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Days to Germination and Effect of Growth Regulator on Rhizome Growth in *Cymbidium goeringii* Hybrid

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ABSTRACT

Germination efficiency, such as days to germination and conditions for the hybrid seeds, was tested after hybrid seeds were sown on the medium. Seeds were germinated from 67 to 126 days after seeding in all cross combinations, and germination condition was different among hybrid combinations. There was big difference on days to germination based on the different media, that is, days to germination in the hyponex medium was shorter than that in the Knudson C medium. Mutants such as MMS and NaN_3 were used to cause mutation. Germination occurred with 0.1% NaN_3 and MMS. However, even though germination occurred in other treatments (0.01% and 0.2% of NaN_3 and MMS), brownish phenomenon was intense, or did not proceed and got worse after 4-5 months of seeding. In addition, it was performed to choose appropriate medium for the growth of *Cymbidium* rhizome whose media adaptability is more different than other orchids. Different concentration of NAA and Kinetin was used. As a result, *C. goeringii* hybrid showed difference for the concentration of NAA and Kinetin combination.

Key words : Cymbidium goeringii, rhizome, germination, mutation

INTRODUCTION

The number of people loving oriental orchids is increasing because *Cymbidium* species have high value for ornamental plants in Korea, Japan, and China. Therefore, the people cultivating the oriental orchids are rapidly increasing in Korea, and the only method to meet this is to develop and improve the effective method for propagation. In addition, it is necessary to develop new species by using hybrid breeding and to distribute them.

The seed of orchids is very minute, and immature embryo is composed of several hundreds of fertilization eggs, sometimes, there is no embryo sac. Because of this kind of seed characteristics, the germination rate is very low in natural condition (Cheong, 1979; Knudson, 1951; Osabako, 1976), and it requires symbiosis with mycorrhiza to germinate. Therefore, the method for micropropagation in rapid time has to be searched.

There are many popular species of *Cymbidium* in terms of ornamental value. However, most of them form rhizomes after sowing them on medium or culturing shoot tip. For rhizome growth of *Cymbidium kanran*, Kokubu et al. (1980) used MS medium (Murashige and Skoog, 1962) containing 2.0 mg/l of NAA. Cheong and Cheon (1983) reported that the

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medium containing 3 g/l of hyponex and 4 g/l of peptone supplemented with 2.0 mg/l of NAA and 0.2 or 2.0mg/l of kinetin has good capacity for rhizome growth. Besides, about 25 different media were used for the germination of orchid seeds (Kano, 1965; Knudson, 1951; Garde, 1929; Murashige and Skoog, 1962; Sideris, 1950; Vacin, 1950; Vacin and Went 1949; Wynd, 1933).

This experiment was also performed to cause mutation by treating with the mutants such as MMS and NaN_3 as well as to make hybrid seeds by crossing between *C. goeringii* and the other orchids. After those treatments, germination status and days to germination were investigated. In addition, it was performed to choose appropriate medium for the growth of *Cymbidium* rhizome whose media adaptability is more different than other orchids.

MATERIALS AND METHODS

1. Pretreatment of seeds

For speeding up germination, seed pretreatment was performed. Seeds were collected 206 days after fertilization between *C. goeringii* and *C. forrestii* including the other 14 different cross combination. After seed pod was sterilized, the seeds were sterilized in 8% $Ca(ClO)_2$ for 30 min. After sterilization, glass filter was used for filtration followed by treatment with ultrasonic waves for 150 min.

2. Days to germination for hybrid seeds

Days to germination and germination status were tested to know the difference among 15 hybrids including *C. goeringii* × Chungumnan by using seed germination medium (3g/L Hyponex + 4g/L Peptone + 0.1mg/L NAA + 0.01mg/L Kinetin).

3. Days to germination for seeds treated by mutation - causing chemicals

Seeds were submerged in NaN_3 and MMS solutions in 0.01%, 0.1% and 0.2% concentration for 10, 30, 60, 120, and 140 min. After chemical treatment, days to germination and conditions were investigated.

4. Rhizome growth

Rhizomes of six inter-hybrid species including *C. goeringii* × Chungumnan were used to improve rhizome and shoot growth ability. For rhizome growth medium, six different hormone combinations of H_3PO_4 medium supplemented with 0.1, 1.0 or 2.0mg/l of NAA and 0.1, 0.2 or 1.0 mg/l of kinetin were used. In addition to different combinations of hormone, 30g/l of sucrose and 8g/l of agar were added, and pH was adjusted to 5.0 to all six different media. Fifteen rhizomes cut by 0.7cm including tips were placed on the medium with four replications. Those were cultured at 25℃ with light irradiation of 1,000 lux, and the developmental condition was investigated after three months.

RESULTS AND DISCUSSION

Germination Efficiency

This experiment was performed to test germination efficiency after hybrid seeds were sown on the medium. Days to germination and conditions for the hybrid seeds for which different *C. goeringii* were crossed are as table 1. Germination occurred from 67 to 126 days after seeding in all cross combinations, and germination condition was different among cross combinations. In case of *C. faberi* × *C. goeringii* or *C. sinensis* × *C. goeringii*, it took 67-92 days to germinate after seeding, and germination condition was also very good, whereas, in case of Chungumnan × *C. goeringii* or Baekwhabose × *C. goeringii*, the germination condition was worse as well as it took more days to germinate than the other hybrids. Therefore, distinguished difference was found among hybrid combinations.

Table 1. Ratio of fruition and seed germination of F_1 hybrid seeds between *Cymbidium* species

Cross Combination	Number of Pollination	Number of fruit set	Ratio of fruition(%)	Days after Pollination (days)	Days to germination (days)	Germination Status
C. goeringii × Chungumnan	2	2	100	211	126	++
Chungumnan × *C. goeringii*	3	1	33	286	99	+
C. goeringii × Ojachi	3	1	33	282	78	+++
Ojachi × *C. goeringii*	2	2	100	271	93	++
C. sinensis Willd × *C. goeringii*	1	1	100	271	96	+
C. goeringii × *C. sinense* Willd	1	1	100	261	98	++
C. sinense Willd × *C. goeringii*	1	0	0	0	0	0
C. goeringii × *C. sinensis*	3	3	100	243	99	+++
C. sinensis × *C. goeringii*	3	3	100	251	92	++++
C. goeringii × *C. latifolium*	2	2	100	244	97	+++
C. latifolium × *C. goeringii*	2	2	100	244	108	++
C. goeringii × Baekwhabose	6	3	50	242	110	+
Baekwhabose × *C. goeringii*	2	2	100	248	99	±
C. goeringii × *C. faberi*	2	2	100	206	82	+++
C. faberi × *C. goeringii*	2	2	100	216	67	++++

Paek et al. (1989) reported that it took 99-453 days to germinate by using Knudson C. However, when hyponex (H_3PO_2) was used, it took only 67-126 days according to this experiment. Therefore, there was big difference on days to germination based on the different media. This is maybe because of the difference among hybrid combinations, however, more consideration goes to medium selection and seed pretreatment method. As a result, it is assumed that seeding medium and seed pretreatment method presented in this experiment are effective for the seed germination of *Cymbidium*.

When 0.01, 0.1, and 0.2% of NaN_3 and MMS solutions were treated to seeds, germination occurred only with 0.1% NaN_3 and MMS. However, even though germination occurred in the other treatments, browinish phenomenon was intense. In the treatment of 0.01%, germination did not proceed and got worse after 4-5 months of seeding. In case of submerging, *Cymbidium* seeds such as *C. misericors* and *C. goeringii* into solution containing NaN_3 or MMS, germination status

Table 2. Germination status and days to germination when seeds were treated with NaN_3 solution

Concentration (%)	Time (min)			Remarks Column
	0.01	0.1	0.2	
10	-	77 (brownish)	- (brownish)	Extremely bad germination status after 4-5 months in all treatments
30	98	77 (brownish)	- (brownish)	
60	98	77 (brownish)	- (brownish)	
120	98	77 (brownish)	- (brownish)	
240	- (brownish)	- (brownish)	- (brownish)	

Table 3. Germination status and days to germination when seeds were treated with MMS solution

Concentration (%)	Time (min)			Remarks Column
	0.01	0.1	0.2	
10	120	-	105	Extremely bad germination status after 4-5 months in all treatments
30	120	-	-	
60	100	116	-	
120	108	-	-	
240	99	-	-	

and days to germinatation are as Table 2 and 3, respectively. When NaN_3 was treated to *C. misericors* and *C. rubrigemmum*, all seeds showed browinish color with more than 0.1mg/l, and no germination was found with 0.2mg/l. When Jeokumnan was submerged into the 0.01mg/l solution, more days to germination was required as submerging time gets less between 99-120 days.

Rhizome growth

This experiment was performed to have the source necessary for rhizome regrowth (Fig. 1) and shoot micropropagation, using rhizome of *Cymbidium* species. The rhizome derived from seed culture of *C. goeringii* was cultured with H_3PO_4 supplemented with different concentration of NAA and BA combination.

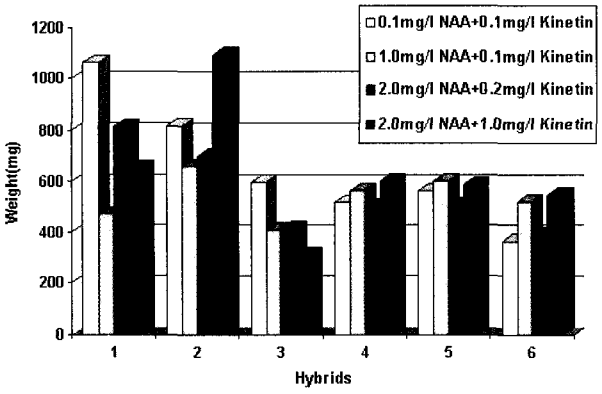

Fig. 1. Effect of growth regulator on the rhizome weight in F1 hybrids. 1) *C. goeringii* × Ojachii, 2) *C. latifolium* × *C. goeringii*, 3) *C. goeringii* × *C. latifolium*, 4) *C. sinensis* × *C. goeringii*, 5) *C. goeringii* × *C. sinensis*, 6) *C. goeringii* × Chunkeomnan

As a result, *C. goeringii* × Ojachui, *C. latifolium* × *C. goeringii* showed the biggest weight at 0.1mg/l NAA+0.1mg/l Kinetin, *C. goeringii* × *C. sinensis* 1.0mg/l NAA +0.1mg/l Kinetin, and *C. goeringii* × Chungeomnan, *C. sinensis* × *C. goeringii*, *C. latifolium* × *C. goeringii* 2.0mg/l NAA +1.0mg/l Kinetin. Therefore, *C. goeringii* hybrid showed difference for the concentration of NAA and Kinetin combination.

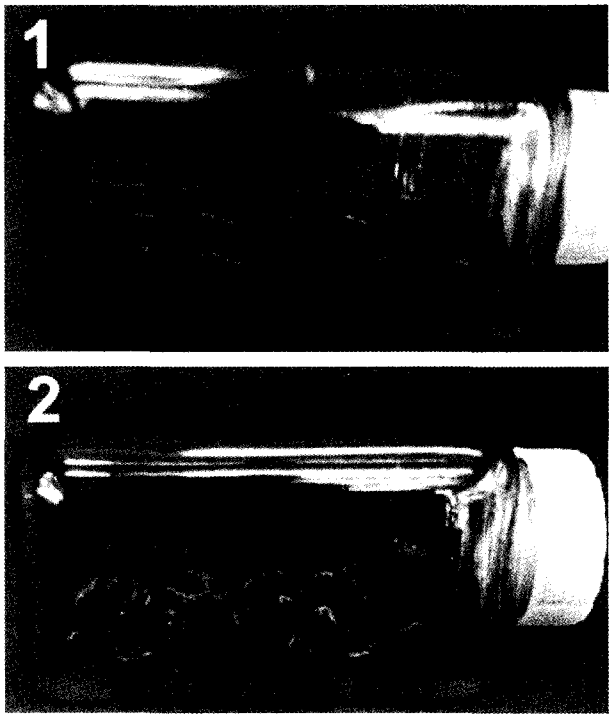

Fig. 2. Rhizomes growing on the media supplimented with different combination of growth regulators. 1) Rhizomes placed on media, 2) Rhizomes grown after three months.

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