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SEED GERMINATION OF NORTH AMERICAN ORCHIDS. II. NATIVE CALIFORNIA AND RELATED SPECIES OF APLECTRUM, CYPRIPEDIUM, AND SPIRANTHES

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Seeds of several terrestrial orchid species native to the United States were germinated on a number of culture media under differing conditions. Germination rates and seedling development varied considerably.

Introduction

Seeds of terrestrial orchids, particularly those from north temperate climates, are generally difficult to germinate in vitro (CURTIS 1936, 1943; DOWNIE 1940, 1949; KNUDSON 1941; VERMEULEN 1947; LIDDELL 1954; ARDITTI 1967; HARVAIS 1973, 1974; FAST 1976; CLEMENTS and ELLYARD 1979; LINDEN 1980). Their requirements, although varied, seem to be exacting and specific. Special and often different media are required even for species within a genus (ARDITTI 1967, 1979, 1982; STOUTAMIRE 1974; WARCUP 1975; WRIGLEY 1976; CLEMENTS 1982; FAST 1982; HADLEY 1982; NISHIMURA 1982).

This paper extends the list of species that have been germinated in vitro.

Material and methods

Mature and immature seeds as well as ripe and unripe fruits were received from several collectors. Immature seeds from unripe capsules were placed in culture immediately on receipt. Mature seeds were stored at 4 C in small paper envelopes (ARDITTI et al. 1979, 1980, 1981).

Unripe capsules were surface sterilized by immersion in a filtered calcium hypochlorite solution (7 g/100 ml water) for 10 min before being opened under sterile conditions. The immature seeds were scraped out and placed on the agar surface (ARDITTI et al. 1981).

Ripe seeds were sterilized by immersion in the sterilizing solution for 10 min. Glass tubes, stuffed with cotton at both ends and fitted onto repipetting bulbs, were used to sterilize, wash with sterile distilled water, and dispense the seeds into culture flasks. The seeds to be germinated on the Hyponex medium were sterilized and then soaked with agitation (60 oscillations/min) in sterile water for 45 days (HARRISON 1970; HARRISON and ARDITTI 1970; ARDITTI et al. 1981). Seeds were germinated and seedlings maintained under several combinations of illumination and pH, as well as composition and concentrations of culture media (tables 2, 3) at 23 ± 3 C (ARDITTI et al. 1981).

Full- or half-strength and modified Curtis media (CURTIS 1936) were used for asymbiotic germination of *Cypripedium*, *Aplectrum*, and *Spiranthes* seeds (ARDITTI et al. 1981). *Cypripedium* seeds were also germinated on a medium developed especially for this genus (CURTIS 1943) as well as on NORSTOG (1973) and Hyponex (TSUKAMOTO et al. 1963) media (table 1). An oat medium (oats and agar autoclaved in water) developed for Australian terrestrial orchids (CLEMENTS and ELLYARD 1979; CLEMENTS 1982) was used for symbiotic germination. Strips of filter paper were placed on the surface following solidification of the medium. The seeds were distributed at one end of the paper. Inocula of *Ceratobasidium* sp. and *Tulasnella* sp. (provided by MARK CLEMENTS, National Botanic Garden, Canberra, Australia) were placed on the other end.

We have defined germination as the appearance of green or white protocorms and are describing seedling development (tables 2, 3) in terms of the appearance of absorbing trichomes, chlorophyll, rhizomes, shoots, and roots (ARDITTI 1967, 1979, 1982; ARDITTI et al. 1981).

Results

All seeds germinated by first forming protocorms. Approximately 90% of the protocorms of each species were initially white, even under illumination, but turned green with time (tables 2, 3). Some protocorms of *Cypripedium* were green from the outset (table 3).

The best overall germination of mature *Cypripedium* seeds was on the Hyponex medium; *C. reginae* also germinated well on the modified Curtis solution. Germination of *C. californicum* and *C. montanum* was enhanced by full- and half-strength Curtis media when the pH was 7.0–7.5 (table 3). Seeds of *C. calceolus* germinated more rapidly on the Curtis *Cypripedium* medium (CURTIS 1943) than on any other solution (tables 1, 3). Immature seeds of *C. acaule* and *C. calceolus* var. *pubescens* germinated well on the Norstog, Hyponex, and full-strength Curtis media (table 3).

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TABLE 1

COMPOSITION OF MEDIA USED FOR THE GERMINATION OF SEEDS OF NATIVE CALIFORNIA AND RELATED ORCHIDS

COMPONENT	AMOUNT OF COMPONENT PER LITER OF MEDIUM			
	CURTIS[a]		CLEMENTS and ELLYARD[b]	Modified Hyponex[c]
	Modified	*Cypripedium*		
Macroelements:				
$CaCl_2 \cdot 2H_2O$, mg	...	100	...	...
$CaCO_3$, mg	148	...	...	...
$FeSO_4 \cdot 7H_2O$, mg	11.06	.01	...	...
KH_2PO_4, mg	240	1,000	...	...
$MgSO_4 \cdot 7H_2O$, mg	520	300	...	...
NaCl, mg	...	100	...	...
Urea, mg	250	...	...	...
Microelements:				
$AlCl_3$, mg	.03[d]	...	...	...
$CoCl_2 \cdot 6H_2O$, mg	.025[e]	...	...	...
$CuSO_4 \cdot 5H_2O$, mg	.03,[d] .025[e]	...	...	...
$FeCl_3 \cdot 6H_2O$, mg	1.0[c]	...	...	...
H_3BO_3, mg	1.0,[c] 6.2[e]	...	...	...
KI, mg	.01,[c] .83[e]	...	...	...
$MnSO_4 \cdot H_2O$, mg	.1,[c] 22.3[e]	...	...	...
$Na_2MoO_4 \cdot 2H_2O$, mg	.25[e]	...	...	...
$NiCl_2 \cdot 6H_2O$, mg	.03[d]	...	...	...
$ZnCl_2$, mg	3.93[e]	...	...	...
$ZnSO_4 \cdot 7H_2O$, mg	1.0[d]	...	...	...
Fertilizer:				
Hyponex, mg	...	...	...	3,000
Hormone:				
Wuchstoff 66,[f] ml	.1	.1	...	...
Nucleic acids,[g] mg	...	500.0	...	...
Complex additives:				
Coconut water from unripe nuts, ml	100.0	...	...	...
Banana homogenate, g	75.0	...	...	...
Peptone, g	...	.050	2.0	...
Oat, g	...	...	2.5	...
Sugar:				
Glucose, g	10.0	5.0	...	...
Sucrose, g	...	10.0	...	...
Darkening agent:				
Graphite, g	2.0	...	...	...
Solidifier:				
Agar, g	14.0	14.0	10.0	15
pH[h]	5.0	5.0	5.2–5.5	5

NOTE.—For composition of all other media, see ARDITTI et al. (1981).

[a] CURTIS (1936, 1943).

[b] CLEMENTS and ELLYARD (1979).

[c] Modified from TSUKAMOTO et al. (1963).

[d] Heller's microelements (ARDITTI 1977); listing of two microelement solutions indicates similar results.

[e] Murashige and Skoog (GAMBORG and WETTER 1975), listing of two microelement solutions indicates similar results.

[f] Wuchstoff 66f—courtesy of EDWARD GERLACH, Gmbh Chemische Fabrik, D4490 Lubbecke 1, Postfach 1165, West Germany.

[g] At this time it is difficult to determine which "Na nucleinate" CURTIS used. We used 250 mg RNA salt and 250 mg DNA salt.

[h] Except in experiments designed to test the effects of pH.

Roots and shoots generally appeared together in most *Cypripedium* seedlings. Two exceptions were *C. acaule*, where shoots formed after the roots, and *C. californicum*, where the reverse was true (table 3).

Protocorms and rhizomes formed simultaneously on seedlings of *C. californicum*. Rhizome formation followed the appearance of protocorms in *C. reginae* (table 3). No rhizomes were formed by the other *Cypripedium* species (table 3). Plant formation in *Cypripedium* occurred anywhere from 5 to 30 mo after the seeds were placed in culture (table 3).

Seeds of *Aplectrum* germinated on all three Cur-

TABLE 2

SEED GERMINATION AND SEEDLING DEVELOPMENT OF APLECTRUM AND SPIRANTHES

SPECIES AND MEDIUM[a]	CULTURE CONDITIONS[b]	PERCENTAGE GERMINATION[c]	MONTHS FROM PLACING SEED IN CULTURE TO APPEARANCE OF					REMARKS
			Absorbing hairs	Protocorms	Shoots	Green color	Roots	
Aplectrum hyemale:								
FC	L	1	...	20	...	...	...	...
FC, HC	D → L (10)	1	...	10	...	...	10	Protocorms, five with black roots.
MC	D → L (10)	10	...	10	10	...	...	Green protocorms.
Spiranthes gracilis:								
FC	D → L (4)	30	...	4	7	7	7	Green plantlets, 3 cm tall in 7 mo. Growth to 5 cm at 20 mo.
	D → L (2)	20	7	2	7	7	7	Green plantlets, 1 cm in 7 mo. Continued germination for 20 mo, numerous 2–10-cm plantlets.
S. romanzoffiana:								
FC	L	20	...	15	15	15	15	Plantlet 5 cm high after 15 mo. Plantlets 6–7 cm have well-developed roots after 18 mo. Germination of *S. romanzoffiana* is not as rapid, but greater root development pushed plantlets above agar surface. Plantlets were 10 cm tall and had 10-cm roots after 27 mo.

[a] FC = full-strength Curtis medium; HC = half-strength Curtis medium; MC = modified Curtis medium. For composition of media, see table 1. The listing of two or more media separated by commas is an indication of similar results.

[b] D = dark; L = light; numbers in parentheses indicate the time in months after inoculation when cultures were moved from dark to light.

[c] Estimates made when protocorms appeared. The number of seeds per culture varied between 100 and 300.

tis media (table 2). The protocorms did not produce rhizomes and formed only small roots after 10 mo in culture in both light and dark (table 2).

Spiranthes seeds germinated only on full-strength Curtis solution (table 2). Germination of *S. gracilis* occurred only in the dark; *S. romanzoffiana* germinated only under illumination. These species did not form rhizomes. *Spiranthes gracilis* produced protocorms (some with absorbing trichomes) and numerous 2–10-cm-tall plantlets in 7 mo. In contrast, 15 mo were required for the formation of 5-cm-tall plants of *S. romanzoffiana*. These plantlets reached a height of 7 cm after 3 mo in culture. At 27 mo the plantlets had well-developed root systems (table 2).

Discussion

Seeds of *Aplectrum hyemale* (eastern United States) cannot germinate asymbiotically on a modified Knudson C medium (STOUTAMIRE 1964). We found that this species germinates very poorly on full- and half-strength Curtis medium and much better on a modification that contains urea, which suggests that *A. hyemale*, like other orchids (BURGEFF 1936), may require or at least benefit from nitrogen in this form. Seeds of *Spiranthes sinensis* have been germinated on the Knudson C and Karasawa media (NISHIMURA 1982). *Spiranthes cernua* (North America) germinated asymbiotically on a modified Knudson C medium (STOUTAMIRE 1964), but it produced only white protocorms on the Norstog medium (HENRICH et al. 1981), which supports germination of *S. romanzoffiana*, found in the British Isles and North America (FAST 1982; HADLEY 1982).

In our studies, *S. gracilis* (North America) and *S. romanzoffiana* germinated and developed well on full-strength CURTIS (1936) medium. The differences among earlier reports (STOUTAMIRE 1964; HENRICH et al. 1981) and between them and our work are not surprising in view of the very specific germination requirements of seeds of north temperate terrestrial orchids.

Only the medium that supported germination of *S. cernua* contained peptone (STOUTAMIRE 1964). This complex additive contains many amino acids, several amides, and a number of vitamins (POWELL and ARDITTI 1975; ARDITTI 1982), and its com-

TABLE 3

SEED GERMINATION AND SEEDLING DEVELOPMENT OF CYPRIPEDIUM

SPECIES AND MEDIUM[a]	CULTURE CONDITIONS[b]	PERCENTAGE GERMINATION[c]	MONTHS FROM PLACING SEED IN CULTURE TO APPEARANCE OF: Absorbing hairs	Protocorms	Rhizomes	Shoots	Green color	Roots	REMARKS
Cypripedium acaule (immature):									
N, Hyp ...	L, D	0	...	...	...	...	...	...	Immature capsules. No germination or growth after 1.5 yr.
MC	L	5	6.75	6.75–11	...	24	6.75	6.75	Shoots, 1 cm with 13-cm roots.
MC	D → L (6–11)	5–10	...	6.75	...	...	...	6.75	White plantlets with roots 7.5 cm long after 2 yr.
CE + fungus, HC, FC	L, D	1	...	...	...	...	...	...	White protocorms form and senesce.
C. acaule × *C. californicum*:									
FC, MC, HC	L, D	0	...	...	...	...	...	...	...
C. acaule × *C. pubescens*:									
FC	L	1	...	11	...	...	...	...	White protocorms form and senesce.
FC	D → L (6)	1	...	26.5	...	26.5	26.5	26.5	Green shoots, 1.5 cm with very curly, hairy roots after 4 yr.
HC	L, D	1	...	26.5	...	...	...	...	Several small white protocorms formed in dark culture.
C. calceolus									
CC	L	0	...	...	...	...	...	...	
	D → L	1–5	...	7–12	...	...	...	...	White protocorms, one small white shoot.
FC	D → L (10)	1	...	14	...	...	...	...	Small brown protocorm with very small leaves.
C. calceolus var. *pubescens*:									
CC	L	0	...	...	...	...	...	...	
	D	1	...	24	...	...	...	...	Small brown and white protocorms, no further growth after 3 yr.
Hyp	L	1	14	14–26	...	...	14	14	Small 1-cm roots and shoots at 2.5 yr.
	D → L (4)	1	...	4	...	...	...	18	White protocorms with roots up to 2 cm long.
MC	L	1	...	27	...	...	...	...	No subsequent growth.
FC[c]	D → L (6)	1	...	30	...	30	30	...	Green plantlets 2 cm tall with curly roots after 2.5 yr.
HC	D → L (5.5) pH 7–7.5	1	...	5.5	...	...	5.5	...	Small green protocorms with 1-cm roots after 2.5 yr.
C. californicum:									
CC	L	20–50	...	13.25	...	13.25	13.25	17	Green plantlets 1–2 cm tall after 1.5 yr.
CC	D → L (7)	20	...	13.25	...	17.0	...	17	White plantlets 0.5–1 cm tall.
CC	D → L (15)	20	...	12	...	24	...	17	Green plantlets 0.5 cm tall.
Hyp	L, D → L (3)	10	...	3	...	14	14	14	Green plants 5–18 cm tall.
N	L	10	...	24	...	...	...	...	White protocorms.
	D → L (23)	10	...	24	...	...	28	...	Green plantlets 1 cm tall.

TABLE 3 (*continued*)

Species and medium[a]	Culture conditions[b]	Percentage germination[c]	Months from placing seed in culture to appearance of: Absorbing hairs	Protocorms	Rhizomes	Shoots	Green color	Roots	Remarks
FC	D → L (21)	10 ...	21	...	...	21.5	21.5		Green plantlets 2.5 cm tall.
HC	L	5–10 ...	24	...	43	34	43		Small green plantlet 3.5 yr after inoculation.
pH 7.0 ..	D → L (10)	5–10 ...	10	...	30	30	30		Green plantlets 2.4 cm tall.
pH 7.5 ..	D → L (17)	5–10 ...	29.5	29.5	30	30	30		Green plants 2–4 cm tall with rhizomes.
FC	D → L (21)	5 ...	21	...	...	21.5	21.5		Green plantlets 2 cm tall.
	D → L (21.75)	5 ...	21.75	...	33	33	33		Plantlets 1 cm tall after 3 yr.
MC	L, D	1 ...	...	...	...	...	...		Seeds germinate, but there is no subsequent growth.
CE + fungus ..	L, D	... ...	...	...	...	...	...		No germination on oat medium.
C. reginae:									
Hyp	L	1 ...	2	5.25	5.25	5.25	5.25		Green plantlet 1 cm tall after 5 mo. Only brown protocorms in dark cultures.
MC	L	50–80 ...	3	...	...	...	...		Excellent germination but no further development.
	D → (1–5)	80 ...	3–5	...	...	...	...		
CE + fungus ..	L, D	50–80 ...	8	...	...	...	...		Brown protocorms after 8 mo.
CC	L, D	50–80 ...	2	...	...	...	...		Brown protocorms only.
CE + Australian fungus ..	D	1 ...	17	...	...	...	...		Protocorms only.
MC, N, FC, HC	L + D	1 ...	16.0–24.5	...	...	...	...		White protocorms only.
C. montanum:									
N, HC	L, D	1 ...	...	...	...	...	...		Poor germination, no further development.
FC, pH 5.5 ..	D → L (3–35)	5 30	3–35	...	30	42	30		1–2 cm after 30 mo. White plantlet, roots are hairy; green color appears after 42 mo.
pH 6.0 ..	D → L (3)	1 ...	3–30	...	30	30	30		Plantlets are 0.5 cm tall after 30 mo, 1–2 cm at 38 mo, 2–4 cm at 42 mo.
pH 7.0 ..	D → L (3.5)	1 ...	3.5	...	30	30	30		Two green plantlets 8 cm tall after 42 mo. Higher pH values seem to promote growth, but not germination.
C. pubescens var. *parviflorum*									
FC, HC ...	D	1 ...	6	...	...	...	...		Protocorms only.

[a] CC = Curtis *Cypripedium* medium, CE = Clements and Ellyard (1979), FC = full-strength Curtis medium, HC = half-strength Curtis medium, Hyp = Hyponex medium, MC = modified Curtis medium, N = Norstog medium.

[b] D = dark, L = light; numbers in parentheses indicate the time in months when cultures were moved from dark to light.

[c] The number of seeds per culture vessel varied between 100 and 300.

position can vary with batch and manufacturer. Therefore, it is not possible to determine from the available information whether *S. cernua* has any special requirements for one or more organic compounds.

Reports on the germination of *Cypripedium* seeds are contradictory (ARDITTI 1967, 1979, 1982; STOUTAMIRE 1974; FAST 1982; HADLEY 1982). According to HENRICH et al. (1981), *C. acaule* and *C. arietinum* did not germinate on the Norstog medium, whereas *C. calceolus* var. *pubescens*, *C. calceolus* var. *parviflorum*, *C. reginae*, and *C. candidum* did. Some of these species, as well as *C. calceolus*, *C. passerinum*, and *C. reginae*, germinate on media containing glucose and fructose, sucrose, coconut water, peptone, yeast, with or without other additives (CURTIS 1943; STOUTAMIRE 1964; HARVAIS 1973, 1974; LINDEN 1980).

In our experiments, illumination seems to have had no significant effect on the germination of *Aplectrum*, *Spiranthes*, or *Cypripedium* and other native orchids (ARDITTI et al. 1981). Germination of some species may be improved in the dark (ARDITTI 1979; ERNST 1982). The reasons for these differences are not clear. Therefore, generalizations regarding the effects of light on the germination of such seeds are not possible.

Altogether, our findings and previous reports provide few clues regarding the germination requirements of seeds of North American orchids. Differences in the maturity of the seeds, characteristics of the species, composition of media, purity of chemicals, culture conditions, and difficulties in obtaining enough seeds for extensive experiments may be some reasons for this.

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