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Effect of Heavy Water on the Germination of a Number of Species of Seeds

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Summary. The effect of deuteriation on the germination rate of seeds is studied as a possible screening technique prior to the cultivation of a plant in high concentrations of D_2O. There appears to be a simple relationship between size of the seed and germination capacity in high deuterium concentrations. Larger seeds may be more successful in germinating because of greater hydrogen-containing food reserves.

Introduction

Although many species of algae have been successfully grown (Crespi et al., 1960) in 99% D_2O, no seed-bearing plant has as yet been cultivated in a medium containing more than 70% D_2O (Blake et al., 1964a, b; Crane et al., 1964; Uphaus et al., 1965). The effect of D_2O on the germination of seeds thus acquires importance as a screening technique in the search for plants that may be adapted to high concentrations of D_2O. Lewis first noted in 1933 that tobacco seeds did not germinate in pure D_2O. Crumley and Meyer (1950) made an extensive early study on the effects of three concentrations of D_2O on the seeds of 4 species of plants. More recently, Siegel et al. (1964) studied the germination of 11 species of seeds in high concentrations of D_2O, and found that only rye seeds were capable of germination in such concentrations.

Methods

In the earlier work reported in the literature, little consideration was given to possible isotopic dilution of the D_2O medium by exchange with H_2O in the atmosphere. In our experiments, germination was conducted in special chambers that were continuously flushed with filtered air of controlled humidity and isotopic composition, obtained by passage through gas-washing bottles containing the appropriate concentrations of D_2O and H_2O. All seeds were treated for 5 min with a 1% solution of the fungicidal agent Orthocide 75. The seeds were then washed thoroughly with H_2O, air dried, and counted into groups of 100. Germination took place in Petri dishes holding filter paper that was saturated with the appropriate concentration of D_2O. The D_2O levels studied were 0, 25, 50, 80, 90, and 100%. Daily counts of germinated seeds were made. Counting was continued up to 3 months or until it became apparent that there would be no further germination.

Table 1. *Percent of seeds which germinated in different D_2O concentrations*

Plant species	Weight per seed[a] (mg)	D_2O					
		0	25%	50%	80%	90%	100%
1. *Secale cereale* (rye)							
var. Abruzzi	25.08	62	72	79	84	84	86
var. Balbo	20.57	62	73	68	82	79	90
var. Pierre	20.33	66	87	86	86	83	85
var. Tetra Petkus	22.86	85	77	84	88	92	88
2. *Convolvulus tricolor* (morning glory)	44.00	81	76	63	36	16	7
3. *Digitalis purpurea* (foxglove)	0.094	95	95	90	—	—	—
4. *Ruta graveolens* (rue)	2.36	94	100	100	41	—	—
5. *Helleborus niger* (black hellebore)	7.07	62	61	8	—	—	—
6. *Linum usitatissimum* (flax)	6.69	94	58	100	88	33	32
7. *Pisum sativum* (pea, var. Alaska)	250.00	90	90	94	88	92	100
8. *Zea mays* (corn, maize)	216.30	100	100	84	90	96	94
9. *Avena sativa* (oats)	32.18	63	69	85	—	—	—
10. *Cucurbita pepo* (squash, var. Hubbard)	269.00	100	100	96	96	86	8
11. *Atropa belladonna* (deadly nightshade)	1.13	79	88	75	—	—	—
12. *Lactuca sativa* (lettuce)	1.14	74	73	87	64	59	16
13. *Phaseolus vulgaris* (bean)	1,197.00	94	90	88	96	94	50
14. *Lobelia inflata* (Indian tobacco)	0.035	100	100	—	—	—	—
15. *Festuca rubra* (fescue grass)	1.65	100	100	100	86	91	91
16. *Triticum aestivum* (wheat)	44.4	88	89	85	86	57	57

[a] Based on the weight of 10 seeds selected at random.

Results and Discussion

The data obtained are shown in Tables 1 and 2. For 7 of the species examined, germination was severely inhibited in high concentrations of D_2O. Referring to Table 1, rye (species 1, four varieties), peas (species 7), corn (species 8), and fescue (species 15) germinated well at all levels of D_2O. Flax (species 6), lettuce (species 12), beans (species 13), and wheat (species 16) were moderately inhibited in germination. Squash (species 10) germinated well in 90% D_2O, but poorly in pure D_2O.

Table 2 shows the time required for half the seeds to germinate (of the total that finally did germinate). Those plant species that were less inhibited by high D_2O levels also showed smaller changes in germination rate. Generally, the half-times of germination in pure D_2O were about double those of the controls in H_2O. However, flax and lettuce, which have very short half-times in H_2O, showed a 7-fold and 13-fold increase in half-times, respectively. *Convolvulus* (species 2) and squash, while severely inhibited in germination at high levels of D_2O, showed less of an effect in half-time of germination. The present study thus confirms the observation of SIEGEL et al. (1964), with respect to germination of rye. However, the 4-day test period used by these investigators appears to be too short for testing germination at high D_2O levels. This fact is

Table 2. *Time in days for 50% of the seeds to germinate in different D_2O concentrations*

Plant species	D_2O					
	0	25%	50%	80%	90%	100%
1. *Secale cereale*						
var. Abruzzi	2.4	2.5	3.2	4.3	4.2	5.3
var. Balbo	2.7	2.9	3.6	4.5	5.0	5.6
var. Pierre	3.2	2.4	3.5	4.7	5.0	5.4
var. Tetra Petkus	2.5	3.4	3.2	4.2	4.8	5.3
2. *Convolvulus tricolor*	4.3	1.8	2.5	4.5	6.5	7.0
3. *Digitalis purpurea*	5.3	6.9	9.4	—	—	—
4. *Ruta graveolens*	5.4	6.2	6.5	11.0	—	—
5. *Helleborus niger*	7.9	6.0	10.0	—	—	—
6. *Linum usitatissimum*	0.7	0.8	2.2	4.6	5.3	4.8
7. *Pisum sativum*	4.6	4.8	4.9	9.6	9.0	9.8
8. *Zea mays*	2.6	3.0	3.0	3.2	4.3	4.8
9. *Avena sxtiva*	6.2	22.0	22.3	—	—	—
10. *Cucurbita pepo*	5.7	5.6	5.0	8.6	9.8	10.2
11. *Atropa belladonna*	33.0	47.0	65.0	—	—	—
12. *Lactuca sativa*	2.5	4.8	10.0	20.8	23.0	33.0
13. *Phaseolus vulgaris*	4.2	3.3	4.2	4.7	4.7	6.1
14. *Lobelia inflata*	7.5	9.4	—	—	—	—
15. *Festuca rubra*	6.2	5.0	6.3	9.4	13.5	16.0
16. *Triticum aestivum*	3.8	3.0	4.0	5.0	7.0	7.8

clearly demonstrated in Table 2, where the half-times of germination are seen to be significantly increased for all species tested.

The data in Table 1 show a clear relation between seed size and the effects of D_2O on seed germination. Larger seeds such as peas and corn germinated well at high D_2O levels, while *Digitalis* and *Lobelia* did not germinate under such conditions. This implies that the initial availability of essential metabolites to the seed is decisive in germination, and the metabolic effects of deuterium do not become important until the hydrogen reserves are exhausted.

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