Note

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Polyvinylpyrrolidone Enhances Seed Germination and Development of *Cypripedium macranthos* Sw. *in vitro*

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Cypripedium macranthos Sw., one of the endangered orchids in Japan, is well known for its poor germination and slow growth of seedlings. This could be due to the browning and subsequent necrosis of the cultures [1, 2]. This phenomenon is recognized among orchid cultures *in vitro*, and is thought to be caused by oxidation of polyphenolic substances [3]. It has been reported that the inhibitory effects of browing can be reduced by using gellan gum instead of agar [4, 5], and by adding activated charcoal or polyvinylpyrrolidone (P.V.P.) to the medium [1-3, 5-7]. However, there is no report on the effect of solidifier on germination of *Cypripedium* species. In the present study, the effects of P.V.P. and gellan gum on both germination and early growth stage of seedling from immature seeds of *Cyp. macranthos* were investigated.

Immature capsules of *Cyp. macranthos* were collected in June 1995, seven weeks after self-pollination by hand. Seeds were sown aseptically on a testing medium (20 m*l* medium in 25×150 mm test tube) by the method previously described [8]. For each treatment at least 4 replicate tubes were used, each containing from 50 to 150 seeds. The basic composition of the medium used was 1/2 Norstog [9] medium with the addition of 10 g·l^{-1} sucrose which was suitable for germination of this species [8].

Influence of solidifier: The basal medium was solidified with 10 g·l^{-1} agar (Wako Pure Chemical Industries, Ltd. JAPAN) or 3 g·l^{-1} gellan gum (Scott Laboratories, Inc., U.S.A.).

Effects of P.V.P.: Basal medium solidified with 10 g·l^{-1} agar was used alone or with polyvinylpyrrolidone (P.V.P., M.W. 40,000. Wako Pure Chemical Industries, Ltd. JAPAN) at concentrations of 100, 316, 1,000, 3,160, and 10,000 mg/*l*.

The pH of all media was adjusted to 5.5 before autoclaving. After sowing, all tubes were placed in the dark at 20°C. Germination and development were recorded 16 weeks after sowing, and classified into the four developmental stages as follows: I) embryo swollen and testa cracking (germination); II) protocorm stage and embryo longer than the testa; III) rhizoids apparent and bud initiation; and IV) root differentiated. Duncan's multiple range test was adopted for statistical analysis of each developmental stage and total germination percentages.

The effects of solidifier were not obvious (data not shown). There were no significant differences in percentage germination and protocorm growth between the two gelling agents tested. The color of both media surfaces was also brown at the time of investigation. However, the protocorm growth on gellan gum was slightly superior to that on agar medium. On gellan gum medium, 4.6% of seeds was at stage IV, compared to only 0.8% on agar medium. Development appeared to be slightly faster on gellan gum medium.

Although agar is the most frequently used gelling agent for culture media, an inhibitory effect on the growth of some plants has been reported [4]. In this experiment, agar had no negative effect on germination, but seedling growth on gellan gum media was slightly more vigorous than on agar medium. The physical nature of gellan gum may promote protocorm development by allowing a more rapid diffusion of nutrients and browning substances than agar [4].

The effects of P.V.P. on the early stages after germination are shown in **Table. 1.** The addition of P.V.P. in concentrations between 100 to 1,000 mg/*l* increased both germination percentages and accelerated the growth of protocorms. Higher concentrations of P.V.P. (3,160 and 10,000 mg/*l*) had inhibitory effects on germination percentages and protocorm growth. Addition of P.V.P. reduced the degree of browning of the medium slightly. Moreover, the degree of browning of the testa of seeds cultured on P. V.P. media was less than that of the control (data not shown). Miyoshi and Mii [3] reported that a high concentration of P.V.P. (10% w/v = 100,000 mg/*l*) had inhibitory effects on seed germination of *Calanthe discolor*, and they suggested that this was the result of P.V.P. increasing the osmotic pressure of the medium. In the present study the inhibitory effect of P.V.P. on germination of *Cyp. macranthos* was observed at a lower concentration of P.V.P. (3,160 and 10,000 mg/*l* equivalent to about 80μM and 250μM respectively). The inhibitory effect in this experiment might be for

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Table 1. Effects of P.V.P. (m.w.40,000) at various concentrations on germination and seedling growth of *Cyp. macranthos* on agar supplemented media (sixteen weeks after sowing).

P.V.P. concentration (mg/*l*)	Percent of seedlings at each developmental stage				Total germination %
	I	II	III	IV	
0	4.0 [a]	9.1 [ab]	24.1 [ab]	0.8 [b]	38.0 [b]
100	2.5 [a]	14.1 [a]	31.8 [a]	1.5 [ab]	49.8 [a]
316	2.6 [a]	12.4 [ab]	22.4 [ab]	3.4 [a]	40.8 [ab]
1,000	1.5 [ab]	10.1 [b]	24.2 [ab]	2.7 [ab]	38.4 [b]
3,160	2.0 [a]	6.8 [c]	6.8 [c]	0.7 [b]	16.3 [c]
10,000	0.6 [ab]	0.0 [d]	0.0 [d]	0.0 [c]	0.6 [d]

Key: I : embryo swollen and testa cracking; II : protocorm stage and embryo longer than the testa; III: rhizoids apparent and bud initiation; and IV: root differentiated.

Mean separation within column by Duncan's multiple range test at 5 % level. Values in the same column with the same suffixed letter are not significantly different.

a different reason from *Calanthe discolor*.

In the present study we showed that the optimum concentration of P.V.P. (m.w. 40,000) was 100 mg/*l*. The optimum concentrations of P.V.P. to obtain the growth stimulation of plants *in vitro* vary with the P. V.P. molecular weight [5]. Thus, further experimentation is needed to determine the most suitable concentrations and molecular weight of P.V.P.. To establish an *in vitro* culture system of *Cypripedium* spp., more studies must be carried out to determine suitable culture conditions for germination and initial growth.

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