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TABLE 2

EFFECT OF MILK SOLIDS AND/OR SUGARS ON EXTRACELLULAR PROTEASE ACTIVITY IN USTILAGO VIOLACEA RACE UWO-1

Medium	Halo around colony (when milk present)	Protease activity in isolated plugs taken from immediate vicinity of colony
WA	...	tr
WA + milk	Yes	+++
MM	...	0
MM + milk	No	0
CM	...	0
CM + milk	No	0
WA + milk + 10 g/liter dextrose	Slight	tr
WA + milk + 20 g/liter dextrose	No	0

NOTE.—WA = 2% Difco Bacto-Agar in distilled water; +++ = high activity: plug rapidly causes large halos; tr = trace activity: very small halo formed; 0 = no activity.

agar contained active proteases, but agar plugs isolated from similar positions next to colonies on CM or MM + milk agar were inactive (table 2). The sugars in these media prevented the appearance of halos. Active plugs of agar were not inhibited by similar levels of sugars, and it appears therefore that sugars repress enzyme production in the cells rather than inhibit the activity of the enzyme. We cannot yet determine whether proteases are induced in *U. violacea* by gelatin or casein or whether these enzymes are produced on skim milk agar merely as a result of the lack of sugar repression. Experiments using nonsugar compounds as carbon sources are necessary to clarify this point. The isolates of *U. violacea* varied in their sensitivity to sugar repression, isolate 2C415 being the most insensitive strain.

These results establish that smut fungi vary greatly in the extracellular enzymes produced and confirm the results of GARBER et al. (1978) that there is much polymorphism both within and between species. These properties may prove useful in distinguishing morphologically similar species; e.g., *U. scabiosae* and *U. violacea* typically differ in several extracellular enzymes. Furthermore, these traits are easily scored and, therefore, ideal genetic markers.

Both natural variants and induced mutants may be utilized in mapping and in investigations of gene regulation, especially when combined with electrophoretic studies similar to those of KIRBY and MULLEY (1982) and of GARBER's group for several enzymes (BRADFORD et al. 1975; BAIRD and GARBER 1979, 1981). How such large molecules are passed through the cell wall of smut sporidia remains uncertain. We are investigating the possible role of the prominent surface fimbriae (POON and DAY 1974; GARDINER et al. 1981, 1982) for their transport.

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LITERATURE CITED

BAIRD, M. L., and E. D. GARBER. 1979. Genetics of *Ustilago violacea*. IV. An electrophoretic survey for urease variants in wild strains. BOT. GAZ. **140**:84–88.

———. 1981. The genetics and biochemistry of urease in *Ustilago violacea*. Biochem. Genet. **19**:1101–1114.

BRADFORD, L. S., R. J. JONES, and E. D. GARBER. 1975. An electrophoretic survey of fourteen species of the fungal genus *Ustilago*. BOT. GAZ. **136**:109–115.

DAY, A. W., A. J. CASTLE, and J. E. CUMMINS. 1981. Regulation of parasitic development of the smut fungus, *Ustilago violacea*, by extracts from host plants. BOT. GAZ. **142**:135–146.

DAY, A. W., and J. K. JONES. 1968. The production and characteristics of diploids in *Ustilago violacea*. Genet. Res. **11**:63–81.

GARBER, E. D., M. L. BAIRD, and L. M. WEISS. 1978. Genetics of *Ustilago violacea*. II. Polymorphism of color and nutritional requirements of sporidia from natural populations. BOT. GAZ. **139**:261–265.

GARDINER, R. B., M. CANTON III, and A. W. DAY. 1981. Fimbrial variation in smuts and heterobasidiomycete fungi. BOT. GAZ. **142**:147–150.

GARDINER, R. B., C. PODGORSKI, and A. W. DAY. 1982. Serological studies on the fimbriae of yeasts and yeastlike species. BOT. GAZ. **143**:534–541.

HANKIN, L., and S. L. ANAGNOSTAKIS. 1975. The use of solid media for detection of enzymes production by fungi. Mycologia **67**:597–607.

KIRBY, G. C., and A. M. MULLEY. 1982. Inheritance of isozyme mobility differences between two races of the head smut of grasses, *Ustilago bullata* Berk. BOT. GAZ. **143**:243–248.

POON, N. H., and A. W. DAY. 1974. Fimbriae in the fungus, *Ustilago violacea*. Nature **250**:648–649.

BOT. GAZ. 145(4):487–494. 1984.

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SEED POLYMORPHISM AND GERMINATION RESPONSES TO SALINITY STRESS IN ATRIPLEX TRIANGULARIS WILLD.

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Seeds of *Atriplex triangularis* exhibited a very pronounced morphological and physiological seed polymorphism. Seed size varied from 1.0 to 2.8 mm and predicted the likelihood of successful establishment through its effect on germination and seedling vigor. Large seeds had a mean dry weight of 2.44 ± 0.16 mg and a mean length of 2.45 ± 0.24 mm; medium seeds, mean dry weight of 1.21 ± 0.10 mg and mean length of 1.78 ± 0.19 mm; small seeds, mean dry weight of 0.64 ± 0.04 mg and mean length of 1.27 ± 0.10 mm. The degree of salt tolerance increased progressively with increasing seed size. Seeds from all size classes that were initially treated with 2%–5% NaCl had from 85% to 100% germination after being immersed in distilled water for 6 days, indicating a transitory adverse effect of salt stress on germination. The amount of water absorbed by all seeds is influenced by change in media salinity but not by hormonal treatments. Small seeds contain more Na^+ and Cl^- than medium and large seeds. Seedling dry weight was related to initial seed size. Salt stress inhibited seedling growth. Gibberellic acid alleviated some of the dormancy in seeds induced by high salt concentrations.

Introduction

The halophyte *Atriplex triangularis* Willd. (*A. patula* var. *hastata* [L.] Gray) (Chenopodiaceae) is an annual of wide distribution, commonly found in inland saline and coastal marshes (OSMOND et al. 1980). Both light and dark seeds were found in bracteoles: the dark seeds had a hard black testa, while light seeds appeared yellowish brown (FRANKTON and BASSETT 1968; UNGAR 1971). TASCHEREAU (1972) reported seed dimorphism, indicating a greater abundance of black seeds. DRYSDALE (1973), however, reported that seed size is lognormally continuous rather than bimodal; he also indicated that seed polymorphism may be developmental as seeds of both colors and major categories are found on the same plants. BAKER (1974) found that *A. triangularis* plants arising from large seeds had faster root and shoot growth than seedlings from small seeds that germinated at the same time.

Change in the properties of membranes plays a prominent role in controlling seed germination by affecting the rate of hydration, enzyme release, ion transport and concentration, pH, and inhibitor content (TAO and KHAN 1977). Gibberellic acid (GA_3), kinetin, and fusicoccin promote proton extrusion, K^+ uptake, and a decrease in transmembrane electric potential in seeds (ILAN et al. 1971; WOOD and PALEG 1972; MARRÈ et al. 1974). Seed germination in various halophytes was enhanced by GA_3 treatment (UNGAR and BINET 1975; UNGAR and BOUCAUD 1975; BOUCAUD and UNGAR 1976; UNGAR 1978).

We investigated the germination responses of *A. triangularis* seeds to various environmental factors. Water uptake and ion content of seeds were measured under various salt concentrations and growth regulator treatments. The effect of seed size and salinity on seedling growth was also determined.

Material and methods

Seeds were collected in October 1981 from an inland salt marsh on the property of the Morton Salt Company, Rittman, Ohio. The lengths of 747 seeds were measured with an ocular micrometer, and seeds were sorted into three groups: small, <1.5 mm; medium, between 1.5 and 2.0 mm; and large, > 2 mm. Air-dry weights of 25 mature seeds were determined using 25 replicates each of small, medium, and large seeds. For all germination and growth experiments, seeds were placed in 50 × 9 mm Gelman no. 7234 sterile, tight-fitting plastic petri dishes containing 6 ml of test solution. Each dish was placed in a 9-cm-diameter glass petri dish to reduce water evaporation. These dishes were placed in programmed refrigerated incubators using a 12-h photoperiod and alternating regime of 5/25 C with a 12-h night/12-h day temperature.

Effects of salinity on the germination of various size classes of *Atriplex triangularis* seeds and subsequent seedling growth were studied in light. Four replicates of 25 seeds each were used under six salinity regimes of 0%, 1%, 2%, 3%, 4%, and 5% NaCl (wt/vol). Germination was recorded at 2-day intervals for 20 days, and the percentage of germination was calculated. After 20 days, seedling growth was measured, and ungerminated seeds from the 2%, 3%, 4%, and 5% NaCl treatments were transferred to distilled water to study the recovery

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TABLE 1

ABSORPTION OF WATER (mg) FROM NaCl SOLUTIONS BY ATRIPLEX TRIANGULARIS SEEDS

NaCl	Seed weight (mg)	6 h[a]	24 h[a]	48 h[a]	72 h
1%:					
Small	.64	.05	.17	.01	.04
Medium	1.21	.21	.13	.04	−.01
Large	2.44	.48	.62	.23	.04
3%:					
Small	.64	.04	.12	.11	.01
Medium	1.21	.27	.19	.08	.06
Large	2.44	.44	.40	.41	.02

NOTE.—LSD (salinity) = 0.04; LSD (time) = 0.05; LSD (seed size) = 0.04.
[a] = Increase in seed weight (mg) during respective time.

small seeds was 0.22 mg; for medium seeds, 0.35 mg; and for large seeds, 0.37 mg.

RATE OF ABSORPTION

During 6 h of soaking there was very little absorption by small seeds (7% of dry weight), whereas large seeds soaking in 1% NaCl increased to ca. 20% of their dry weight (table 1). There was a slightly lower absorption in the 3% NaCl treatment, but the two treatments were not significantly different from each other. Small seeds soaking in 1% and 3% NaCl for 24 h showed a significant enhancement in the amount of absorption: 33% in 1% NaCl and 24% in 3% NaCl. Medium and large seeds also had a significant increase in the amount of absorption ($P = .01$). No significant absorption occurred in small and medium seeds soaking in 1% NaCl for 48 and 72 h, whereas absorption was considerably reduced in large seeds after 48 h. However, for medium seeds imbibing in 3% NaCl, the amount of absorption decreased substantially after 48 h.

Only the results of the 3% NaCl treatments are reported in table 2 since the 1% NaCl treatment did not yield significantly different results. Small seeds had a higher concentration of Na^+ than medium and large seeds (table 2). When soaked for 6 h in 1% NaCl, the sodium ion concentration substantially increased, but it progressively decreased to the level of the nontreated seeds after soaking for 72 h. In medium and large seeds, there was some increase in Na^+ concentration when seeds were presoaked for 6 h in NaCl, but this was not as prominent as that of small seeds, and it also decreased after 72 h of soaking. Change in media salinity does not seem to have any effect on Na^+ content of seeds.

When the amount of Na^+ was expressed as a percentage of tissue water, there was a very high concentration of Na^+ in the small seeds when soaked for 6 h in 1% NaCl, but, as the soaking time increased, the Na^+ concentration decreased substantially after 24 h of soaking. The Na^+ concentration was also higher after 6 h in medium and large seeds,

TABLE 2

CONCENTRATION (% ± SE) OF Na^+, Cl^-, AND K^+ IN POLYMORPHIC SEEDS OF ATRIPLEX TRIANGULARIS AFTER SOAKING IN 3% NaCl FROM 6 TO 72 h

Ion	Air-dry seed[a]	6 h[a]	24 h[a]	48 h[a]	72 h[a]
Small seed:					
Na^+	.67 ± .04	1.02 ± .03	.93 ± .08	.73 ± .04	.61 ± .05
K^+	.37 ± .02	.27 ± .01	.27 ± .04	.19 ± .01	.19 ± .02
Cl^-	1.56 ± .13	1.34 ± .19	.83 ± .06	.76 ± .10	.86 ± .12
Medium seed:					
Na^+	.43 ± .02	.64 ± .02	.53 ± .03	.46 ± .02	.52 ± .03
K^+	.37 ± .01	.30 ± .02	.31 ± .03	.29 ± .01	.27 ± .03
Cl^-	.72 ± .03	.91 ± .05	.40 ± .05	.48 ± .06	.81 ± .19
Large seed:					
Na^+	.38 ± .04	.35 ± .05	.29 ± .02	.33 ± .01	.32 ± .01
K^+	.32 ± .02	.37 ± .01	.33 ± .02	.39 ± .01	.36 ± .02
Cl^-	.98 ± .02	.42 ± .12	.32 ± .02	.33 ± .01	.50 ± .08

NOTE.—Na^+: LSD (time) = 0.09; LSD (seed size) = 0.07; K^+: LSD (time) = 0.03; LSD (seed size) = 0.02; Cl^-: LSD (time) = 0.03; LSD (seed size) = 0.11.
[a] = Ion content from a 1:1 (seed:distilled water) extract.

TABLE 3

PERCENTAGE INCREASE IN WEIGHT (mg ± SE) AND CONCENTRATION (% ± SE) OF Na^+, Cl^-, AND K^+ IN SEEDS TREATED WITH GA_3 AND NaCl FOR 24 h

TREATMENT	0% NaCl				3% NaCl			
	Wt	Na^+	Cl^-	K^+	Wt	Na^+	Cl^-	K^+
Small seed:								
H_2O ...	77.70 ± 5.85	.61 ± .16	.80 ± .11	.43 ± .14	45.30 ± .00	.68 ± .15	.90 ± .04	.23 ± .02
GA_3 ...	64.70 ± 5.10	.24 ± .03	.78 ± .08	.30 ± .08	36.80 ± 6.15	.22 ± .03	.57 ± .15	.29 ± .04
Medium seed:								
H_2O ...	44.40 ± 2.52	.49 ± .16	.87 ± .26	.41 ± .03	46.00 ± 1.09	.35 ± .08	.42 ± .02	.29 ± .02
GA_3 ...	48.40 ± 1.81	.28 ± .06	.44 ± .03	.59 ± .09	44.40 ± 2.63	.52 ± .17	.47 ± .02	.35 ± .02
Large seed:								
H_2O ...	50.60 ± 4.60	.28 ± .11	.23 ± .02	.44 ± .03	39.00 ± 1.38	.26 ± .04	.23 ± .01	.38 ± .02
GA_3 ...	48.50 ± 1.54	.22 ± .09	.17 ± .01	.44 ± .01	37.80 ± 1.79	.32 ± .09	.36 ± .15	.46 ± .07

NOTE.—Wt: LSD (seed size) = 2.98; LSD (treatment) = 3.44; LSD (salinity) = 2.98; Na^+: LSD (seed size) = 0.15; LSD (treatment) = 0.17; LSD (salinity) = 0.15; Cl^-: LSD (seed size) = 0.17; LSD (treatment) = 0.19; LSD (salinity) = 0.19; K^+: LSD (seed size) = 0.04; LSD (treatment) = 0.05; LSD (salinity) = 0.04.

but much lower than in small seeds, and ion content decreased significantly with an increased soaking time.

Chloride, as a percentage of dry weight, was initially (6 h) unchanged and then progressively decreased with time for small, medium, and large seeds that were soaked in 1% and 2% NaCl (table 2). Chloride as a percentage of tissue water was very high in small seeds during the 6-h treatment (28%) but decreased to 2% after 72 h of soaking in 1% NaCl. Similar results were obtained when seeds were exposed to 3% NaCl. In medium and large seeds, initial Cl^- concentration was much lower than that of small seeds, but the Cl^- concentration, when calculated as a percentage of tissue water, progressively decreased with increasing exposure time.

Potassium as a percentage of tissue water decreased with increase in soaking time for small, medium, and large seeds in various concentrations of NaCl. When K^+ is expressed as percentage of dry weight for small and medium seeds, it decreased in concentration with increase in soaking time in both salinity treatments. However, in large seeds, the concentration did not change in either salinity treatment (table 2).

EFFECT OF GA_3 ON THE ABSORPTION OF NaCl SOLUTION

GA_3 treatments applied to small seeds reduced their absorption with or without salt, whereas in medium and large seeds there was no significant hormonal effect on the absorption of water and NaCl solution (table 3). Seeds in nonsaline medium, when soaked for 24 h without GA_3, contained more Na^+ than seeds treated with GA_3 at all salinities and seed sizes (table 3). Small seeds had higher Na^+ concentrations than medium and large seeds. In Cl^-, the pattern was similar to that in Na^+ (table 3). Concentration of K^+ decreased with an increase in NaCl in the medium. Inclusion of GA_3 did not have any effect on the K^+ concentration of seeds (table 3).

TABLE 4

EFFECT OF PRESOAKING OF SEEDS IN DISTILLED WATER ON THEIR SUBSEQUENT GERMINATION (% ± SE) IN 2% NaCl

Time of soaking (h)	2 days	10 days	20 days	Velocity of germination
Small seed:				
6	.0 ± .0	18.0 ± .3	41.0 ± 1.0	8.5 ± .5
12	.0 ± .0	24.0 ± 3.7	47.0 ± 5.5	11.6 ± 1.8
24	1.0 ± 1.0	20.0 ± 1.6	48.0 ± 3.7	10.2 ± 1.1
Medium seed:				
6	.0 ± .0	16.0 ± 2.8	30.0 ± 3.5	7.5 ± 1.2
12	4.0 ± 2.8	10.0 ± 2.6	33.0 ± 1.9	7.1 ± 1.7
24	4.0 ± .6	18.7 ± .03	33.3 ± .9	9.1 ± .4
Large seed:				
6	.0 ± .0	9.0 ± 1.9	27.0 ± 3.0	6.1 ± 1.3
12	6.0 ± 3.5	18.0 ± 3.5	41.0 ± 2.6	10.6 ± 1.6
24	10.0 ± 2.6	24.0 ± 1.6	31.0 ± 1.0	11.7 ± 1.3

TABLE 5

EFFECT OF SALINITY ON THE VELOCITY OF GERMINATION OF POLYMORPHIC SEEDS

NaCl (%)	Small seed	Medium seed	Large seed
0.0	36.5	40.1	44.1
1.0	18.7	24.9	40.3
2.0	9.0	14.3	18.7
3.0	.0	3.1	3.8
4.0	.0	2.6	1.1
5.0	.0	.3	.9

NOTE.—Velocity of germination = $\Sigma G/t$, where G = percentage of germination at 2-day intervals, t = total germination period (20 days).

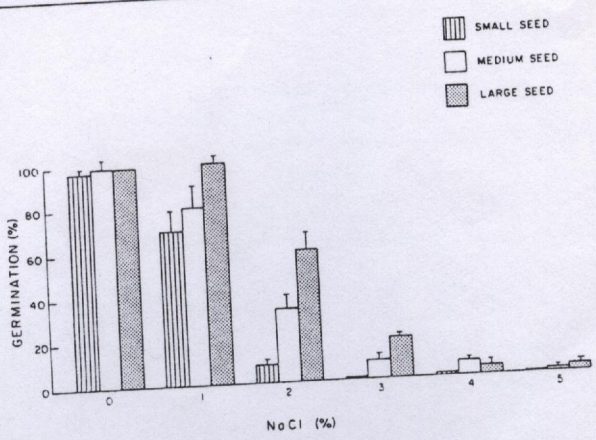

FIG. 3.—Final germination percentage of *Atriplex triangularis* seeds after a 20-day treatment with 1%, 2%, 3%, 4%, and 5% NaCl.

EFFECT OF PRESOAKING ON GERMINATION

Presoaking for 12 and 24 h resulted in a faster rate of germination than 6 h of soaking (table 4). Final germination percentages for all seed sizes in all treatments were not significantly different from each other. Presoaking significantly increased the final germination percentage of small seeds compared with the nonsoaked controls.

SALT TOLERANCE

The velocity of germination of small seeds substantially decreased with increase in salinity (table 5). No small seeds germinated in 3%, 4%, and 5% NaCl. Medium seeds appeared to be a little more tolerant and have a higher velocity of germination than small seeds at all NaCl treatments, with medium seeds germinating in 5% NaCl. Large seeds were most tolerant to salinity. Velocity of germination for large seeds was higher at all NaCl concentrations than for small and medium seeds. However, an increase in salinity concentrations progressively decreased germination percentages for all seed sizes.

Without salt, seeds of all sizes had ca. 100% germination (fig. 3). Salt in the medium induced dormancy, which increased with increased salt concentration. Salinity is more effective in inducing dormancy in small seeds than in large seeds. Seeds of all sizes in all treatments recovered very quickly (table 6). Germination was above 90% in almost all treatments after 6 days except for the small seeds that were initially treated with 5% salt, which had an 87% recovery of germination. Increase in salt stress inhibited the growth of seedlings, more in small seeds than in large seeds (table 7).

EFFECT OF SALINITY AND GA_3 ON GERMINATION

GA_3 promoted the germination of small, medium, and large seeds compared to the controls in distilled water and the dark (table 8). GA_3 completely reversed the inhibitory effect of 1% NaCl on germination in all three seed sizes. With an in-

TABLE 6

GERMINATION (% ± SE) PERCENTAGE OF ATRIPLEX TRIANGULARIS SEEDS IN DISTILLED WATER AFTER A 20-day NaCl TREATMENT

NaCl (%)	2 days	4 days	6 days	Total germination
Small seed:				
2	79.5 ± 3.3	94.5 ± 2.9	97.7 ± 2.9	98.0 ± 2.0
3	69.0 ± 6.4	82.0 ± 5.3	98.0 ± 1.0	98.0 ± 2.0
4	62.0 ± 5.3	82.0 ± 3.5	98.0 ± 1.0	98.0 ± 2.3
5	52.0 ± 9.1	78.0 ± 8.1	87.0 ± 5.5	87.0 ± 11.0
Medium seed:				
2	53.7 ± 3.5	78.1 ± 7.8	100.0 ± .0	100.0 ± .0
3	71.7 ± 3.5	88.0 ± 3.3	97.8 ± 1.2	98.0 ± 2.3
4	61.7 ± 5.2	88.3 ± 4.8	96.8 ± 1.2	97.0 ± 4.0
5	62.6 ± 6.4	86.8 ± 5.1	97.9 ± 2.0	98.0 ± 4.0
Large seed:				
2	65.9 ± 7.1	97.6 ± 2.3	100.0 ± .0	100.0 ± .0
3	79.3 ± 3.5	97.6 ± 5.3	100.0 ± .0	100.0 ± .0
4	72.2 ± 3.5	91.8 ± 1.8	97.9 ± 1.2	98.0 ± 2.0
5	71.1 ± 8.7	93.8 ± 5.2	95.9 ± 1.6	96.0 ± 3.2

TABLE 7

EFFECT OF SALINITY ON THE GROWTH OF SEEDLINGS FROM POLYMORPHIC SEEDS OF ATRIPLEX TRIANGULARIS

VARIABLE	SALINITY (%)			
	.0	.5	1.0	1.5
Small seed:				
Hypocotyl length (mm ± SE)	25.2 ± .8	20.1 ± .9	12.8 ± .5	5.0 ± .1
Root length (mm ± SE)	20.6 ± .6	8.7 ± .7	4.2 ± .4	4.0 ± .8
Dry weight (mg ± SE)	.4 ± .1	1.2 ± .2	.7 ± .2	.6 ± .1
Medium seed:				
Hypocotyl length (mm ± SE)	24.9 ± 1.0	25.1 ± 1.3	16.6 ± 1.1	12.8 ± 2.9
Root length (mm ± SE)	23.1 ± 1.2	15.8 ± 2.2	2.8 ± .2	2.6 ± .8
Dry weight (mg ± SE)	.7 ± .1	0.8 ± .1	.6 ± .1	.4 ± .1
Large seed:				
Hypocotyl length (mm ± SE)	32.9 ± 1.1	35.8 ± 1.3	18.8 ± 2.0	18.4 ± 1.8
Root length (mm ± SE)	27.0 ± 1.1	17.7 ± 1.7	8.7 ± .5	8.5 ± .9
Dry weight (mg ± SE)	1.9 ± .1	2.9 ± .1	2.2 ± .0	1.6 ± .1

crease in salinity, germination was progressively reduced, but inclusion of GA_3 improved germination.

Discussion

Several *Atriplex* species growing under saline conditions were reported to have seed dimorphism and polymorphism (KOLLER 1957; FRANKTON and BASSETT 1968; UNGAR 1971; DRYSDALE 1973; GUSTAFSSON 1973; BAKER 1974). GUSTAFSSON (1973) reported that the rate and percentage germination of black and brown seeds of *A. triangularis* and *A. longipes* were scarcely different, and black seeds of *A. longipes* showed greater germination percentages in some experiments. UNGAR (1984) indicated that plant size affects both the number of seed produced per plant and the ratio of small to large seeds.

Polymorphic seeds of *A. triangularis* are physiologically different in their response to salinity. Germination of small seeds is prevented by NaCl exposure, but this is clearly temporary. Distilled water removes the inhibition of seed germination caused by NaCl. Seeds of several species, including *A. halimus* and *Salicornia europaea,* remain dormant at low water potential, and these seeds do not lose their viability and will germinate when returned to distilled water treatment (ZID and BOUKHRIS 1977; UNGAR 1982).

TABLE 8

EFFECT OF SALINITY AND GA_3 (1,000 mg/liter) ON THE GERMINATION (% ± SE) OF POLYMORPHIC SEEDS OF ATRIPLEX TRIANGULARIS IN THE DARK

NaCl	Small Seeds	Medium seeds	Large seeds
0%:			
$-GA_3$	14 ± 2.6	28 ± 2.8	63 ± 6.6
$+GA_3$	30 ± 8.1	34 ± 1.2	76 ± 3.3
1%:			
$-GA_3$	1 ± 1	7 ± 3	36 ± 2.5
$+GA_3$	18 ± 3	35 ± 6	61 ± 7.2
1.5%:			
$-GA_3$	0	0	20 + 4.3
$+GA_3$	9 ± 3	15 ± 1.9	27 ± 4.4
2%:			
$-GA_3$	0	1 ± 1	12 ± 3.7
$+GA_3$	2 ± 2	3 ± .5	14 ± 5.3

Germination in *A. halimus* was not permanently inhibited by 4% and 5% NaCl, and seeds germinated when returned to distilled water (ZID and BOUKHRIS 1977). IGNACIUK and LEE (1980) found that germination of *A. glabriuscula* and *A. laciniata* was not inhibited after 30 days of soaking in 600 mM NaCl. This common response among halophytes implies that it is of some ecological significance within highly saline environments, reflecting a physiological response that is strongly selected for during the evolution of these species.

GA_3 was effective in promoting germination of *A. triangularis* seeds in 1% NaCl but stimulated less than 5% germination at higher salinities. This inability to reverse the inhibitory effect of NaCl at very high salinities indicates that metabolic processes are being directly affected by salt stress. The more effective promotion of seed germination by GA_3 in the dark than in light also was reported by BOUCAUD and UNGAR (1973, 1976) and UNGAR and BINET (1975).

Presoaking of polymorphic seeds improved the rate of germination, particularly of small seeds. Similar effects of presoaking on germination were reported in *A. semibaccata* and *A. lentiformis* (YOUNG et al. 1980). UCHIYAMA (1981) related seed germination to the rate of absorption of water. He believed that, irrespective of temperature, when the water content of the seeds of *A. nummularia* reached 70%, seeds would germinate.

In *A. triangularis* the absorption rate of seeds from NaCl solutions was affected by an increase in the salinity of the medium. Uptake of water by the dry seeds is characterized by an initial phase

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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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