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AN ANALYSIS OF "DARK-OSMOTIC INHIBITION" OF GERMINATION OF LETTUCE SEEDS [1]

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The lettuce seed has been chosen frequently for germination studies, because its behavior may be controlled by a large number of factors and thus should furnish clues for understanding the physiology of seed germination in general. Since lettuce seeds may be extremely sensitive to light, they have also been useful for obtaining information on the mechanism of light action characteristic of many photomorphogenic responses in plants.

The operational definition of germination employed in basic studies is emergence of the radicle from the seed or fruit. Thus one observes all or none events, and percent germination is used as a measure of the physiological activity of the processes that lead to germination.

For most lots of lettuce seeds, a temperature can be found which permits full germination in darkness (8, 9). As this temperature is raised to 30 or 32° C, germination usually decreases to zero (4, 8). By the regulation of temperature, seeds can be made to germinate at any desired level from zero to 100 %. The same can be done with diverse classes of chemical compounds (9). Many substances inhibit dark germination at temperatures optimal for germination on distilled water. Some of these, for instance some auxins and unsaturated lactones, are highly active at concentrations of 0.002 M or lower (9, 17); others that have no toxic or injurious effects at such relatively high concentrations were found by Weintraub to inhibit germination in darkness at 0.1 or 0.15 M (17). When non-germinating control seeds have been required to study the effects of promotive environmental or chemical factors, supra-optimal temperatures for germination (25 or 26° C) have usually been employed.

In all the above cases where germination is inhibited in darkness, white light may promote germination. For some of the cases it has been shown that a brief illumination with red light is also sufficient to release the inhibition (2, 9). When the red irradiation is followed by a brief far red irradiation, the action of red light is reversed (2).

The compounds that Weintraub (17) found inhibitory to germination of Grand Rapids lettuce seeds at concentrations of 0.1 or 0.15 M were all organic, and among them were sugars, sugar alcohols, amino acids, and ascorbic acid. They inhibited germination to a greater extent in darkness than in continuous red or white light varied over a 100-fold range in intensity. Weintraub suggested that the inhibitory action of solutions of these substances may at least in part be osmotic.

It will be shown here that this type of solute inhibition of lettuce seed germination found by Weintraub functions in 2 steps, the 1st osmotic and the 2nd non-osmotic. The effects of light and some other factors on solute inhibition of germination in darkness have also been investigated. The use of ordinarily innocuous compounds can be applied as a routine experimental procedure and may often be advantageous for reducing dark germination of lettuce seeds.

MATERIALS AND METHODS

SEEDS: The data reported in this paper were secured with 1 lot of Grand Rapids lettuce seed (*Lactuca sativa* L.). This lot was obtained in 1953 from Ferry-Morse Seed Co., Mountain View, California, and stored at 4° C. Its maximum germination in darkness at 21°C on water-saturated filter paper increased from 70 % at the beginning of this study to 95 % later on.

GERMINATION CONDITIONS: The standard experimental procedure will be described below. All departures from this procedure are noted in the descriptions of individual experiments.

To constitute 1 replicate of a treatment, 100 seeds were sprinkled onto two 5.5 cm disks of Whatman no. 1 filter paper which had been pressed into a 5 cm Petri dish and saturated with an experimental solution or distilled water. The dishes were placed into moist chambers wrapped with light-proof cloth and kept at 21± 1° C. Times allowed for germination were sufficient to permit maximum germination attainable under the particular experimental conditions. Sowing the seeds and all subsequent operations re-

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quiring vision were performed rapidly in dim green light from Westinghouse Ken-Rad green 10 or 25 W, 120 V lamps with the intensity sometimes reduced with a Variac. According to Borthwick et al (2), green light has relatively little effect on lettuce seed germination. Controls were always exposed to the green light when its use was necessary for experimental manipulations, and on the two occasions when such use of green light was tested, there was little or no effect on germination.

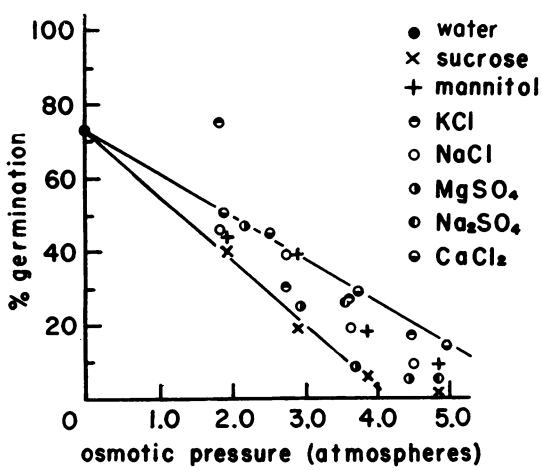
Red and Far Red Light: Red light was obtained by filtering the light from a bank of 8 Sylvania 48T12 Standard cool white fluorescent tubes through 2 sheets of DuPont red cellophane. Far red light was obtained by passing light from a bank of nine 300 or 500 W reflector photoflood lamps through Corning glass H.R. red purple ultra filters (no. 5874). These systems for securing red light relatively free of far red light and far red light free of red light were suggested by Borthwick et al (1). Temperature during irradiations was not controlled, since both the red and far red light reactions have a temperature coefficient near unity (3). Temperature shifts imposed by the irradiations were not extreme or long enough to markedly affect germination, according to data of Cohen (5). Energies of irradiances were not measured and are not considered to be important for the conclusions drawn from these experiments.

Chemicals: Sucrose, mannitol, and the salts used in this study were C.P. or reagent grade.

Characterization of "Dark-Osmotic Inhibition"

Relation of Osmotic Pressure To Solute Inhibition in Darkness: The following experiment was designed to relate inhibition of germination in darkness by solutions of 3 binary salts, 2 ternary salts, an organic metabolite and an organic non-metabolite to osmotic pressure over a range of 5 atmospheres. The osmotic pressures of solutions within this concentration range may depart considerably from ideality. For the salts used, it was necessary to make appropriate corrections. The osmotic coefficients and basic formula were obtained from Stokes (14) and from Robinson and Stokes (12, 13). Approximations made in the calculations were: 1) the ratio of the molecular weight of water to its partial molal volume was considered to equal unity; 2) molarity to equal molality; 3) osmotic coefficients obtained at 25° C to equal osmotic coefficients at 21° C; 4) since the osmotic coefficients of a substance vary with concentration, it was necessary to interpolate between values of osmotic coefficients recorded in the literature to obtain osmotic coefficients for concentrations used here; 5) osmotic coefficients for the sodium sulfate and the lowest magnesium sulfate concentrations were secured by limited extrapolations, and must be regarded with some degree of uncertainty. No useful values for osmotic coefficients of mannitol solutions were found and osmotic coefficients for sucrose were all between 1 and 1.017 for the concentrations employed. Consequently, osmotic pressures of the organic solutions were not corrected for departure from ideality.

Figure 1 shows the relationship of inhibition of germination in darkness to the osmotic pressure of the solutions used. Each point represents the average percent germination of 2 replicates, except for the water control which is based on 4 replicates. Within the range of 2 to 5 atmospheres, the trend is for germination to be suppressed in direct proportion to the osmotic pressure, regardless of the solute used. Therefore it is reasonable to conclude that the primary action of this solute inhibition is osmotic.



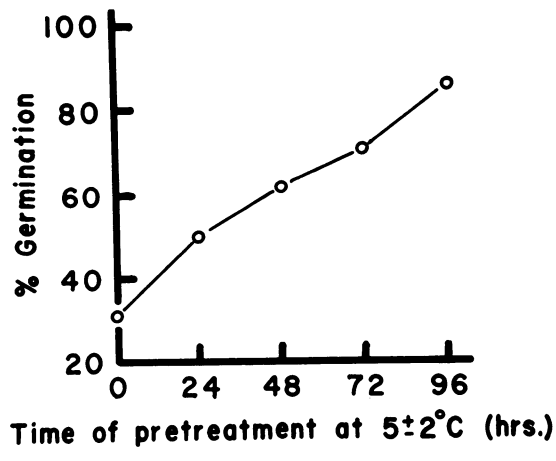


Fig. 1 (*left*). Effect of osmotic pressure on germination in darkness. The lines are drawn approximately through the points representing the effects of sucrose and calcium chloride solutions, respectively. The lines also describe the spread of the experimental points.

Fig. 2 (*right*). Negation of dark-osmotic inhibition by cold.

Sucrose is somewhat more inhibitory than solutions of the other compounds at equal osmotic pressures. This is a reproducible result and is also evident in the data of Weintraub (17). The reason for this is not known, but increase in osmotic pressures of the solutions by hydrolysis of sucrose is a possible explanation. Calcium chloride was somewhat less inhibitory than solutions of other compounds at equal osmotic pressures. Since the solutions were prepared without previously drying the solutes, the concentrations of calcium chloride, which is deliquescent, may have been overestimated. The apparent stimulatory effect of potassium chloride at the lowest concentration upon germination in darkness was not found in two further experiments. However, there was an indication that potassium chloride in low concentrations may not be quite as inhibitory as other compounds at the same osmotic concentrations.

EFFECTS OF LIGHT UPON SOLUTE INHIBITION IN DARKNESS: Six compounds at similar, but not equal, osmotic concentrations were employed to produce inhibition of germination in darkness. They were sodium, potassium and calcium chloride; sodium sulfate: sucrose; and mannitol. Table I shows that the solutions all inhibited germination in darkness to similar levels. Three minutes of red light given 5 hours after the beginning of imbibition were sufficient to prevent reduction of germination by the solutions. Far red light (2 minutes) supplied immediately after the red irradiation reversed the action of the latter. The effects of light in this experiment are identical with those observed by Borthwick et al. (2, 3) on lettuce seeds that were dark dormant for reasons other than inhibition by germination solutions.

The type of inhibition by solutions described above will be called "dark-osmotic inhibition" because firstly, the suppression of germination by solute correlates well with the osmotic pressure of the solution; secondly, the solute inhibition has little or no relation to the nature of the solute; and thirdly, at osmotic pressures lower than 5 atmospheres, the solutions do not reduce germination when seeds receive a sufficient irradiance of red or white light. The term "dark-osmotic inhibition" is intended to describe conveniently the treatment when one supplies darkness and an osmoticum and produces the end result of reduced germination. The term is inadequate for describing all the inhibitory processes occuring within the seed, as will be shown subsequently.

The quantitative response of different seed lots to dark-osmotic inhibition varies, and, as a particular seed lot ages in storage, its level of dark germination at 21° C may increase on osmotica as it does on water. The osmotic pressure required to suppress dark germination during this study increased steadily as dark germination on water increased.

SECONDARY INHIBITION INDUCED BY OSMOTIC INHIBITION: Since in the previous experiments only 2 days were allowed for germination, one might think that dark-osmotic inhibition merely delays germination, and if seeds were kept for long intervals, osmotic inhibition would disappear. To check this possibility, germination on 0.18 M mannitol was followed for a longer period of time. Part of the results of 2 time course experiments are given in table II. The 1st group of data shows that inhibition by 0.18 M manni-

TABLE II

DELAY AND PREVENTION OF GERMINATION BY DARK-OSMOTIC INHIBITION

TREATMENT	GERMINATION PERIOD (HRS)	PERCENT GERMINATION	
		REPLICATES	AVERAGE
Water	31	70, 72	71
0.18 M mannitol	31	0, 0	0
0.18 M mannitol	73	24, 28	26
Water	48	92, 88	90
0.18 M mannitol	48	30, 26	28
0.18 M mannitol	96	37, 21	29
0.18 M mannitol	144	30, 32	31

TABLE I

EFFECT OF BRIEF IRRADIATIONS WITH RED AND FAR RED LIGHT UPON INHIBITION OF GERMINATION BY SOLUTIONS IN DARKNESS

SOLUTION	MOLARITY	PERCENT GERMINATION				
		CONTINUOUS DARKNESS		RED LIGHT (3 MIN)*	RED LIGHT (3 MIN) PLUS FAR RED LIGHT (2 MIN)*	
		REPLICATES	AVERAGE	AVERAGE	REPLICATES	AVERAGE
Water	...	91, 97	94	**	71, 82	77
NaCl	0.075	49, 41	45	**	29, 32	31
KCl	0.075	53, 53	53	**	19, 29	24
$CaCl_2$	0.05	49, 38	44	**	34, 40	37
Na_2SO_4	0.05	57, 58	58	**	42, 36	39
Sucrose	0.15	31, 23	27	92	15, 20	18
Mannitol	0.15	40, 38	39	95	27, 27	27

* Administered 5 hours after the beginning of imbibition.

** Not counted; germination approximately 100 %.

tol does delay the germination of those seeds that manage to germinate on this medium. However, the 2nd group of data indicate that seeds which do not germinate on 0.18 M mannitol before 48 hours are permanently inhibited.

If dark-osmotic inhibition is a purely osmotic phenomenon, transferring the seeds from an osmoticum to water would release the inhibition. Results of such experiments with mannitol are given in table III, the four groups of data representing 4 separate experiments. Similar results were obtained with sodium chloride as the osmoticum. The 1st group of results in table III shows that seeds which had been kept on 0.22 M mannitol for 18 hours and then were transferred to water germinate at the same percentage as seeds kept on water throughout. Shifting the seeds in dim green light with a spatula from mannitol to mannitol did not abolish the effect of dark-osmotic inhibition. Thus, the transfer procedure as such was not responsible for the germination of seeds that were pre-treated with mannitol for 18 hours and then shifted to water. The 3 other groups of data in table III show, however, that when seeds are maintained on 0.18 M mannitol for 31 hours or longer, the inhibition of most of the seeds can no longer be released by transfer to water that would release a simple osmotic inhibition. Washing seeds with water at the time of the shift from mannitol to water for 4 or 8 hours does not result in increased germination.

One of the reasons why the term dark-osmotic inhibition is descriptive only of the treatment and not of the inhibitory seed processes can now be stated. When seeds are kept on a sufficiently concentrated solution to prevent germination for some period between 18 and 31 hours, osmotic inhibition is replaced or augmented by a secondary, non-osmotic inhibition in most of the seeds.

It was shown previously that red light given 5 hours after the beginning of imbibition nullifies the effect of dark-osmotic inhibition. In that case the red irradiation could either prevent primary osmotic inhibition or prevent or release the secondary inhibition by means of a "stored" effect. The following experiment was done to determine whether red light can reverse the secondary inhibition. Seeds were kept on 0.18 M mannitol for 92 hours, a period that assures the establishment of secondary inhibition. Half of them received a red irradiation (10 minutes) after 92 hours, and the other half remained in continuous darkness. Germination counts were made after an additional 48 hours. Table IV gives the results of this experiment which show that red light does release secondary inhibition. Whether red light operates on the primary osmotic inhibition will be discussed subsequently.

TABLE IV

RELEASE OF SECONDARY INHIBITION BY RED LIGHT

SOLUTION	RED LIGHT (10 MIN) *	PERCENT GERMINATION	
		REPLICATES	AVERAGE
0.18 M mannitol	—	28, 44	36
0.18 M mannitol	+	86, 90	88

* Administered 92 hours after the beginning of imbibition.

TIME OF THE OSMOTIC BLOCK IN THE GERMINATION PERIOD: The physiological events of seed germination are considered to be an orderly, sequential series of processes. To gain information about these processes by inhibition studies, it would be important to know at what point in the sequence an inhibitory factor functions. It is sometimes possible to determine whether inhibition blocks a process that occurs early or late in the total period of time required for germination. An experiment was designed to find out how long seeds may imbibe water and yet remain sensitive to dark-osmotic inhibition. Seeds were

TABLE III

EFFECTS OF TRANSFER OF SEEDS FROM MANNITOL TO WATER ON GERMINATION

INITIAL SOLUTION	TIME (HRS) OF SHIFT	SECOND SOLUTION	TOTAL GERMINATION PERIOD (HRS)	PERCENT GERMINATION	
				REPLICATES	AVERAGE
Water	18	Water	33.5	93, 92	93
0.22 M mannitol	18	0.22 M mannitol	46	21, 30	26
0.22 M mannitol	18	Water	46	89, 94	92
Water	...	...	52	89, 96	93
0.18 M mannitol	...	...	165	15, 20	18
0.18 M mannitol	52	0.18 M mannitol	165	33, 26	30
0.18 M mannitol	52	Water	165	50, 42	46
0.18 M mannitol	20	Water	68	71, 76	74
0.18 M mannitol	48	Water	96	48, 49	49
0.18 M mannitol	68	Water	116	41, 38	40
0.18 M mannitol	...	...	140	44, 28	36
Water	...	...	36	85, 85	85
0.18 M mannitol	31	0.18 M mannitol	67	25, 23	24
0.18 M mannitol	31	Water	67	52, 50	51

transferred from water to 0.3 M mannitol at 2-hour intervals from the time of sowing until 16 hours thereafter. The results indicated that after 16 hours of water uptake, germination of about half of the seeds could still be inhibited osmotically. To check this finding, the following experiment was carried out. Groups of seeds were allowed to take up water for 16 hours and then were shifted to water or 0.3 M mannitol. Other groups of seeds remained on water or 0.3 M mannitol throughout the germination period. Still another group of seeds was irradiated with red light (10 minutes) after 16 hours on water and then immediately transferred to 0.3 M mannitol. The results of this experiment are summarized in table V. Transferring the seeds was not itself inhibitory, since the germination of seeds shifted from water to water, or from water to mannitol following a red irradiation was as high as the germination of seeds that remained continuously on water in darkness. Nevertheless, transfer to mannitol strongly inhibited germination of unirradiated seeds even after they were kept for 16 hours on water.

TABLE V

INHIBITION OF GERMINATION BY 0.3 M MANNITOL AFTER 16 HOURS OF WATER UPTAKE BY SEEDS

INITIAL SOLUTION	RED LIGHT (10 MIN) BEFORE SHIFT	SHIFT AFTER 16 HOURS	PERCENT GERMINATION	
			REPLICATES	AVERAGE
0.3 M mannitol	—	...	11, 3	7
Water	—	...	95, 95	95
Water	—	to water	92, 88	90
Water	—	to 0.3 M mannitol	51, 47	49
Water	+	to 0.3 M mannitol	93, 98	96

The data in table V permit an additional conclusion. Red light can produce an effect when administered before dark-osmotic inhibition, for seeds that were irradiated while on water subsequently germinated 96 % on 0.3 M mannitol, while unirradiated seeds treated in the same manner germinated 49 %. Further experiments confirmed this result. Thus, the action of red light is not confined to the release of an established block in the germination processes. Red light can prevent the formation of a block, or it can open a pathway around a block before inhibition is established.

NEGATION OF DARK-OSMOTIC INHIBITION BY COLD TREATMENT: Borthwick and Robbins (4) found that the lettuce variety New York which normally does not germinate at 30° C will germinate well at that temperature if it first receives a period of low temperature in the imbibed condition. Low temperature pretreatment, during which no seeds germinate, is also capable of negating dark-osmotic inhibition of Grand Rapids lettuce seeds at 21° C. Seeds were sown on 0.18 M mannitol and stored for varying periods in darkness at 5± 2° C. They were then allowed to germinate for 48 hours at 21° C. In figure 2 the promotive and inductive effect of low temperature pretreatment upon subsequent germination at 21° C is shown. Each point represents the average percent germination of 2 replicates. The point representing zero time at low temperature was obtained from seeds remaining on 0.18 M mannitol at 21° C for 144 hours. This result indicates that not time itself, but time at low temperature, is responsible for the promotion of germination.

SEED SURGERY AND DARK-OSMOTIC INHIBITION: Inhibition of lettuce seed germination by supra-optimal temperatures can be prevented by surgery on the coats surrounding the embryo. Borthwick and Robbins (4) and Evenari and Neumann (10) found that removal of the pericarp and testa has no effect on germination, but surgery on the endosperm and on the integumentary membrane which adheres closely to the endosperm strongly promotes germination. Borthwick and Robbins removed the coats, while Evenari and Neumann "opened" the endosperm, an operation which they did not describe. Because of this remarkable influence of the external layers of the lettuce achene on germination, their effect on seeds subjected to dark-osmotic inhibition was studied.

Dry seeds were pierced with a sharp dissecting needle through the middle of their widest portion, so that all the fruit layers received 2 punctures, and both cotyledons were pierced. The criterion of germination in these experiments was the growth of any part of the embryo out of the pericarp and extension of the hypocotyl or radicle. Frequently, when surgery has been performed on seeds, the cotyledons emerge before the rootlet, and root growth may be severely curtailed. Borthwick and Robbins (4) observed the same unusual mode of germination by intact seeds under high oxygen tension at 30° C.

When seeds are punctured by the method described above, germination on 0.18 M mannitol in darkness is much greater than in intact seeds. Results from 3 separate experiments are summarized in table VI.

TABLE VI

PROMOTION OF GERMINATION BY PUNCTURING SEEDS BEFORE SUBJECTING THEM TO DARK-OSMOTIC INHIBITION*

DATE OF EXPERIMENT	SEEDS PUNCTURED	RED LIGHT (10 MIN)	PERCENT GERMINATION	
			REPLICATES	AVERAGE
1/31/57	—	—	20, 25	23
	+	—	63, 67	65
2/7/57	—	—	28, 27	28
	+	—	70, 83	77
4/13/57	—	—	26, 25	26
	+	—	73, 59	66
	+	+	94, 93	94

* All seeds on 0.18 M mannitol.

Thus, seed surgery can prevent dark-osmotic inhibition from functioning, as it can prevent reduction of germination by supra-optimal temperature. However, not all punctured seeds escape from responsiveness to dark-osmotic inhibition. Such punctured seeds will germinate if given sufficient red light, proving that they are viable. This indicates that there must be a subtle difference between an effective and an ineffective puncture or that seeds may or may not respond to the same kind of puncture.

Discussion

Weintraub's (17) findings concerning the inhibition of germination by organic compounds at concentrations of 0.1 and 0.15 M have been extended in this study. It was shown that inorganic salts also produce such an inhibition in darkness and that a brief red irradiance is as effective in preventing a reduction of germination by the solutions as continuous red or white light used by Weintraub. Furthermore Weintraub's suggestion that the inihibition of germination by 0.1 or 0.15 M solutions is at least in part osmotic was confirmed.

Evidence has been presented to show that dark-osmotic inhibition causes a delay in germination of those individuals that are able to germinate on osmotica and permanently prevents the germination of the others. Dark-osmotic inhibition was shown also to affect the seed in two distinct phases, the 1st a primary osmotic inhibition and the 2nd a non-osmotic inhibition that is established as a result of the 1st. This strongly suggests that dark-osmotic inhibition operates in the following manner: 1) The primary action is retardation of water uptake by a tissue crucially concerned with germination processes. This results in a relative reduction in the chemical potential or activity of water, limiting the rate of a germination process and delaying germination. 2) When germination is delayed for a certain period of time, either an inhibitory reaction that is proceeding all the time gains the upper hand or an inhibitory reaction is initiated resulting in the formation of a block that permanently prevents germination in darkness. Alternatively, a substance that is necessary for dark germination becomes exhausted or is not manufactured, if germination is delayed for a sufficiently long period.

Induction of a secondary inhibition or dormancy has been reported previously for a number of plant structures maintained in an environment where one or more factors did not permit growth. After the elapse of sufficient time, these structures were no longer capable of normal activity in an environment that formerly was favorable for growth. Popcov (11) and Davis (6, 7) made such observations on seeds other than lettuce, Thornton (15) on Easter lily bulbs, and Vegis (16) on buds of *Stratiotes aloides*.

Two experiments were performed to determine the time required by the seeds used in this study to germinate at 21° C on water in darkness. The earliest germination was noted after 18 to 20 hours, and germination approached 100 % after 30 to 32 hours. Twenty-six hours after sowing, approximately 50 % of the seeds had germinated. It was established also that about 50 % of the seeds were still responsive to dark-osmotic inhibition after 16 hours of water uptake, while about 50 % of the seeds had escaped from sensitivity to dark-osmotic inhibition during the 16 hours on water. Seeds that have progressed farther along the pathway to germination would be more likely to be past a block than seeds which lag behind. Though this cannot be proved, it is probable that seeds which ordinarily would germinate before 26 hours after sowing are not sensitive to dark-osmotic inhibition after 16 hours on water, while seeds that ordinarily would germinate later than 26 hours after planting remain sensitive to dark-osmotic inhibition until some time after 16 hours. This suggests that despite the advancement of early germination processes, seeds retain sensitivity to dark-osmotic inhibition up to 10 hours before root protrusion would ordinarily occur, and lose their sensitivity thereafter.

Determinations of the time course of water uptake by lettuce seeds showed that during the initial 20 hours mannitol retards water uptake (unpublished data). The retardation appeared equal in light and darkness, though light during that period subsequently caused germination of most of the seeds on mannitol. Therefore, the protective action of light in nullifying dark-osmotic inhibition is likely to be against the secondary, non-osmotic inhibition, though this is not a necessary conclusion, since overall water uptake by the achene may not be representative of uptake by the part directly concerned with germination.

Borthwick et al (2, 3) have shown that when seed lots of some lettuce varieties germinate well in darkness at a particular temperature (20 to 25° C), their germination at that temperature can be reduced by first allowing them to imbibe water and maintaining them at 30 to 35° C for 1 to 4 days. The secondary inhibition produced by dark-osmotic inhibition may be similar to or identical with that induced by high temperature. In both cases, seeds are prevented from germinating at the normal time by inhibitory factors, i.e., high osmotic pressure or high temperature, and they subsequently exhibit a dark dormancy under conditions that ordinarily are favorable for germination. The lower supra-optimal germination temperatures (e.g. 25° C) may also reduce germination by merely delaying germination for a sufficient period of time to permit a secondary inhibition to be established. Dark dormancy resulting from any of the 3 treatments being discussed can be released by a brief red irradiance and can be prevented or released by piercing or removing the coats surrounding a lettuce embryo. Furthermore, pretreatment of imbibed seeds with low temperature prevents subsequent reduction of germination by dark-osmotic inhibition or temperatures that are ordinarily supra-optimal.

Dark-osmotic inhibition should now be sufficiently well understood to be employed routinely as

an experimental tool for study of lettuce seed germination where elevation of temperature or use of toxic compounds is inappropriate or undesirable.

Summary

1. The germination of Grand Rapids lettuce seeds in darkness at 21° C is suppressed in approximately direct proportion to the osmotic pressures (within a limited range) of solutions used.

2. This reduction of germination by osmotica can be negated by irradiating the seeds with a small quantity of red light shortly after they begin to imbibe. Far red radiation supplied after red light reverses the action of red light.

3. Dark-osmotic inhibition delays the germination of some individuals and permanently inhibits that of others.

4. Transferring seeds from a solution that produces osmotic inhibition to water would release a pure osmotic inhibition. However, when germination of seeds has been inhibited osmotically for 30 hours or longer, most of the seeds do not germinate after transfer to water.

5. The secondary inhibition following primary osmotic inhibition can be released by red light and may be similar to dormancies of lettuce seeds produced by high temperature treatments.

6. The germination of seeds that have imbibed water for 16 hours can still be affected by dark-osmotic inhibition, indicating that some early events in germination can proceed with the seeds remaining sensitive to dark-osmotic inhibition.

7. Seeds irradiated with red light while on water show no reduction of germination when subsequently shifted onto an osmotically effective solution. Thus red light does not have to act on an established block, but can produce an effect that is "stored" and later prevents, releases, or circumvents a block in germination processes.

8. Low temperature treatment of seeds negates dark-osmotic inhibition. Puncturing seeds may also nullify dark-osmotic inhibition.

9. It is suggested that dark-osmotic inhibition and perhaps other inhibitory treatments function solely by delaying germination, and that during the delay a process occurs which permanently prevents germination in darkness.

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