Effect of Complex Supplements on Growth of *Ophrys* Seedlings in Vitro©

Masanori Tomita
Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Aomori Pref. 036-8561 JAPAN

Mature seeds of *Ophrys apifera*, *O. fusca, O. lutea,* and *O. sphegodes* were surface-sterilized and immediately sown in vitro. Germination occurred on all tested media. Among eight tested media, Norstog medium gave the highest germination percentage for these four *Ophrys* species. The effect of complex supplements (organic nitrogen sources) on germination of *O. apifera* was tested. Among the four complex supplements tested, organic supplements of Norstog gave the highest germination percentage.

INTRODUCTION

Ophrys species are European native terrestrial orchids. Although they have high horticultural value, it is difficult to establish a cultivation system for these species because little is known about their seed germination requirement(s). In this experiment, the effects of medium nutrient composition on both germination and seedling growth of four *Ophrys* species were studied.

MATERIALS AND METHODS

Mature seeds of *O. apifera, O. fusca, O. lutea,* and *O. sphegodes* were used in the seed germination trials. Seeds were surface sterilized with sodium hypochlorite solution (1% available chlorine), then rinsed in sterile distilled water. After sterilization,

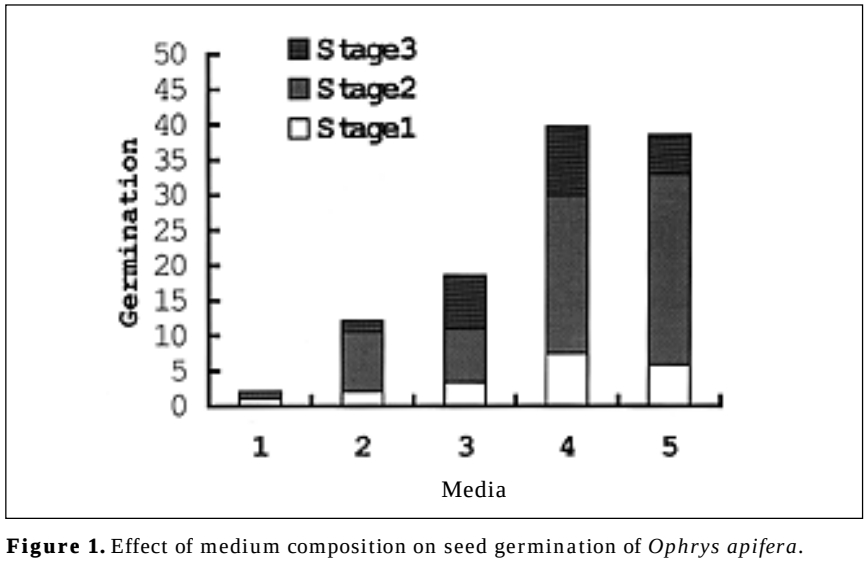

Figure 1. Effect of medium composition on seed germination of *Ophrys apifera*.

some seeds were used for an evaluation of their viability by the TTC test (Van Waves and Debergh, 1986).

Experiment 1. Hoppe (Hoppe and Hoppe, 1987), Kew-A, Knudson C (Knudson, 1946), Malmgren (Malmgren, 1992), ½ MS (Murashige and Skoog, 1962), Norstog (Norstog, 1973), Phytamax, and T (Tsutsui and Tomita, 1990) were tested as culture media (Kew-A and Phytamax: M. M. Ramsay, pers. comm.). To each nutrient medium 30 g liter^{-1} sucrose and 4 g liter^{-1} Gellan gum were added; the pH was adjusted to 5.2. All cultures were incubated in the dark at 20±2^{o}C. After 16 weeks of culture germination rate was determined.

Experiment 2. Mature seeds of *O. apifera* were used as material. Inorganic salts of Norstog medium (plus 30 g liter^{-1} sucrose and 4 g liter^{-1} Gellan gum) were used alone or with each organic complex supplement as follows: casamino acids 200 mg liter^{-1}, coconuts water 100 ml liter^{-1}, yeast extract 200 mg liter^{-1}, or organic supplements of Norstog medium (Table 1). Other conditions were the same as Experiment 1. After 16 weeks, the cultures were assessed on a scale of I to III as follows: (I) embryo swollen, testa splitting (germination); (II) protpcorm stage, embryo as long or longer than the testa; (III) rhizoids apparent, bud beginning to differentiate (Tomita and Tomita, 1997).

RESULTS AND DISCUSSION

The results of Experiment 1 are summarized in Table 2. In Experiment 1, germination had occurred on all tested media within 3 weeks of inoculation. There were no differences among the four *Ophrys* species on the tendency regarding the effect of medium on germination. Among eight tested media, Norstog medium gave the highest germination percentage. The major difference in composition between Norstog and other tested media used in this experiment is nitrogen source—Norstog medium lacked inorganic nitrogen. In Experiment 2, the effect of complex supplements (organic nitrogen source) on germination of *O. apifera* was tested (Fig. 1). The germination rate on the media containing only inorganic salts was low. When

Table 1. Composition of media used for seed cultures of *Ophrys apifera* (Experiment 2).

Medium no.	Casamino acids 200 mg liter^{-1}	Coconut water 20 ml liter^{-1}	Norstog's organics	Yeast extract 200 mg liter^{-1}
1	-	-	-	-
2	+	-	-	-
3	-	+	-	-
4	-	-	+	-
5	-	-	-	+

The basic composition of the medium used was Norstog (1973) medium without organics.

All media contained sucrose 30 g liter^{-1} and gellan gum 4 g liter^{-1}, and adjusted pH 5.2 before autoclaving.

Table 2. Effects of media composition on seed germination (%) of four *Ophrys* species.

Medium	*O. apifera*	*O. fusca*	*O. lutea*	*O. sphegodes*
Hoppe(1987)	31.6ab*	14.6b*	26.4b*	3.2NS**
Kew-A	21.3b	11.3b	22.3b	2.6
KnudsonC	18.6b	15.5b	19.1b	2.2
Malmgren(1992)	34.8ab	22.8ab	30.6ab	5.5
½MS	16.6b	9.9c	18.1b	1.9
Norstog(1973)	43.5ab	21.1ab	36.4ab	9.6
Phytamax	12.1b	6.6c	22.6b	1.3
T	27.2ab	13.6b	23.5b	6.4
Colored embryo with TTC test	59.6	31.1	42.1	21.1

Cultured for 16 weeks.
*Mean separation within column by Duncan's multiple range test at 5% level.
**NS:Not significant

the complex supplements were combined with inorganic salts of Norstog, both the germination rates and seedling growth improved. Among the four complex supplements tested, organic supplements of Norstog gave the highest germination percentage. However, germination percentages with yeast extract were not significantly different to those with organic supplements of Norstog.

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