foil covering the petridish were used to compare between light and dark culture conditions of seed germination.

The percent germination were examined by a microscopy ($\times 40$) on 10, 17, 24 day after sowing. Protocorms with small leaves were counted as germinated seeds. The fresh weight of seedling was measured by chemical balance on 40 days after sowing. At the same time, the number of leaves, the number of roots, leaf length, and root length of seedlings were surveyed. Each treatment was repeated five times.

Three months after in vitro sowing, the seedlings were subcultured onto four different medium. The basic medium were Hyponex and additioned substances were yeast extract (2g/L), trypton(2g/L) or peptone(2g/L). The final growth survey was measured on 12 Mar. 1998.

Results and Discussion

The sucrose has increased the germination rate of *B. striata* seeds until its concentration up to 10g/L. Especially the seed onto the $\frac{1}{2}$ MS medium with sucrose 10g/L showed two times as high as germination rate than the seed without sucrose in the early 10 days (Table 1). Sucrose has been reported to play an important role in the growth and development of seedling of in vitro cultures as energy sources (Arditti and Harison, 1977). The seed germination of *B. striata* may be dependent upon the concentration of sucrose and its high concentration (above 20g/L) injured the germination rate. As a rule, it has taken 15~17 days for seeds of *B. striata* to germinate in vitro.

It was not showed a significant difference among the treatments of yeast extract in $\frac{1}{2}$ MS medium in germination rate of *B. striata* seeds. At the concentration of yeast extract 1g/L, the germination rate was highest of all the treatments. The yeast extract is composed of nitrogen and vitamin B and reported to help the growing of seedlings. But it seemed not to be necessary factor in seed germination of *B. striata* under this experiment. Choi and Chung (1991) reported that yeast extract had not affected on the seed germination of *Cymbidium goeringii*. Hyponex media showed two times as high as germination rate than $\frac{1}{2}$ MS. Germination rate responses to light was surveyed. The seeds on Hyponex media showed the highest germination rate under dark condition (0 hr pho-

Table 1. The effect of sucrose concentration on the germination of *Bletilla striata*

Medium	Sucrose(g/L)	Percent germination(%)		
		10 days	17 days	24 days
$\frac{1}{2}$ MS	0	1.82 ^C	2.08c	2.39c
	5	2.54b	3.81b	3.81b
	10	4.00a	5.45a	5.45a
	20	2.90b	5.09a	5.09a

²Duncan's multiple range test(5%)

Table 2. The effect of light or dark culture condition on the germination of *Bletilla striata* for 25 days after treatment

Hyponex(g/L)	Peptone(g/L)	Trypton(g/L)	Percent germination(%)	
			Light	Dark
3	-	-	12.6a ²	22.8a
3	2	-	5.9b	8.8b
3	5	-	3.7bc	7.7b
3	-	2	12.8a	8.4b
3	-	5	6.3b	3.7c

²Duncan's multiple range test(5%)

Table 3. The effect of peptone or tryptone on the germination of *Bletilla striata* in light condition

Hyponex(g/L)	Peptone(g/L)	Trypton(g/L)	Percent germination(%)		
			10 days	17 days	24 days
3	-	-	10.9a ²	11.8a	12.6a
3	2	-	3.3c	5.9b	5.9b
3	5	-	0d	0c	3.7c
3	-	2	5.1b	12.8a	12.8a
3	-	5	4.0b	6.4b	6.4b

²Duncan's multiple range test(5%)

toperiod) and its rate was 22.8% for 25 days(Table 2). Under light environment(16 hrs photoperiod), its rate was 12.6% and it was superior to that of on MS. This coincided with the results of Kang and So(1990) and they concluded that dark condition was poor to the germination of *Bletilla striata* seed. The addition of tryptone or peptone on Hyponex media didn't increase the germination rate of the seeds(Table 3). However, *Dendrobium monile* effectively germinated on the Hyponex media addicted tryptone 2g/L(Chung *et al.*, 1981).

The early growth of the seedlings increased depending on the concentration of sucrose (Table 4). Especially the root length of the seedling on the media contained sucrose 20g/L was 3.0mm and it was 6 times as long as root length than that of sucrose 10g/L. Sugar was reported to be a carbon source for germinating orchid seeds(Arditti and Harison, 1977). The seedlings of orchid of which leaf or root was not developed were heterotrophic in uses of carbon. Arditti and

Ernst(1984) reported that exogeneous carbohydrate is not a requirement for growth and development after the first leaf has appeared or there is potential to generate it.

The fresh weight is commonly used as a indicator factor of plant growth. The seedling onto the $\frac{1}{2}$ MS media containing 20g/L of sucrose had 4.7mg of fresh weight for 40 days in light condition(Figure 1). If it being considered germination rate and early growth, the media containing 20g/L sucrose was seemed to be a most proper. But above all, the hyponex media containing 2g/L of tryptone showed relatively high germination rate and more growth of seedlings. Development of shoot and root were remarkably increased on the hyponex media additioned 2g/L of tryptone comparing to other treatments(Table 5). Especially shoot growth was 2 times more than other treatment and it was 3.1mm. The germination rate on hyponex media under dark condition was highest of all treatments surveyed, but its growths were very poor and undevel-

Table 4. Effect of sucrose concentrations on the early growth of *Bletilla striata* seedlings

Media	Sucrose (g/L)	No. of shoot	No. of roots	Shoot length (mm)	Root length (mm)	Fresh weight (mg)
½MS	0	1.7ab ²	0c	2.5b	-	0.4c
	5	2.0a	0.4b	2.2b	0.3b	1.6b
	10	2.1a	0.6a	2.6b	0.5b	1.8b
	20	2.2a	0.7a	3.7a	3.0a	4.7a

²Duncan's multiple range test(5%)

Table 5. Effect of peptone and tryptone on the early growth of *Bletilla striata* seedlings

Media	Peptone (g/L)	Tryptone (g/L)	No. of shoot	No. of roots	Shoot length (mm)	Root length (mm)	Fresh weight (mg)
Hyponex ²	0	0	1.7b ^y	0.2a	1.1b	0.1b	0.5c
	2	0	1.9a	0b	1.6b	0c	1.1b
	5	0	0c	0b	0c	0c	0.2c
	0	2	2.1a	0.6a	3.1a	1.0a	2.6a
	0	5	2.0a	0.3a	1.8b	0.3b	1.2b

²Composition: Hyponex 3g/L, sucrose 30g/L, agar 20g/L

^yDuncan's multiple range test(5%)

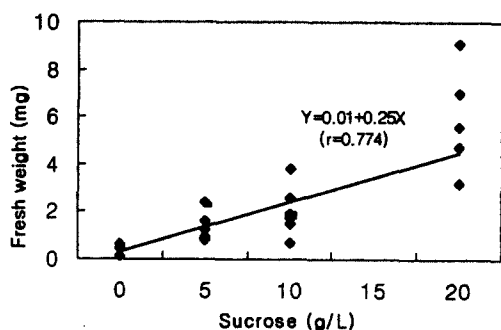


Figure 1. Effect of sucrose on the fresh weight of *Bletilla striata* seedlings in the early stage

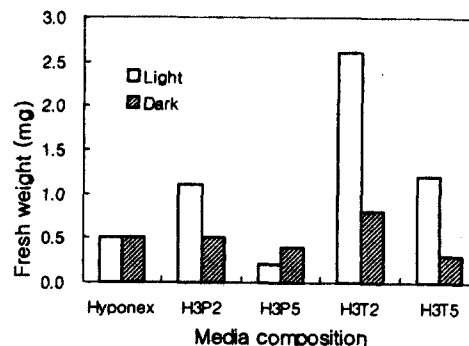


Figure 2. Effect of various composition of media and light on the fresh weight of *Bletilla striata* seedlings in the early stage

oped into seedling(Figure 2). The leaves were chlorosis and the root didn't emerged at all. Light environment(16 hrs photoperiods) may be necessary for the seed germination and early growth of the orchids. The effect of tryptone on the seedling growth of *B. striata* in early stage may be very important but lit-

tle was known about its pathway in orchids. Peptone or tryptone were often used in culture media with vary results (Arditti and Ernst, 1982). Organogenesis and subsequent development in *Cymbidium* are enhanced by tryptone. Peptone didn't increased the germination rate and early growth of *B. striata* in

Table 6. Effect of supplementary substance to the medium on the growth of *Bletilla striata* seedlings after subculture

Supplementary substance	Leaf			Root		Fresh weight (mg)
	Number (ea)	Length (cm)	Width (cm)	Number (ea)	Length (cm)	
Control	2.95b ²	18.53c	3.35b	2.90b	9.95b	73c
Yeast extract(2g/L)	3.00ab	21.85b	3.13b	2.65b	10.02b	110b
Tryptone(2g/L)	3.15a	18.28c	3.00b	2.70b	10.68b	65c
Peptone(2g/L)	3.25a	26.25a	5.05a	3.55a	15.40a	180a

²Duncan's multiple range test (5%)

this study.

The growth after subculture were surveyed and the seedling onto medium including peptone(2g/L) was 15.4mm in root length and 180mg in fresh weight. Compared with others, their growth were much best. Yeast extract or tryptone did not increase the leaf number, leaf length, leaf width, root number, root length and fresh weight.

In general, MS and Hyponex media mainly have been used for germination of Orchidaceae plants. The most proper media for germination of *B. striata* in this study was hyponex and addition of tryptone notably enhanced the development and differentiation of its seedlings. After subculture, supplement of peptone(2g/L) increased the growth of seedling, especially leaf(26.25mm) and root length.

Acknowledgement

This research was carried out in Post-Doctoral Fellowship Program(1997) supported by Korean Research Foundation. We are thankful to them for their financial supporting.

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