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The Roles of Inorganic Nitrogen Salts in Maintaining Phytochrome- and Gibberellin A_3-Mediated Germination Control in Skotodormant Lettuce Seeds

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Abstract. Skotodormant seeds of *Lactuca sativa* Grand Rapids imbibed in darkness for 10 days (10-day DS) germinated poorly upon terminal treatment with red light (R) or gibberellin A_3 (GA_3). Inorganic nitrogen salts in the imbibition solutions reduced seed skotodormancy. Ten-day DS seeds, imbibed in 25 mM salt solutions followed by terminal R, germinated 99% if imbibed in NH_4NO_3, 70% if imbibed in KNO_3 or NH_4Cl, and 55% if imbibed in $NaNO_3$. Seeds imbibed in higher salt concentrations germinated fully upon terminal R treatment. Seeds imbibed in 25 mM NH_4Cl or in 50 mM NH_4NO_3 germinated completely upon GA_3 treatment. Osmotic effects of imbibition media accounted for only part of the effect, since seeds imbibed in 50 mM $CaCl_2$ or NaCl germinated poorly following R or GA_3 treatment. Seeds imbibed in 500 mM polyethylene glycol (PEG) 1000 or mannitol solutions for 10 days still exhibited skotodormancy. Treatments of R or GA_3 did not stimulate germination in seeds imbibed in mannitol, but germination was complete if seeds were given 1-h acid immersion plus a water rinse before the terminal R or GA_3 treatment. Seeds imbibed in 50–500 mM PEG during 10-day DS germinated significantly better in response to terminal R. Terminal GA_3 significantly improved germination only in seeds imbibed at 500 mM PEG. P_{fr} appeared to function in mannitol-imbibed seed only after an acid treatment. Seed exposure to inorganic nitrogen salts during the 10-day DS maintained seed sensitivity to terminal R or GA_3 treatment. The depth of seed skotodormancy was related to the availability of inorganic nitrogen and also involved the levels of P_{fr} or endogenous GA_3.

Key Words. Germination—Gibberellin—Imbibition—Lettuce—Nitrogen—Phytochrome—Skotodormant

Skotodormancy (secondary dormancy) is induced in water-imbibed lettuce seeds (*Lactuca sativa* L. cv. Grand Rapids) given a 5-min far red light (FR) irradiation 0.5 h after the onset of imbibition, followed by extended dark storage (DS) at 20°C (Hsiao 1992, Hsiao and Vidaver 1989, Hsiao et al. 1984, Vidaver and Hsiao 1975). Terminal treatments of red light (R) or growth promoters such as gibberellin A_3 (GA_3) do not normally initiate appreciable germination in skotodormant seeds. Less than 10% germination occurred in 10-day imbibed skotodormant seeds following a single acid treatment (1-h immersion in 1 M HCl + water rinse) or after treatment with red light (R) or GA_3. Seeds rendered skotodormant by 30-day DS at 20°C germinated 90% or higher following an acid treatment, plus an R or GA_3 treatment. Although some P_{fr} persists for up to 30 days DS, P_{fr} cannot induce germination in skotodormant seeds upon R or GA_3 stimulus unless seeds are given an acid treatment.

Skotodormancy induced by 4-day imbibition in a −1.2-MPa PEG solution at 15°C was markedly reduced in seeds exposed to 1 μM HCN or 10 mM salicylhydroxamic acid (SHAM) during osmotic treatment (Khan and Zeng 1985). Nitrate promotes the germination of a wide range of species (Goudey et al. 1988, Vincent and Roberts 1977). As is the case with phytochrome, the effect of nitrogen is best realized in combination with other factors such as temperature changes, nutritional status, light, or GA_3 (Roberts and Benjamin 1979, Saini et al. 1985, Williams 1983). This study examines the ability of terminal R and GA_3 treatments to induce the germination of skotodormant lettuce seeds imbibed for 10 days in inorganic nitrogen salts or osmotica.

Abbreviations: FR, far red; DS, dark storage; R, red; GA_3, gibberellin A_3; PEG, polyethylene glycol; SHAM, salicylhydroxamic acid; ANOVA, analysis of variance; GLM, general linear model; LSD, least squares difference; P_{fr}, far-red absorbing form of phytochrome.

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Materials and Methods

L. sativa cv. Grand Rapids seeds were from the same seed lot as those used in earlier studies (Hsiao and Vidaver 1989, Hsiao et al. 1984). They had been stored at −20°C and had maintained light sensitivity. Unless otherwise stated, each treatment had three replicates of 50 seeds with initial 5-min FR to suppress germination given 0.5 h after the onset of imbibition at 20°C. The light sources and filters for R and FR treatments were the same as in previous studies (Hsiao and Vidaver 1989, Hsiao et al. 1984). Except for germination counts, all operations were carried out in darkness or under dim green safelights (Hsiao and Vidaver 1971, Vidaver and Hsiao 1974).

Unless otherwise stated, all light treatments were for 5 min, and the concentration of GA_3 was 0.5 mM. Dry seeds were placed in 5.0-cm-diameter Petri dishes containing two 5.5-cm Whatman No. 1 filter paper discs. Imbibition at 20°C was carried out with 1.5 mL of distilled water, inorganic salt solutions, or osmotic chemicals. In two experiments seeds imbibed in the dark with 500 mM mannitol or PEG 1000 for 10 days were given 5 min of initial R rather than the usual FR treatment. The 10-day period of imbibition in darkness following initial light treatment is referred to as dark storage (DS). Any germinated seeds present after DS were discarded, and subsequent germination percentages were based on the remaining skotodormant seeds. These seeds were immersed for 1 h in 125 mL of distilled water to remove residual imbibition chemicals from the seed surface and were then transferred to fresh 5.0-cm Petri dishes for incubation in the dark at 20°C in the appropriate incubation medium and given light or GA_3 treatment. In the acid treatment of seeds a 1-h immersion in 3 mL of 1 M HCl (pH 0.1) was followed by 1-h rinse in 125 mL of distilled water. The control seeds received 1 h in 3 mL of distilled water followed by 1 h in 125 mL of distilled water. Incubation was as described above. Seeds were scored as germinated if the radicle had visibly penetrated the pericarp, and 90% germination was considered complete.

Electrolytic conductivity was determined as follows. Three 500-mg replicates (about 500 dry seeds) were each imbibed in a 9.0-cm Petri dish containing two 9.0-cm Whatman No. 1 filter paper discs and 5.0 mL of distilled water or of 50 mM KNO_3 or NH_4NO_3. After initial FR irradiation and 10-day DS, samples of 50 skotodormant seeds were taken from each of the three replicates. Each sample was flushed with 500 mL of distilled water, soaked in 10 mL of water for 1 h, and then transferred to 10 mL of water. Electroconductivity (μmho/50 seeds/10 mL) measurements were made of electrolytes released from the seeds into the 10 mL of soaking water and into the immersion water at 3, 6, and 18 h. A portable conductivity meter was used (Cole-Parmer, Niles, IL). Total electroconductivity was the sum of electroconductivity values after soaking seed for 1 h plus the value measured after the 18-h immersion.

All experiments were randomized complete block in design and were repeated at least once with similar results. Germination percentages were arc sine transformed and subjected to analysis of variance (ANOVA) by the general linear model procedure (GLM) of Statistical Analysis System (SAS Institute Inc., Cary, NC). A Fisher's protected LSD test was used for a two-way comparison of means at 5% probability.

Results

Seeds without Dark Storage

The FR-treated seeds without DS germinated completely at 20°C if given a terminal treatment of R or GA_3 (Table 1). The main effects of chemical, light, or GA_3 treatment and their interaction were all significantly different at $P < 0.01$. Imbibition concentrations of up to 50 mM KNO_3 or NH_4NO_3 had no effect on the germination of seeds given terminal R or GA_3 treatments, whereas germination of seeds in a 100 mM concentration of either chemical was inhibited by 20–50%. Seeds imbibed in the dark in 50 mM KNO_3 or NH_4NO_3 with no light treatment germinated even more poorly than seeds imbibed in water. Seeds incubated in 500 mM KNO_3 or NH_4NO_3 for up to 10 days did not germinate even following terminal R or GA_3 treatments.

Table 1. Effects of various concentrations of KNO_3 and NH_4NO_3 and subsequent treatment with FR, FR + R, or FR + GA_3 on the germination of lettuce seeds 48 h after the onset of imbibition.[a]

Chemicals and concentrations (mM)	Light or GA_3 treatments		
	FR	FR + R	FR + GA_3
H_2O	16 g[b]	99 a	98 a–c
KNO_3			
10	14 gh	98 a	99 a
25	4 hi	99 a–c	99 a–c
50	4 i	98 ab	99 a–c
100	4 i	60 ef	76 d
NH_4NO_3			
10	8 g–i	99 a	99 a–c
25	5 hi	94 bc	98 a–c
50	3 i	93 c	98 a–c
100	4 i	52 f	73 de

[a] Dry seeds were imbibed for 0.5 h in H_2O, KNO_3, or NH_4NO_3 with or without GA_3, given FR or FR + R, and germinated in darkness for 48 h. Values are percent germination.

[b] A 9 × 3 factorial ANOVA was performed. The effects of chemical, light or GA_3 treatment, and their interaction are all significantly different at $P < 0.01$. Values that are followed by the same letter are not significantly different at $P < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

Seeds with Dark Storage for 10 Days

Experiment 1. Seeds imbibed in 50 mM salt solutions during the 10-day DS, transferred to water, and then held in darkness for 48 h germinated poorly (Table 2). If given terminal R the seeds imbibed in 50 mM KNO_3, NH_4NO_3, or NH_4Cl germinated completely. Complete germination followed GA_3 treatment of the seeds imbibed in NH_4NO_3 or NH_4Cl but not with KNO_3. The main effects of chemicals, terminal treatments, and interactions were all significantly different at $P < 0.01$.

Experiment 2. Seeds were treated as in experiment 1 but imbibed in a wider range (10–100 mM) of nitrogen compounds during the 10-day DS and then given a terminal treatment with R or GA_3 (Table 3). The main effects of

Table 2. Effects of 50 mM KNO_3, NH_4NO_3, and NH_4Cl imbibed during 10-day DS and subsequent treatment with R or GA_3 on the germination of lettuce seeds.[a]

Chemicals during 10-day DS	Terminal treatment on day 10		
	D	R	GA_3
H_2O	2 de[b]	22 c	8 d
KNO_3	0 e	100 a	7 d
NH_4NO_3	0 e	99 ab	97 ab
NH_4Cl	0 e	93 b	100 a

[a] Dry seeds were imbibed for 0.5 h in H_2O or 50 mM KNO_3, NH_4NO_3, and NH_4Cl, given FR, imbibed for 10 days during DS, rinsed in H_2O for 1 h, and then transferred to fresh dishes with H_2O, GA_3, or given R, and germinated in darkness for 48 h; D, dark control. Values are percent germination.

[b] A 4 × 3 factorial ANOVA was performed. The effects of chemical, terminal treatment, and their interaction are all significantly different at $P < 0.01$. Values that are followed by the same letter are not significantly different at $P < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

Table 3. Effects of various concentrations of NO_3^- or Cl^- compounds imbibed during 10-day DS and subsequent treatment with R or GA_3 on the germination of lettuce seeds.[a]

Chemical during DS	Terminal treatment	Concentrations of chemical during DS (mM)			
		10	25	50	100
KNO_3	R	11 m–p[b]	74 c	100 a	100 a
	GA_3	1 rs	7 n–p	7 n–p	38 e–h
$NaNO_3$	R	27 h–j	55 de	100 a	100 a
	GA_3	1 rs	12 k–p	48 ef	50 ef
NH_4NO_3	R	25 h–k	96 ab	97 ab	99 ab
	GA_3	7 n–p	28 g–j	97 ab	98 ab
NH_4Cl	R	21 i–n	71 cd	95 b	98 ab
	GA_3	11 l–p	97 ab	100 a	99 ab
$CaCl_2$	R	16 j–o	29 g–j	44 e–g	34 f–i
	GA_3	0 s	5 p–s	4 p–s	7 n–p
NaCl	R	24 h–l	19 i–m	29 g–j	37 f–h
	GA_3	3 q–s	2 q–s	4 p–s	7 n–p

[a] Dry seeds were imbibed for 0.5 h in various concentrations of NO_3^- or Cl^- compounds, given FR, imbibed for 10 days during DS, rinsed in H_2O for 1 h, and then transferred to fresh dishes with H_2O, GA_3, or given R, and germinated in darkness for 48 h. Values are percent germination.

[b] A 6 × 2 × 4 factorial ANOVA was performed. Effects of chemical, terminal treatment, concentration, and their interactions are all significantly different at $P < 0.01$. Values that are followed by the same letter are not significantly different at $P < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

the chemicals, terminal treatments, concentrations, and their interactions were all significantly different at $P < 0.01$. Ten-day DS seeds imbibed in 10 mM salts remained unresponsive to either terminal R or to GA_3. The germination response to terminal R in 10-day DS seeds imbibed at concentrations of 25 mM differed with the salt. Seeds imbibed in 25 mM NH_4NO_3 during DS germinated completely following terminal R, but only 70% of the seeds imbibed in 25 mM KNO_3 or NH_4Cl. Terminal R treatment stimulated complete seed germination if DS occurred in a 50 mM concentration of any of the nitrogen salts (compare Table 3 with Table 2).

The effect of terminal GA_3 treatment on salt-imbibed seeds followed a different pattern. Skotodormancy was overcome by terminal GA_3 in those seeds exposed to 25 mM NH_4Cl. Skotodormant seeds imbibed in any of the ammonium salts at 50 mM responded to terminal GA_3 with complete germination. Terminal GA_3 stimulated only 50% germination in seeds imbibed in 50 mM $NaNO_3$, and GA_3 had no promotive effect on germination of seeds imbibed in KNO_3. Seeds imbibed in DS with 100 mM KNO_3 germinated no better than 38% following terminal GA_3 treatment. In other experiments (data not shown) imbibition of seeds during the 10-day DS with some other nitrogen salts also gave complete germination with subsequent treatment of R or GA_3 or both (treatment indicated in the bracket after each chemical): 100 mM $Pb(NO_3)_2$ [R]; 100 mM $Ca(NO_3)_2$ [R]; 25 mM urea $[CO(NH_2)_2]$ [R]; 50 or 100 mM choline chloride $[C_5H_{14}ClNO]$ [GA_3]; and 10 mM hydroxylamine hydrochloride $[(NH_3OH)Cl]$ [R, GA_3].

Experiment 3. If dry seeds were initially imbibed in 25 mM PEG 1000 and given FR followed by R or GA_3 they germinated completely after 48 h (data not shown). If the PEG concentration was increased to 50 or 100 mM PEG germination was 3% or less. In this experiment, the procedure was similar to that in experiment 2, but mannitol or PEG 1000 was substituted for the nitrogen salts (Table 4). The main effects of imbibition chemical, terminal treatment, concentration, and their interactions were all significantly different at $P < 0.01$. Terminal R promoted germination in seeds imbibed in 50–500 mM PEG, but terminal GA_3 was effective only in seeds imbibed in 500 mM PEG. Neither terminal R nor GA_3 had any promotive effect on the germination of seeds imbibed in the mannitol solutions.

Experiment 4. Seed samples were imbibed in 500 mM PEG, given initial R or FR 0.5 h after the onset of imbibition, and held for 10-day DS followed by terminal treatments of R, FR, GA_3, or FR + GA_3 (Table 5). The main effects of initial and terminal treatment and their interactions were all significantly different at $P < 0.01$. Given R at day 0, 10-day DS seeds germinated 41% in darkness in water, after removal of the PEG. They responded to terminal R, GA_3, or FR + GA_3 with 83–95% germination regardless of the initial light treatments. In a separate experiment, similar germination responses oc-

Table 4. Effects of various concentrations of mannitol and PEG 1000 imbibed during 10-day DS and subsequent treatment with R or GA_3 on the germination of lettuce seeds.[a]

Chemical during DS	Terminal treatment	Concentrations of chemical during DS (mM) 0	10	25	50	100	500
Mannitol	R	11 c–e[b]	2 f	20 cd	6 ef	9 c–e	21 c
	GA_3	7 d–f	6 ef	8 c–f	14 c–e	5 ef	11 c–e
PEG 100	R	8 c–f	8 c–e	21 cd	53 b	63 b	83 a
	GA_3	5 ef	5 ef	7 ef	8 c–f	7 d–f	84 a

[a] Dry seeds were imbibed for 0.5 h in various concentrations of mannitol or PEG 1000, given FR, imbibed for 10 days during DS, rinsed in H_2O for 1 h, and then transferred to fresh dishes with H_2O, GA_3, or given R, and germinated in darkness for 48 h. Values are percent germination.
[b] A 2 × 2 × 6 factorial ANOVA was performed. Effects of chemical, terminal treatment, concentration, and their interactions are all significantly different at $P < 0.01$. Values that are followed by the same letter are not significantly different at $P < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

Table 5. Effects of 500 mM PEG 1000 imbibed during 10-day DS and subsequent treatment with R or GA_3 on the germination of lettuce seeds.[a]

Initial light treatment at day 0	Terminal light or GA_3 treatment at day 10: D	R	FR	GA_3	FR + GA_3
FR	1 d[b]	83 b	5 d	84 b	89 ab
R	41 c	89 ab	2 d	95 a	88 ab

[a] Dry seeds were imbibed for 0.5 h in 500 mM PEG 1000, given R or FR, imbibed for 10 days during DS, rinsed in H_2O for 1 h, and then transferred to fresh dishes with H_2O, GA_3, or given R or FR, and germinated in darkness for 48 h; D, dark control. Values are percent germination.
[b] A 2 × 5 factorial ANOVA was performed. Effects of initial and terminal treatment and their interaction are all significantly different at $P < 0.01$. Values that are followed by the same letter are not significantly different at $P < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

Table 6. Effects of 500 mM mannitol imbibed during 10-day DS and 1-h acid immersion (H^+) and subsequent treatment with R or GA_3 on the germination of lettuce seeds.[a]

Initial treatment at day 0: Light	Chemical	Terminal H^+ immersion, light or GA_3 treatment at day 10: H^+	D	R	GA_3
FR	H_2O	+	12 fg[b]	99 ab	99 ab
		–	2 h	11 fg	7 g
FR	Mannitol	+	10 fg	96 bc	100 a
		–	0 h	20 f	16 fg
R	Mannitol	+	96 bc	100 a	100 a
		–	44 e	70 d	93 c

[a] Dry seeds were imbibed for 0.5 h in 500 mM mannitol, given R or FR, imbibed for 10 days during DS, given a 1-h H^+ treatment (+, with; –, without), rinsed in H_2O for 1 h, and then transferred to fresh dishes with H_2O, GA_3, or given R, and germinated in darkness for 48 h; D, dark control. Values are percent germination.
[b] A 3 × 2 × 3 factorial ANOVA was performed. Effects of initial treatment, H^+, terminal R or GA_3 treatment, and their interactions are all significantly different at $P < 0.01$. Values that are followed by the same letter are not significantly different at $P < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

curred in 10-day DS seeds that had been imbibed in 500 mM KNO_3 or NH_4NO_3 and given the same initial and terminal treatments (data not shown).

Experiment 5. Following initial R or FR and a 10-day DS imbibition in 500 mM mannitol, an acid treatment was given before terminal treatments (Table 6). The main effects of initial treatment, acid treatment, terminal R or FR treatment, and interactions were significantly different at $P < 0.01$. Without the terminal acid treatment, 10-day mannitol-imbibed seeds exposed to R at day 0 germinated 44% in darkness after removal of the mannitol, as with seeds imbibed in PEG (compare Table 6 with Table 5). Seeds given initial FR had limited response to R or GA_3, terminal GA_3 being more effective than terminal R in promoting germination (Table 6). Neither R nor GA_3 treatment was very effective in promoting germination in the 10-day mannitol-imbibed seeds given initial FR treatment, whereas the same initial and terminal treatments had been effective in the 10-day PEG-imbibed seeds (compare Table 6 with Table 5). Skotodormant seeds imbibed in 500 mM mannitol for 10 days and given an acid treatment germinated as completely following terminal R or GA_3 as did water-imbibed seeds without any DS (compare Table 6 with Table 1). Responses were similar to the 10-day DS PEG-imbibed seeds given R or GA_3 (compare Table 6 with Table 5). The 10-day DS mannitol-imbibed seeds with initial R treatment also gave complete germination with terminal acid treatment only (Table 6).

Table 7. Effects of 50 mM KNO_3 or NH_4NO_3 imbibed during 10-day DS on the electroconductivity (μmho/50 seeds/10 mL) of electrolytes released from skotodormant seeds into soaking water for 1 h and immersion water for 3–18 h.[a]

Chemical	Soaking water	Hours in immersion water 3	6	18	Total[b]
H_2O	26.6 c[c]	30.6 b	43.6 c	587.7 c	614.3 c
KNO_3	1253.2 a	558.4 a	852.0 a	1193.0 a	2446.2 a
NH_4NO_3	1001.1 b	41.2 b	653.1 b	829.3b	1830.4 b

[a] Details of procedures are given in the Materials and Methods section.
[b] Total electroconductivity = soaking water + 18-h immersion.
[c] An ANOVA was performed. Effects of chemicals are significantly different at $P < 0.01$. Values in each column which are followed by the same letter are not significantly different at $P < 0.05$ as determined by Fisher's protected LSD test.

Experiment 6. The electroconductivity (μmho/50 seeds/10 mL) of electrolytes released from the seeds after 10-day DS was determined for the 1-h water soak and for the immersion in water for 3, 6, and 18 h (Table 7). The electroconductivity of soaking or immersion water from seeds originally imbibed in water was significantly lower than that from seeds imbibed in 50 mM KNO_3 or NH_4NO_3 ($P < 0.01$), with higher electrical conductivity ($P < 0.01$) present in the washes from KNO_3-imbibed seeds.

Discussion

Hsiao and Vidaver (1989) suggested that the initiation of skotodormancy in seeds might result from a buildup of inhibitors within the system, a decrease in endogenous GA activity, a decrease in levels of P_{fr}, or several of these factors occurring concurrently. They concluded that skotodormancy was the single most important factor determining whether an external stimulus such as R or FR acting on the P_{fr} already existing in skotodormant seeds would be able to affect germination.

In the present study, during the 10-day DS process, the FR-treated seeds were imbibed in a variety of inorganic nitrogen salts or osmotic compounds. The seeds were then rinsed and immersed in water for 1 h to remove any residual chemicals on seed surfaces before transferring them to fresh dishes for terminal R or GA_3 treatment. Thus the effectiveness on germination of the interactions between terminal R or GA_3 treatment and the chemicals imbibed during the 10-day DS had to be due to either changes brought about by nitrogen-containing compounds or to osmotic changes. FR-treated seeds incubated in water with no other treatment were unable to germinate. However, exposure to terminal R treatment induced complete germination in seeds previously imbibed in 50 or 100 mM KNO_3, NH_4NO_3, or NH_4Cl (Tables 2 and 3). Terminal GA_3 induced complete germination after 48 h only in seeds imbibed in the presence of ammonium ions (Tables 2 and 3). Development of skotodormancy was impeded when DS was carried out in 500 mM PEG or mannitol, PEG being more effective than mannitol (Tables 4–6). This difference may be due to the greater toxicity of mannitol or the slower movement of PEG through cell membranes (Greenway 1970).

In DS seeds imbibed in 500 mM PEG or mannitol, an initial R pretreatment reduced the degree of skotodormancy, an effect possibly associated with dark reversion of P_{fr}. Terminal GA_3 caused higher germination in such seeds than did terminal R (Table 6), indicating synergism between GA_3 and R in breaking skotodormancy. The 10-day DS seeds imbibed in 50 mM KNO_3 or NH_4NO_3 yielded higher electrical conductivity in the soaking or immersion water than did the 10-day DS seeds imbibed in water (Table 7). The chemicals in the imbibition medium possibly affected levels of certain seed substrates that were required to maintain sensitivity to terminal R or GA_3. It is concluded that P_{fr} and GA_3 can overcome skotodormancy in 10-day DS FR-treated seeds imbibed in inorganic nitrogen salts at concentrations of 50 mM or higher or in 500 mM PEG. The kind and concentration of salts or osmotica present during DS influenced seed sensitivity to terminal R or GA_3 treatments (Tables 2 and 6).

The deeper dormancy of skotodormant over primarily dormant lettuce seeds may be due to reduced levels of preexisting P_{fr} and endogenous GA_3 in skotodormant seeds (Hsiao et al. 1984). Such seeds normally require both systems to induce complete germination (Hsiao and Vidaver 1989, Hsiao et al. 1984, Speer et al. 1974, Vidaver and Hsiao 1974), whereas either one of the phytochrome or GA promotion systems is sufficient to terminate primary dormancy (Table 1). The skotodormancy of 10-day DS seeds imbibed in inorganic nitrogen salts (Tables 2 and 3) or in PEG (Tables 4 and 5) can be overcome by either terminal R or GA_3. Note also that acid treatment restored the effectiveness of the phytochrome- and GA-promoting systems in skotodormant seeds imbibed in water or mannitol (Table 6). The results suggest that the imbibing of osmotically active compounds during DS may influence the loss of certain constituents from the seeds or may maintain at higher levels both the phytochrome and the GA promotion systems.

Inorganic nitrogen salts imbibed during the 10-day DS period were more effective in lessening skotodormancy than a number of other salts (data not shown). The interaction of nitrate and light in improving germination of many seeds is well known (Evenari 1965, Hilhorst et al. 1986). In *Sisymbrium officinale* the germination response appears to be determined by the product of the concentrations of P_{fr} and nitrate, indicating a multiplicative interaction (Hilhorst et al. 1986, Hilhorst and Karssen 1988). Decline of germination in water of *S. officinale* seeds was correlated with the rate of KNO_3 leaching out

of the seeds (Hilhorst 1990) leading to the suggestion that secondary dormancy was induced by loss of nitrate from seeds. In our study the germination of the FR-treated Grand Rapids lettuce seeds without DS was reduced significantly by imbibition in either KNO_3 or in NH_4NO_3 at a 25 mM or higher concentration, but this reduction was alleviated by terminal R or GA_3 in seeds imbibed in concentrations of either chemical up to 50 mM (Table 1).

Nitrate plays a vital osmotic role in the regulation of plant growth and development (Blom-Zandstra and Lampe 1985, Steingrover et al. 1986). McIntyre et al. (1996), using an *Avena fatua* model, have shown that the seed coat of the intact caryopsis prevents the uptake of water by the embryo in the amount required for induction of germination. They postulated that nitrate, when supplied to the dormant caryopsis, stimulated germination by accumulating in the embryo, thereby rendering its osmotic potential more negative and promoting the uptake of water. The reduced potential may be generated by the nitrate itself or by the combined effect of the nitrate and other osmotica such as amino-N produced by nitrogen reduction. Amino acids are implicated in the osmotic regulation of plant growth (Takeba 1980). Skotodormant seeds imbibed in NH_4NO_3 or in NH_4Cl germinated more completely in response to terminal R and especially to terminal GA_3 than did seeds imbibed in KNO_3 or $NaNO_3$ (Table 3). The small positively charged ammonium ions appear to penetrate readily to the embryo and may well exert a nutritional effect as well as an osmotic effect on water uptake in the case of nitrate imbibition (McIntyre and Cessna 1991).

There is still no agreement as to the non-osmotic mechanisms by which nitrogen-containing compounds induce germination in dormant seeds. Nitrates and nitrites may promote germination by acting as electron acceptors, thus increasing respiration (Adkins et al. 1984). The hypothesis is consistent with the increase in respiration rate which precedes nitrate-induced germination but is not supported by evidence that KNO_3 can promote germination without being metabolically reduced (Hilhorst 1990).

The phytochrome and GA_3 systems of numerous species are known to be influenced by the presence of inorganic nitrogen salts. Both R and GA_3 treatments successfully overcame skotodormancy in 10-day DS seeds incubated in ammonium salts, but GA_3 was much less effective when incubation was in nitrate salts (Tables 2 and 3). The reason for such differences may involve the formation of active GA-receptor complexes plus phenomena such as affinity, response capacity, and uptake efficiency (Firn 1986, Trewavas 1991). Differential loss of electrolytes during DS may also influence the ability of 10-day DS seeds to respond to GA_3 (Table 7).

This study is the first one to include inorganic nitrogen salts during the 10-day DS incubation to clarify the mechanisms involved in induction and breakage of skotodormancy in lettuce seeds. The study confirmed the findings of Hsiao (1992), Hsiao and Vidaver (1989), and Hsiao et al. (1984) that the capacity for R or FR light sensing can be stored in fully imbibed secondarily dormant Grand Rapids lettuce seeds, but remains inactive until the seeds are acidified and incubated in water. Earlier work (Hsiao et al. 1984) reported that acid treatment increased the sensitivity of skotodormant seeds to subsequent R or GA_3 treatment and also increased amino acid leaching into immersion solutions. The effect of acidification appeared to be due to a scarification-like effect on seed envelope membranes. Mayer (1986) suggested that the cellular membrane was the site at which light and temperature were sensed with changes in the membrane determining the seed response to its environment. Thus, the cycling of phytochrome with repetitive R pretreatment may have decreased the level of skotodormancy to the extent that an acid treatment made it possible for the P_{fr} already present in the seeds to function when seeds were subsequently incubated in water (Hsiao 1992). Solute leakage from seeds has been used to estimate the persistence of functional membrane and cell membrane damage (Woodstock and Tao 1981). By this standard, 10-day DS treatments in nitrogen salts significantly increased electrolyte loss from seeds (Table 7). Even after the initial water flush, conductivity readings in the 1-h soak treatment increased 50-fold over the value for seeds with 10-day DS in water. The KNO_3 imbibition produced significantly greater electrolyte loss than NH_4NO_3 imbibition. Hsiao and Vidaver (1989) formulated a model to interpret the effects of R and growth promoters in breaking primary and secondary dormancy in light-sensitive seeds of Grand Rapids lettuce. The model assumes that (1) whether or not germination occurs is dependent on the sum of the activities of the phytochrome and GA systems (designated X and Y, respectively); with sufficient activity either system alone can promote full germination; (2) the promotive capacities of both systems decrease with time during DS due to the conversion of $X \rightarrow X'$ and $Y \rightarrow Y'$; skotodormant seeds are those in which neither system alone can promote germination; (3) acid immersion fully restores the activities of both phytochrome and GA systems, probably by manipulating the conversion of $X' \rightarrow X$ and $Y' \rightarrow Y$ as essential prerequisites for promoting germination in skotodormant lettuce seeds; and (4) certain growth promoters have a positive but weak effect on the GA system and no effect on the phytochrome system. The results of the present study conform to this model in assumptions (1)–(3) in seeds imbibed in water during DS. We suggest that the inorganic nitrogen salts or PEG present during the 10-day DS block the conversion of $X \rightarrow X'$ and $Y \rightarrow Y'$ so that either system alone can still cause complete germination in seeds so incubated. Thus the germination response is determined by the product of

the concentrations of P_{fr} and GA_3. Each of these systems is sensitive to the presence of inorganic nitrogen salts, especially nitrate or ammonium ions, indicating a multiplicative interaction.

Nitrate concentration in soils is influenced by many factors such as soil type, moisture, temperature, and agricultural practices (Fenner 1985). Seed nitrate levels respond in passive fashion to external NO_3^- levels whether seeds are incubated on filter paper or in soil (Goudey et al. 1988). Changes in the soil or seed NO_3^- or NH_4^+ content might partially account for the sporadic release from dormancy of buried seeds during a growing season. In a telenomic view the nitrate/ammonium dependence of skotodormant seeds allows them to sense the nitrogen status of the ecosystem before germination starts, and thus this capacity may be considered part of the survival strategy for certain plants.

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