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IN VITRO GERMINATION OF *PAPHIOPEDILUM* SEED ON A COMPLETELY DEFINED MEDIUM

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ABSTRACT

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Seed germination and seedling survival of *Paphiopedilum* cultured aseptically for 8 months in darkness or in the light varied with seed source, culture medium and incubation environment. In the light, germination was best and nearly equal on Burgeff EG-1, Thomale GD, and Norstog media, but more seedlings survived on Burgeff EG-1. Comparing incubation environments, the best germination and seedling survival occurred in the dark on either Norstog or Thomale GD, suggesting that increased production of *Paphiopedilum* from seed may require germination and early seedling growth in darkness. Since the Norstog medium is completely defined, it can be used to determine which organic constituents are vital for asymbiotic germination and growth of *Paphiopedilum*.

INTRODUCTION

Depending on the orchid species to be germinated, the in vitro culture medium can be of extreme importance (Yates and Curtis, 1949; Arditti, 1967; Thompson, 1974). The Knudson B or C culture media, or slight modifications of either, have been extensively used for orchid seed germination and seedling growth (Arditti, 1967). Although most orchid species can be germinated on these as well as on a number of other synthetic media (Withner, 1959; Arditti, 1967), *Cypripedium*, *Paphiopedilum*, *Phragmipedium* and *Selenipedium* are considered difficult (Arditti and Harrison, 1977). Two media commonly used for germination of *Paphiopedilum* seeds are Burgeff EG-1 (Richter, 1972; Thompson, 1974) and Thomale GS (Lucke, 1971; Richter, 1972; Ernst, 1975; Flamee, 1978). There are two major groups of orchids based on seed structure, one composed of species with seeds containing mostly differentiated embryos with a rudimentary cotyledon and

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endosperm, and the other, which includes *Paphiopedilum*, is composed of species producing seeds with undifferentiated embryos and little or no endosperm (Arditti, 1967; Ernst, 1974).

Orchid seed germination and subsequent plant development also varies depending on the light or dark requirement of the species. *Cattleya* seeds germinate at different rates in light or darkness, depending on the species (Arditti, 1967). *Cymbidium* seeds germinate in the dark and grow into small protocorms with leaves, but no roots develop (Yates and Curtis, 1949; Kohl, 1962). The *Paphiopedilum* seed germination studies reported in the literature available to us used lighted incubation environments exclusively (Arditti, 1967; Lucke, 1971; Richter, 1972; Ernst, 1974; Flamee, 1978).

Norstog (1973) developed a medium to support the growth of prematurely excised barley embryos. This medium, with incubation in darkness, was used in growing plants from undifferentiated inter-specific hybrid lily embryos (Stimart and Ascher, 1974), and differs from the Burgeff EG-1 and Thomale GD media in that it contains amino acids and vitamins in addition to macro- and microelements and carbohydrate, and is completely defined.

Since the seeds of *Paphiopedilum* contain an undifferentiated embryo similar to the hybrid embryos we excised from the lily, we compared the Norstog medium with 3 media commonly used for orchid seed germination, and incubated the seeds in either light or darkness to determine whether conditions suitable for development of undifferentiated excised embryos would improve *Paphiopedilum* seed germination and seedling survival.

MATERIALS AND METHODS

The 4 semisolid media tested included Burgeff EG-1 (Richter, 1972) and Thomale GD (Thomale, 1954), both currently used for *Paphiopedilum*, Knudson C (Kohl, 1962), widely used to germinate seeds of numerous orchid genera, and Norstog (Norstog, 1973). Because of thermal instability, the Norstog medium was sterilized through a 0.22-μm filter. The other 3 media were autoclaved at 120° C for 15 min. Fifteen ml of medium were dispensed into sterilized 8-dram vials in a laminar-flow hood, the vials were then capped with plastic and cooled in a slanted position. Media were prepared, poured, and held at room temperature (22° C) for 1 week to detect contamination prior to sowing the seed.

Cross- or self-pollinations of *Paphiopedilum* hybrids and species were made to produce seeds. When the fruit matured, 7—8 months after pollination, seeds were removed and placed in 8-dram vials which were corked and stored at 4° C until the seeds were used. Seeds were surface-sterilized in a laminar-flow hood by soaking them for 15 min in 50 ml of a 0.5% sodium hypochlorite solution containing 1 drop of a wetting-agent. After sterilization, seeds were rinsed once in sterilized distilled water, collected on a spatula, and spread over the surface of the slant. Eight replications of each seed source were sown on each medium and the cultures were incubated at 25° C

either in darkness or under a 16-h photoperiod of cool-white fluorescent light at 160 μE m^{-2} s^{-1}. The experiment was repeated twice.

Data were taken 8 months after sowing by determining the percentage of seeds germinated and the percentage of seedlings surviving. A 5-class scoring-system was used to record estimated percentages. Individual culture vials were scored as having 0 or approximately 25, 50, 75 or 100% germination or survival. The scores of vials within a treatment were averaged and the mean converted to the nearest score used in recording the data.

RESULTS

Each of the *Paphiopedilum* seed lots germinated in 1 or more medium—environment combinations (Table I). The amount of germination and seedling survival varied with the seed source, the medium, and the incubation environment. Hybrid seed generally produced higher scores than seeds from self-pollination of the *Paphiopedilum* species, which probably reflects inbreeding-depression in the latter. Although seeds from self-pollination of the 2 individuals from each of the species scored similarly within treatments, differences between these scores further illustrated a genotypic effect.

Totalling the scores in Table I across seed sources provides a comparison of the effect of the media and environments (Table II). In the light, germination scores were roughly equal for Norstog, Burgeff EG-1, and Thomale GD; all nearly double the score for Knudson C. However, many seedlings died at the protocorm stage in the lighted environment. Considering survival as well as germination, the Burgeff EG-1 medium was superior (Tables I and II). In the dark, however, total germination and survival scores for Burgeff EG-1 were lower than in the light. The germination score for Knudson C was the same in both environments, but survival was increased in the dark. Thomale GD produced the highest scores in the dark environment with a doubling of the germination score and a more than 4-fold increase in the survival score. Although the Norstog medium did not score as high as the Thomale GD, its scores from the dark environment were nearly double the best scores from the lighted environment (Tables I and II).

At the conclusion of the experiment, dark-grown seedlings appeared etiolated (Fig. 1). Although the leaves were underdeveloped, these plants greened quickly when placed in the light and grew normally. In a month or so, they were indistinguishable from the plants germinated in the light.

TABLE I

Mean *Paphiopedilum* seeds germination scores (above line) and seedling survival scores (below line) 8 months after sowing on Burgeff, Knudson, Norstog and Thomale media and incubation in the light or in darkness. Scores are estimated percent seeds germinated or seedlings surviving in a 5-class system, 0, 25, 50, 75 or 100% and mean scores involve 16 vials each

Seed source	Incubated in light				Incubated in darkness			
	Burgeff	Knudson	Norstog	Thomale	Burgeff	Knudson	Norstog	Thomale
Paphiopedilum								
'McLaren Park' × 'White Fringe'	100	50	75	100	75	50	100	100
	100	75	50	100	75	100	100	100
'Jack Tonkin Jason' × 'Hellas Westonbint'	50	25	25	50	25	25	50	50
	100	75	25	50	100	100	100	100
P. parishii × *P. curtisii* var. *Sandarae*	25	25	25	50	50	50	100	100
	25	0	0	0	75	75	25	100
P. callosum self-pollinated	25	0	0	25	0	0	100	100
	0			0			100	100
P. callosum self-pollinated	25	0	0	25	0	0	75	75
	25			0			100	100
P. callosum self-pollinated	25	25	75	25	25	25	50	75
	25	0	0	0	0	25	25	100
P. callosum self-pollinated	25	25	100	25	0	0	100	100
	0	0	0	0			25	75

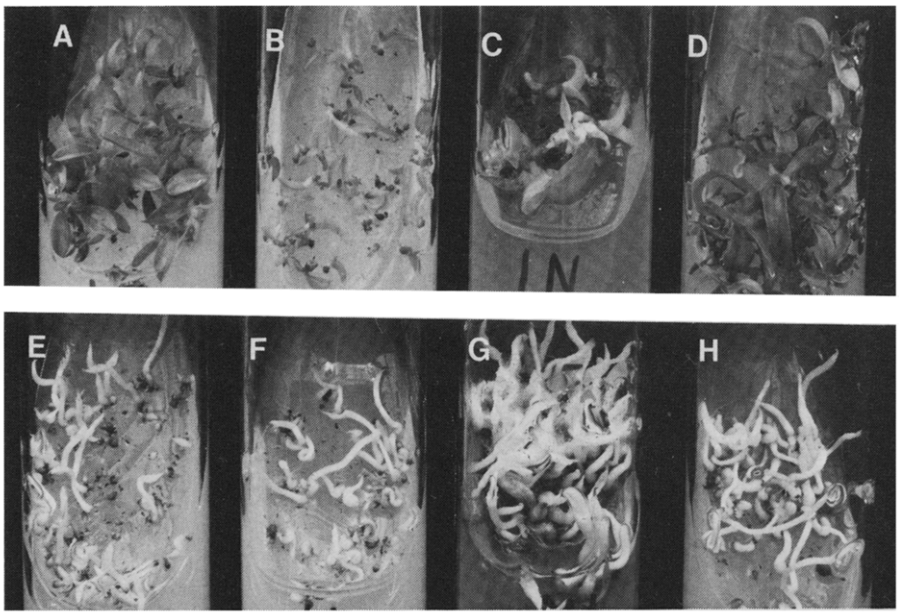

Fig. 1. Germination responses of *Paphiopedilum* seeds from 'McLaren Park' × 'White Fringe' incubated 8 months on selected media in light or darkness. A—D, incubated in the light (L); E—H, incubated in darkness (D). Media, from left to right, Burgeff EG-1 (B), Knudson, C (K), Norstog (N), and Thomale GD (T).

TABLE II

Total seed germination and seedling survival scores across seed sources for *Paphiopedilum* seed sown in vitro on 4 media and incubated 8 months in the light or in darkness

Culture medium	Incubated in light		Incubated in darkness	
	Germination	Survival	Germination	Survival
Burgeff EG-1	275	275	175	250
Knudson C	150	150	150	300
Norstog	300	75	575	475
Thomale GD	300	150	600	675

DISCUSSION

The Burgeff EG-1 or the Thomale GD media and incubation in a lighted environment are currently recommended for germination and seedling growth of *Paphiopedilum* (Lucke, 1971; Richter, 1972; Thompson, 1974; Ernst, 1975; Flamee, 1978). In terms of seedling survival, our data suggest that only the Burgeff EG-1 medium is superior in the light. However, optimum

seed germination and seedling survival occurred in the dark on either the Norstog or the Thomale GD media, indicating that the germination environment may be a critical factor in improving *Paphiopedilum* seed propagation. *Cypripedium*, a genus closely allied to *Paphiopedilum*, has also been reported to germinate better in vitro in the dark (Stoutamire, 1963, 1964).

Although the Norstog medium was not superior to Thomale GD, it is better than Burgeff EG-1 when used in the dark environment. The main difference between Thomale GD and Norstog is that the latter is completely defined while the former contains peptone as a complex organic additive. Therefore, the Norstog medium would appear better suited for research to determine the organic components essential for asymbiotic germination and seedling growth of *Paphiopedilum*.

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