Plant & Cell Physiol., 7 (1966)

DYNAMIC ASPECTS OF WATER-RELATIONS IN GERMINATION OF *HIRSCHFELDIA INCANA* SEEDS

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(Received March 16, 1966)

Seed germination of *Hirschfeldia incana* was found sensitive to substrate hydration, being progressively inhibited in increasingly high levels of hydration. This phenomenon was analyzed kinetically. Water-sensitivity was unaffected by light conditions, though germination was promoted by a short irradiation and to a lesser extent by continuous irradiation and inhibited by darkness. Water-sensitivity was greatly modified by temperature. Kinetics of the germination process (timing of maximal sensitivity to promotive short irradiation) were unaffected by hydration, but were hastened by temperatures which modified water-sensitivity. The equilibrium level of seed moisture-content was strongly dependent on substrate hydration, which also determined the kinetics of water uptake. The level of water sensitivity was determined by the hydration or temperature during the initial 8-16 hrs of incubation. GA hastened the processes of germination and reduced water-sensitivity, the growth regulators Amo-1618, CCC, and coumarin retarded germination, but only the latter two enhanced water-sensitivity. It was concluded that the level of germination in each condition was determined by its effects on the relative rates of the germination processes and the build-up of an inhibition. It is suggested that the latter is due to effects of hydration on the resistance to diffusion of oxygen into the seed through the enclosing mucilaginous seed coat.

The physical and chemical components of water energy of the germination substrate exert different effects on germination responses (*1-4*). Moreover, the requirements of the seed for ample water for rehydration of its embryonic tissues often conflict with its requirement for efficient gaseous exchange. This is apparently the cause for the phenomenon of 'water sensitivity', where germination is inhibited by an over-abundant supply of moisture (*5-11*).

The great majority of laboratory studies on seed germination are carried out in PETRI-dishes, on a substrate made up of filter-paper or agar, moistened with some standard amount of distilled water (or solution made up

Abbreviations: GA, gibberellic acid; Amo-1618, 2-isopropyl-4 dimethylamino-5-methyl-phenyl-1-piperidine carboxylate methyl chloride; CCC, Cycocel, 2-chloroethyltrimethylammonium chloride.

with distilled water). Yet, these moisture relationships are rarely, if at all, encountered by the seed in its natural micro-environment (*12*). Moreover, PETRI-dishes are not gas-tight and lose moisture continuously. This exposes the seeds to a gradual increase in moisture potential on the one hand (*13*), and to a decrease in the resistance to gaseous exchange between the embryo and the atmosphere, on the other. These changes are violently reversed with each irrigation. Such changes may have profound effects on germination (*14-16*).

The studies reported below were made in order to analyze effects of substrate hydration on germination responses of 'water-sensitive' seeds. Preliminary studies showed that seeds of *Hirschfeldia incana* L. Lagrèz-Fossat were suitable for this purpose.

MATERIALS AND METHODS

Fruits of *H. incana* were collected from their natural habitat (roadsides) in the Judaean hills, between June and July, in 1963 and 1964. The seeds were extracted manually. Different levels of hydration were obtained in two ways. One procedure (the 'ladder technique') was to use horizontal steps of a vertical zig-zag strip of filter paper. The filter paper strip was

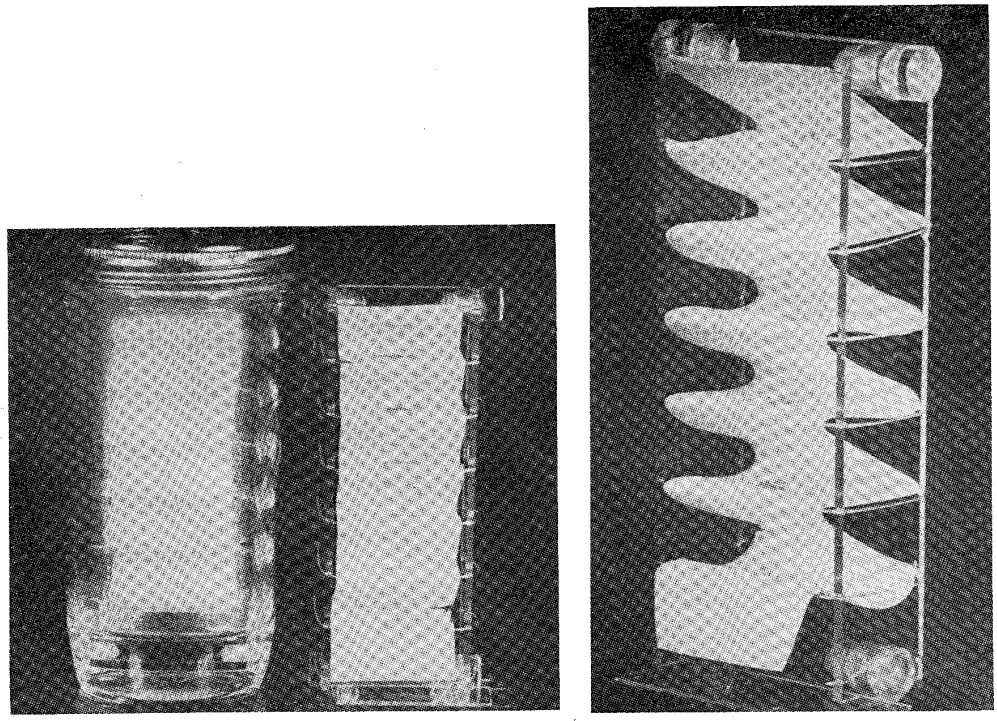

Plate 1. System used in studies on dynamic aspects of water relations in germination. Uprights, spacers and bolts are made of "Perspex". Horizontal supporting wire made of nylon fishing line. Filter strip is Whatman No. 1. Vertical distance between steps —20 mm, steps are 25×50 mm.

supported by a scaffolding which was entirely constructed from "Perspex" and nylon fishing wire, using no adhesive. The base of the filter paper strip was immersed in water and the whole structure was sealed in 'Ball' mason jars (Plate 1). The moisture content of the filter paper was allowed to equilibrate for at least 24 hrs before sowing. The other procedure (the 'filter paper-layer technique') was to use different numbers of filter paper layers in a standard (50 mm diameter) PETRI dish, moistened with a standard amount (2 ml) of water. The filter paper used in both techniques was Whatman No. 1 and the water was double deionized. Illuminated incubators (mixed fluorescent and incandescent sources, giving 2,000-3,000 lux) provided temperatures within 0.5° of the preset values. Dark conditions were obtained by enclosing the jars or dishes in light-proof tin containers. Short irradiation treatments were given by exposing the dark-incubating seeds to the light inside the incubators for 10 min. Forty seeds were used in each dish, or on each step, respectively. Each experiment was carried out in triplicate and was repeated several times. The gibberellin used was the potassium salt of GA obtained from Merck, Rahway, N. J. Amo-1618 was obtained from Rainbow Color and Chemical Co., Northridge, Calif. CCC was obtained from Cyanamide International, Wayne, N. J.

RESULTS AND DISCUSSION

Sensitivity to substrate hydration

Decrease in substrate hydration had promotive effects on germination of *H. incana* seeds, both in the filter paper-layer technique (Table I) and the ladder technique (Fig. 1). Fig. 1 also shows the moisture contents of the various steps. As both techniques gave qualitatively similar results, they could be used interchangeably, as needed.

The fact that under otherwise identical conditions the final level of germination was an inverse function of hydration, indicates (a) that above a

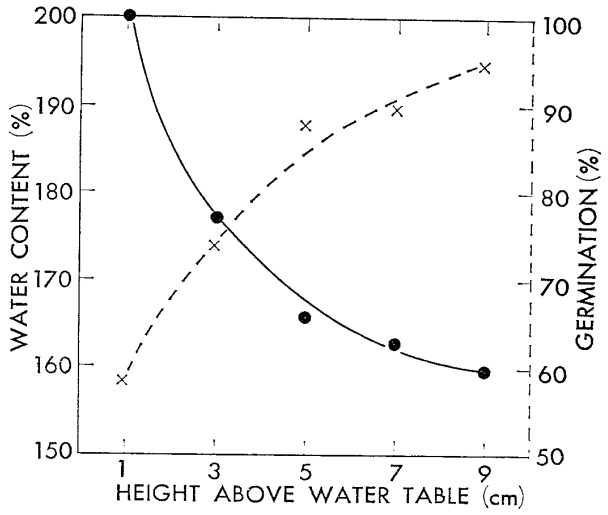

Fig. 1. Effects of height above water-table on germination (×- - -×) and on water content of substrate (●——●) (ladder technique). 20° in the light.

TABLE I

Effects of substrate hydration on germination (filter paper-layer technique)

Number of filter paper layers	Germination percentages[a]
1	47±4
2	61±6
3	64±5
4	68±5
5	77±0

[a] Final percentages ± standard error at 26° in the light.

certain level of hydration, inhibitory processes participate in the control of germination, and (b) that increasing levels of hydration result in a net advantage taken by the inhibitory processes over those which normally lead to germination. This could equally result from effects of hydration on the build-up of the inhibition as from its effects on the promotive processes. In order to study these possibilities, a kinetic analysis was made of the various partial processes, in an attempt to identify those whose rate was hydration dependent in such a way as to result in water-sensitivity.

Kinetic analysis of relationships of light response, temperature response and water sensitivity

One of the time-dependent phenomena in the germination of *H. incana* seeds was light sensitivity. The response to a short irradiation changed with time of incubation, rising to a peak and then declining to zero. The maximal response to a single short irradiation was always greater than to continuous irradiation. Though germination in all cases was improved with decrease in substrate hydration, the maximal response to a single short irradiation occurred at the same time at all levels of hydration, about 12 hr after start of incubation (Fig. 2).

Main effects of time of irradiation and of degree of hydration on germination are shown in Figs. 3 and 4, respectively.

The loss in responsiveness to light with increasing duration of dark-incubation was apparently not a result of dissipation of the photo-receptor. Thus, transfers from darkness even to continuous irradiation had a progressively lesser promotive effect as the initial darkness was extended longer than ten hours (Fig. 5). Loss of viability was also not involved, as responsiveness was completely regained after a 24–96 hr drying period (over granulated $CaCl_2$). These facts suggest that loss of responsiveness resulted from build-up of an unrelated block to the overall process of germination, which was removable by dehydration.

The results in Figs. 2–5 do not support the possibility that the promotive action of reduced hydration is a result of hastening the partial processes of germination which are concerned with changes in sensitivity to light.

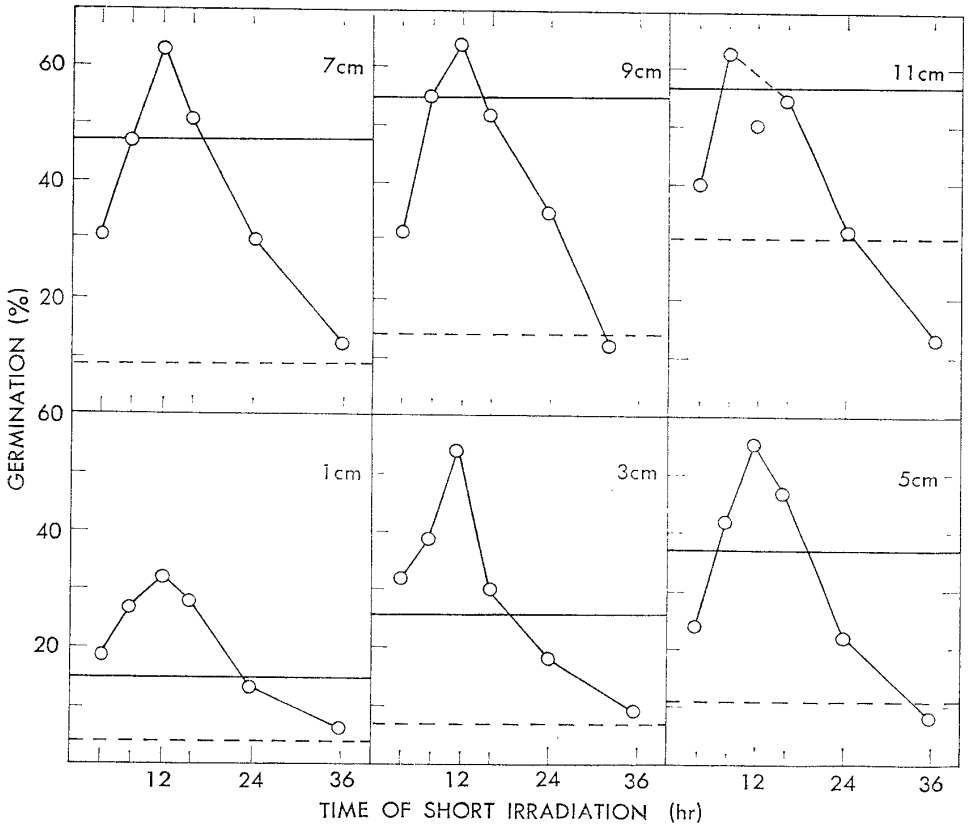

Fig. 2. Effects of substrate hydration on time of maximal sensitivity of germination to a single irradiation, at 20°. Solid and broken horizontal lines are controls in light and darkness throughout, respectively (ladder technique).

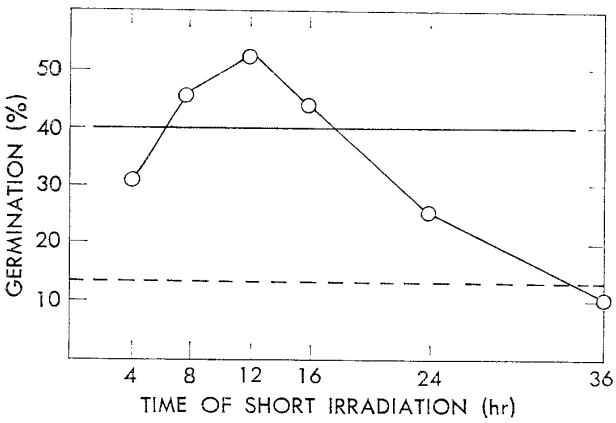

Fig. 3. Main effects of time of irradiation (combining all times of irradiation). Data of Fig. 2. Solid and broken horizontal lines are controls in light and darkness throughout, respectively.

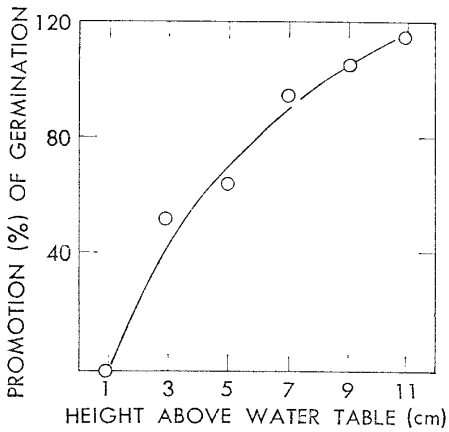

Fig. 4. Main effects of substrate hydration (combining all levels of hydration) on germination resulting from a single short irradiation (percent promotion over germination at 1 cm above water-table). Data of Fig. 2.

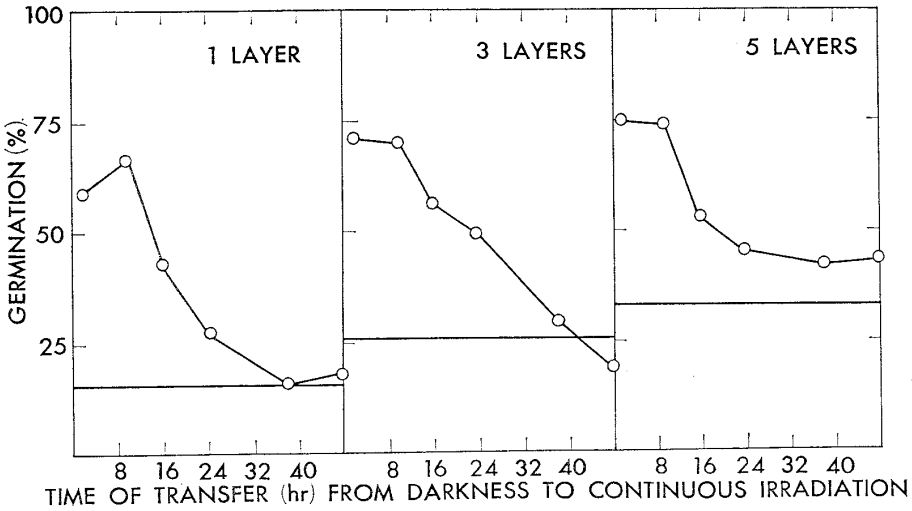

Fig. 5. Responsiveness to continuous irradiation as affected by duration of initial dark-incubation. Filter paper-layer technique, at 23°.

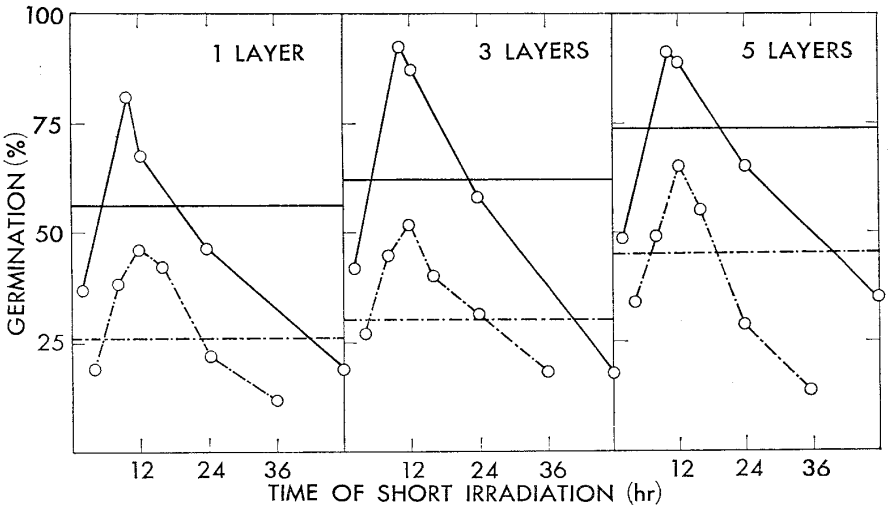

Fig. 6. Effects of temperature (solid lines, 26°; broken lines, 20°) and of substrate hydration (filter paper-layer technique) on germination under continuous irradiation (horizontal lines) or after a single short irradiation applied at different times during incubation. Dark controls at 26° and 20° were 18, 31 and 32 percent, and 4, 3 and 11 percent, on one, three and five layers of filter paper, respectively.

The effects of hydration were strongly modified by temperature. Differences in germination due to substrate hydration, which were quite marked at 20°, even when the seeds were irradiated at time of maximal sensitivity, became almost negligible at 26°, as a result of the combined promotion of temperature and irradiation (Fig. 6).

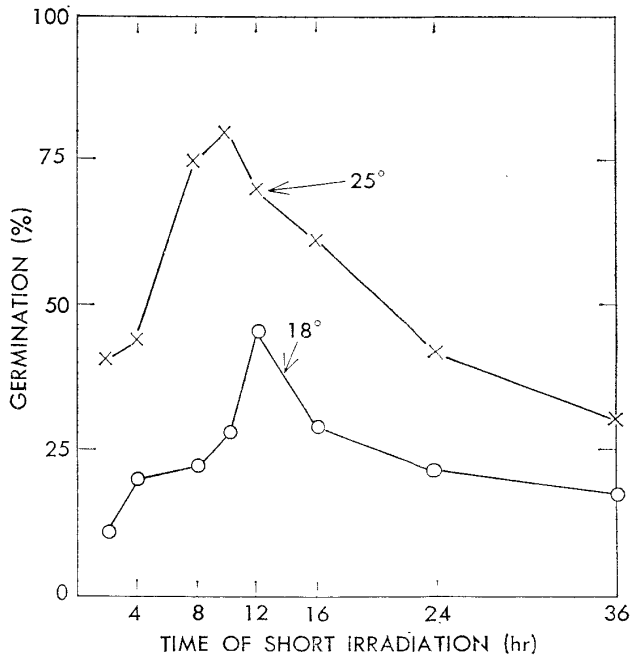

Fig. 7. Effects of temperature on activation of light-sensitivity.

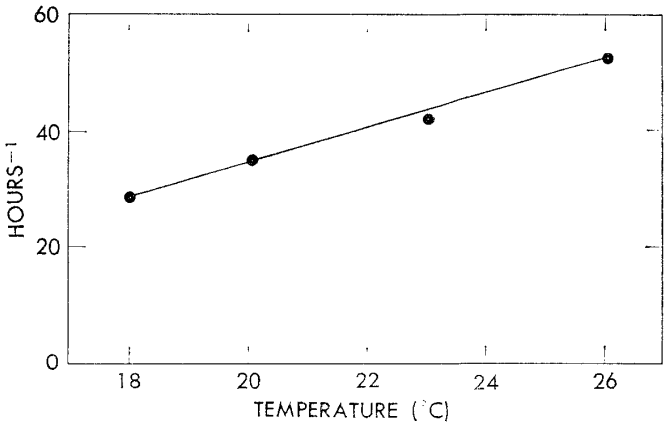

Fig. 8. Hastening of germination by temperature: Relationship of reciprocal of time to 50% of final germination in continuous irradiation.

TABLE II

Effects of temperature on time course of germination

Temperature	Germination[a]					
	Collection A			Collection B		
	S (hr)	R (%/hr)	P (%)	S (hr)	R (%/hr)	P (%)
20°	18	5.1	96±2	24	4.0	78±6
26°	13	3.5	93±3	17	4.0	91±3

[a] Start, S, rate, R, and final percentages, P, after KOLLER (*17*), under continuous irradiation.

Maximal sensitivity to light was attained earlier at 26° than at 20° (Fig. 7). Furthermore, the onset of germination in continuous irradiation was hastened by 5-7 hours when temperature was increased from 20° to 26° (Table II). This is also shown by the analysis in Fig. 8, in which the reciprocal of time from start of incubation to 50 percent of final germination is plotted against temperature (cf. *18*). The promotive effects of high temperature on germination were thus attributable to changes in the kinetics of germination, hastening the activation of the light-sensitive mechanism.

The acceleration of the kinetics of germination by high temperature may be related to its modifying influence on the degree of sensitivity to hydration. It may very well be that by hastening onset of germination, high temperature assists the seeds to 'escape' the inhibition which is gradually building up in the course of incubation. As both the build-up of the inhibition and the 'escape' are time dependent processes, conditions such as high temperature, which would hasten germination without affecting to the same extent the build-up of the inhibition, would increase the 'escape' from inhibition. Analogously, conditions such as low hydration, which may delay the build-up of the inhibition without affecting to the same extent the kinetics of germination, would also increase the 'escape' from inhibition.

Support for this possibility was sought in studies on the effects of transfers between conditions which may affect the rate of each process (germination on the one hand, and build-up of the inhibition on the other), independently of each other. The results of reciprocal transfers between a low and a high temperature (20° and 26°, respectively) at different times during incubation, are presented in Fig. 9. These results show that determination of final germination percentage by temperature occurred during the initial 12 hr after start of incubation, irrespective of the degree of hydration.

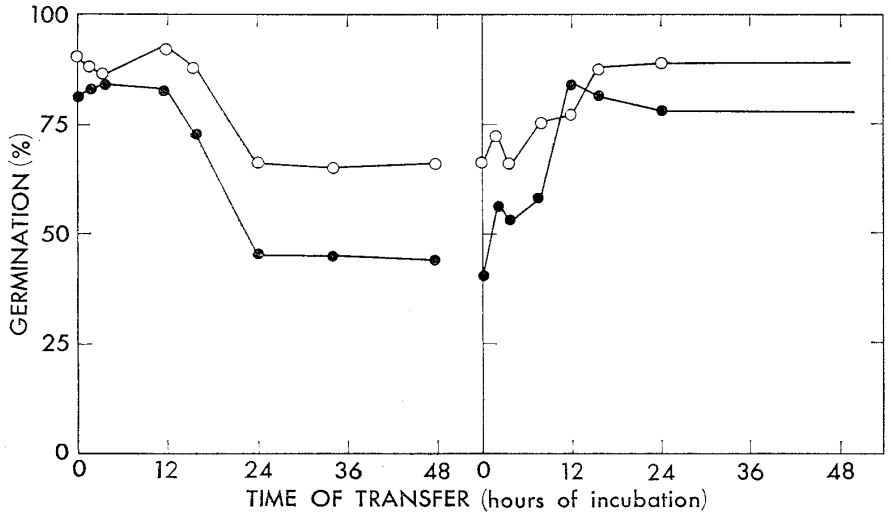

Fig. 9. Effects of a single transfer from 20° to 26° (left) and from 26° to 20° (right) in continuous irradiation, on one (●) and three (○) layers of filter paper moistened with 2 ml water.

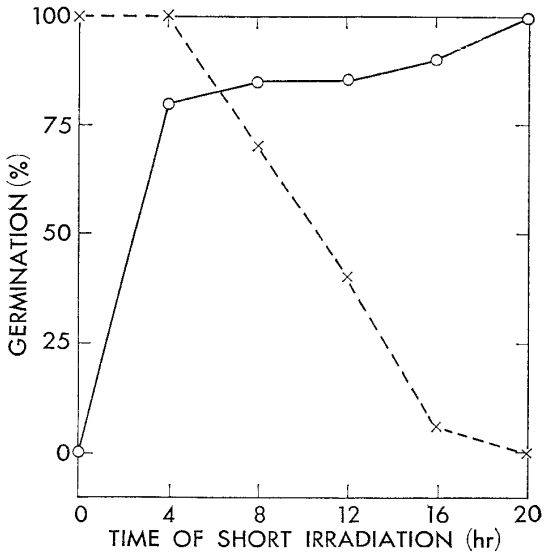

Fig. 10. Germination of 'hydration-sensitive' fraction of seed population at 20° (21% difference between germination on one and five filter-paper-layers) in continuous irradiation, when transferred at various times from low to high hydration (solid line) and vice versa (broken line)). Details in text.

Reciprocal transfers were also made between a low and a high hydration at different times during incubation. The transfer from low to high hydration was accomplished by adding water to dishes containing five layers of filter paper, and initially moistened with 2 ml water. The amount added (1.3 ml) was calculated to provide a hydration equivalent to that of a single layer moistened with 2 ml water. The reciprocal transfer was made by adding four layers of dry filter paper underneath a single layer, originally moistened by 2 ml water. The results in Fig. 10 show that most of the determination of final germination percentage by the lower degree of hydration occurred within four hours from start of imbibition. On the other hand, the determination of final germination by the higher degree of hydration did not occur during the first four hours of imbibition. After that initial period, final germination decreased progressively with increasing initial exposure to high hydration.

From these results it appears that the requirement for low hydration in these hydration-sensitive seeds is of relatively short duration, and occurs during the first four hours of incubation (low to high hydration transfer). When this initial requirement is denied to these hydration-sensitive seeds, they become irreversibly inhibited (except by drying). This irreversibility starts after the first four hours of incubation and is not reached simultaneously in all these seeds, being completed within 20 hr (i.e. over a period of 16 hr, high to low hydration transfer).

Chemical regulation of water-sensitivity

Other methods were tried to modify the sensitivity to hydration by selectively affecting the rate of one of the two opposing, time-dependent processes. It was found that a 200 ppm solution of GA promoted germination both in light and in darkness, at temperatures between 15° and 30°. This

TABLE III

Effects of GA on kinetics of germination, as influenced by level of substrate hydration

Number of filter paper layers	Incubation medium	Germination[a]		
		S (hr)	R (%/hr)	P (%)
1	H_2O	19.0	4.2	57±2[c]
1	GA[b]	16.5	3.7	95±3
3	H_2O	20.5	3.6	76±8[c]
3	GA[b]	18.0	5.4	96±2
5	H_2O	21.5	4.5	74±4[c]
5	GA[b]	19.0	5.6	97±1

[a] Start, S, rate R, and final percentages, P, at 20° under continuous irradiation.
[b] 200 ppm.
[c] The seeds that did not germinate were transferred to new PETRI-dishes on 1, 3 and 5 filter paper layers with GA and germinated to 96, 95 and 95 percent, respectively.

solution completely eliminated all differences in germination percentages at different levels of hydration, but also hastened the onset of germination in all cases, and induced higher final percentages than at the most favorable levels of hydration (Table III).

Seeds were incapable of germinating at 20° in light under a 75 mm layer of water, or a 200 ppm solution of GA. When, after four days, the level of the supernatant was reduced to 5 mm, seeds in GA were the only ones to germinate (100%). The seeds from the water treatment, which had not germinated within four days of reduction of the level of the supernatant, were divided into equal portions. One was transferred to a 200 ppm solution of GA, the other to water. Only the former germinated (100%).

In view of the modifying action of growth promoters, such as GA, on the sensitivity to high hydration, the effects of two growth retardants and of a germination inhibitor were tried, under conditions where such sensitivity was low (26°). The growth retardants Amo-1618 and CCC are both known to inhibit the biosynthesis of gibberellin-like substances in the plant (*19*, *20*). However, only the latter caused the typical sensitivity to hydration, while the former caused increasing inhibition at progressively lower levels of hydration (Tables IV, V). The germination inhibitor coumarin, which increased hydration sensitivity in *Avena fatua* (*11*), also caused the typical hydration-sensitivity in *H. incana* (Table V). This difference between the effects of Amo-1618 on the one hand, and CCC and coumarin on the other, is not clear at present.

Kinetics of water uptake by the seed as affected by the hydration of the substrate

The uptake of water during the imbibition phase (i.e; before appearance

TABLE IV

Effects of Amo-1618 on germination, as influenced by level of substrate hydration

Medium	Germination[a] on indicated number of filter paper layers		
	1	3	5
H_2O	92±2	94±2	96±2
Amo-1618[b]	70±4	54±4	43±8

[a] Final percentages, at 26° under continuous irradiation.
[b] 1,000 ppm.

TABLE V

Effects of CCC and coumarin on time till onset (S), rate (R) and final percentage of germination (P), as influenced by level of substrate hydration

Concentration (ppm)	Germination[a] on indicated number of filter paper layer								
	1			3			5		
	S (hr)	R (%/hr)	P (%)	S (hr)	R (%/hr)	P (%)	S (hr)	R (%/hr)	P (%)
CCC									
0	14	7.1	91	14	8.5	97	16	9.6	94
100	17	4.0	45	16	5.3	59	16	7.0	74
200	18	2.5	41	17	5.6	54	16	5.7	60
Coumarin									
0	15	14.5	98	16	13.1	92	17	8.0	96
50	21	1.5	81	24	1.4	89	24	1.2	86
100	30	1.9	58	38	1.8	80	29	<1	77

[a] At 26° under continuous irradiation.

of visible germination) was studied by kinetic analysis. Weighed seeds were left to imbibe at different heights above the water table (ladder technique). At intervals, seeds were carefully removed and weighed. As the seed coats of *H. incana* become mucilaginous when moistened, care was taken during weighing to interfere as little as possible with the water held by the mucilage. Each replicate consisted of 40 seeds, and four replicates were used for each measurement and then discarded. Water uptake at all heights above the water table approached saturation after 8–12 hours from the start of imbibition, at a level which was directly related to the height above the water table (Fig. 11). Since water uptake ceased at about the same time at all heights, but reached different saturation levels, it was assumed that at these levels the water potential of the seed was in equilibrium with that of the substrate. In this case, the rate at which hydration approaches equili-

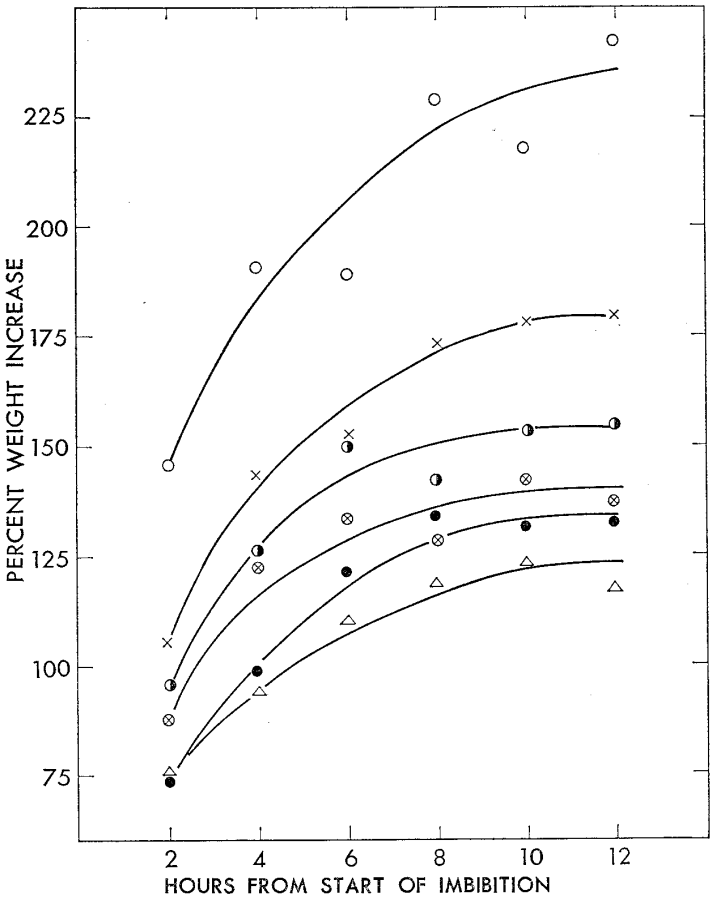

Fig. 11. Course of water uptake (percent of dry weight) in seeds at 26° at different heights above water-table (○, ×, ◑, ⊗, ●, △, representing 1, 3, 5, 7, 9 and 11 cm, respectively).

brium should be the same at all water potentials, implying that rate of uptake, k, at time t from start of imbibition is a function of the saturation water deficit at that moment, D_t (in percent), as follows: $D_t = D_o \cdot e^{-kt}$, where D_o is the initial saturation deficit (= 100%). In that case, log D should be linearly related to t. The regression of log D on t at six different levels above the water table was linear in all cases, with correlation coefficients, r, varying between 0.900 and 0.998. The coefficients of regression of log D against t varied somewhat at the different levels (Table VI), but as no pattern could be found for these variations, they may be assumed to have resulted from experimental errors. On this assumption, the regression of the mean log D was calculated as a function of t, giving the general equation $\log D = 2.0 - 0.17\,t$ ($r = 0.988$).

The same analysis was made of water uptake by the filter paper-layer technique, and similar results were obtained. The question whether the seed colloids are of low potential, or are small in amount can tentatively

TABLE VI

Linear regression parameters of log saturation water deficit (in percent) against time from start of imbibition, at different levels above water-table

Data of Fig. 11.

cm above water table	Regression coefficient	Correlation coefficients
1	−0.13	0.964
3	−0.19	0.980
5	−0.16	0.900
7	−0.15	0.978
9	−0.19	0.992
11	−0.17	0.998

be answered in favor of the second possibility. It seems that by far the greater proportion of the water uptake by imbibition is held in the mucilage. After 18 hr of submersion in water, water content of the entire seed was 200% of its dry weight, while after removal of its mucilage its water content was only 28%.

The results appear to have a direct bearing on the reactions of germination as affected by substrate hydration, since minute differences in water potential cause large differences in the amount of water held in the surrounding mucilage and thus affect the length of the diffusion pathway for the respiratory gases. In *Avena fatua*, submersion in water induced a state of dormancy, which was reversed by increased oxygen and was interpreted as resulting from interference by the water layers with diffusion of oxygen into the embryo (*10*). In further experiments water sensitivity of *H. incana* seeds was not modified by continuous presence of pure oxygen rather than in air. Thus, a five-fold increase in oxygen concentration could not overcome differences in lengths of diffusion pathways resulting from up to a twofold increase in hydration (cf. Fig. 11). However, this does not disprove the possibility that oxygen uptake is the limiting factor, because when the seeds are situated on the moist substrate, adhesion and surface tension attract much more water around them than is left on them when they are picked out for weighing. Thus, when the seeds are in contact with the wet substrate their overall capacity for gaseous exchange may be affected by the hydration of their mucilage coating to a much greater extent than is indicated by the actual thickness of the mucilage. The present interpretation may indicate that the action of gibberellin in modifying water-sensitivity results from reduction in oxygen requirements for germination, possibly by increasing the efficiency of respiration as a source of energy.

This work was supported by PL 480 grant FG-Is-115 from the United States Department of Agriculture. The writers wish to express their gratitude to Mr. E. Ziv for his cooperation and to the Negev Institute for Arid Zone Research, Be'er Sheva, for their hospitality and help.

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