Plant Physiol. (1990)94, 1096-1102
0032-0889/90/94/1096/07/$01.00/0

Received for publication April 6, 1990
Accepted July 12, 1990

Dose-Response Analysis of Factors Involved in Germination and Secondary Dormancy of Seeds of *Sisymbrium officinale*

II. Nitrate

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ABSTRACT

The role of nitrate as a promoter of germination of *Sisymbrium officinale* seeds was examined in optimal light conditions. It was shown that the requirement for nitrate was absolute. This was true for all seed lots used. The probit of germination in water was log-linearly related to the level of endogenous nitrate. Preincubation at 15°C resulted in an immediate decrease in germination, whereas in 25 millimolar KNO_3 the decrease was delayed. The decline of germination in water was strongly correlated with the rate at which nitrate leached from the seeds. The germination response to a range of KNO_3 concentrations was followed during preincubation at 24-hour intervals. During the entire 264-hour preincubation period increasingly higher nitrate concentrations were required to maintain a response. This resulted in a right-hand shift of the dose-response curve parallel to the *x* axis. After 120 hours the high maximum germination level started to decline. The dose-response curves could be simulated by an equation from the receptor-occupancy theory. It is proposed that induction of secondary dormancy is a result of a decrease of the number of nitrate receptors. After 24 and 48 hours of preincubation, the nitrate-response curves were biphasic. The biphasic character could be related to the level of endogenous nitrate and to a differential requirement for nitrate of two fractions of the seed population. Similarities with the behavior of fluence-response curves after prolonged dark incubation led to the hypothesis that phytochrome and nitrate share the same site of action.

Nitrate stimulates the seed germination of a broad range of wild species (16). Several models have been proposed for its role as a dormancy breaking agent. In these models reduction of nitrate and nitrite is required to reoxidize $NADPH_2$ to NADP, either directly by the specific reductases (15, 16) or indirectly by the inhibition of catalase action and thus initiating a hypothetical peroxidase-regulated chain of reactions leading to reoxidation of $NADPH_2$ (4). It was suggested that the stimulation of the pentose phosphate pathway by NADP was essential for the relief of dormancy. However, it has been shown that these models are not valid for seeds of *Sisymbrium officinale* (8). Inhibitors of nitrate reductase activity did not influence the nitrate-stimulated germination. Moreover, nitrate levels in the seeds did not decrease during induction of germination by red light. Hence, a direct regulatory role for the nitrate ion was proposed.

In several species nitrate and light may act synergistically on germination (9, 13). Detailed studies of the light- and nitrate-stimulated germination of *S. officinale* seeds revealed a very close interaction between both factors. Each was only active in the presence of the other. Nitrate steepened the fluence-response curve and it was concluded that nitrate might be regarded as a cofactor for phytochrome action (7). In a study of the fluence-response during prolonged dark incubation it was hypothesized that nitrate had an effect on the phytochrome-receptor X, in that nitrate enhanced the number of active receptors and/or inhibited the inactivation of the receptors (5).

The aim of the present study was to monitor the response to nitrate at saturating fluences and to compare the nitrate-response curves with the fluence-responses obtained under similar conditions but at saturating nitrate concentrations.

MATERIALS AND METHODS

Seeds

Seeds of *Sisymbrium officinale* (hedge mustard) were collected from plants growing in the vicinity of the laboratory. The seed lots were from different years (1985, 1986, and 1987) and from different natural habitats, with the exception of the seed lot with the highest nitrate content. This batch was obtained from plants that were grown on hydroculture with nitrate added to the growth medium (the seeds were a gift from H. J. Bouwmeester). For most experiments a seed lot from 1985 was used. This lot was the same as used before (5). Experiments were performed in 1988. Seeds were cleaned and stored dry at 4°C in plastic containers. No changes in dormancy or viability were observed over the experimental period.

Germination Experiments

Duplicates of 75 seeds were sown in 50-mm Petri dishes on one layer of filter paper (Schleicher and Schull No. 595) and

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moistened with 1.5 mL of water or nitrate solution. After various preincubation periods at 15°C, the seeds were irradiated with R[1] and transferred to 24°C. Germination was counted after 3 d in the dark. Broad-band R (620–700 nm) came from six red fluorescent tubes (Philips TL 20W/17) filtered by 3-mm red Plexiglas (Red 501; Röhm and Haas, Darmstadt, Federal Republic of Germany). Fluence at seed level was $1.5^* 10^{-3}$ mol m^{-2} and the irradiation time was 10 min. All seed handling was done in dim green light from one green fluorescent tube (Philips TL 40W/17) filtered by two layers of yellow (No. 46) and two layers of blue (No. 62) Cinemoid filters (Strand Electric, London, United Kingdom). Experiments were repeated at least once with qualitatively similar results.

Calculations

Nitrate-response curves were calculated by means of weighted regression analysis of the data points in a log-dose probit diagram (2).

Nitrate Measurements

Seed lots of 50 mg, either dry or preincubated and rinsed with 100 mL of distilled water, were transferred to a plastic vial on ice. After freezing and thawing, the seeds were homogenized with a stainless steel rod in 1.5 mL of distilled water. In one experiment extractions were carried out with 0.2% Triton X-100; 50 mM KH_2PO_4, pH 3.7; or 0.2 M NaCl. In addition, extraction was carried out at an elevated temperature (10 min, 100°C). The homogenate was kept on ice for 1 h and centrifuged for 15 min at 16,000*g*. Of the supernatant 500 μL was put on top of a 3-mm layer of Lichroprep RP-8 (particle size 25–40 μm; Merck, Darmstadt, Federal Republic of Germany) in a 2-mL plastic column supported by a MA 25 prefilter (Millipore, Etten-Leur, The Netherlands). The column had been prewashed with three portions of 500 μL methanol followed by 200 μL of the supernatant. Of the filtrate 20 μL was injected into a Model 3500B HPLC system (Spectra Physics, Santa Clara, CA) equipped with a Model 770 spectrophotometric detector (Spectra Physics) set at a wavelength of 210 nm, and a Model C-R1B integrator (Shimadzu, Kyoto, Japan). The column was a stainless steel Lichrosorb 10 NH2 column (Chrompack, Middelburg, The Netherlands). The mobile phase was 25 mM KH_2PO_4, pH 3.7. Nitrate levels were calculated on the basis of the linear relationship between concentration and peak height of pure standards, which received similar treatments as the test samples. Recovery of nitrate added to the samples was generally higher than 95%. Nitrate levels in the incubation medium were measured by direct injection into the HPLC system. Measurements were performed on at least four independent replications. Nitrate levels were expressed as nmol g^{-1} dry weight. This could be done since no growth occurred during dark preincubations. Furthermore, from oxygen-uptake experiments the loss of dry weight as a result of respiration was calculated to be maximally 0.05%, assuming a respiratory quotient of 1 (M. P. M. Derkx, personal communication).

[1] Abbreviations: R, red light (660 nm); LNR, low nitrate response; VLNR, very low nitrate response; *B*, slope of log-dose probit line.

Nitrate Measurements in Dry Seed Parts

To determine nitrate in dry seed parts, dry seeds were treated with abrasive paper of a very fine grade. This treatment caused the seed coats to break. The cotyledons were separated from the rest of the embryo. All collected seed parts were weighed and extracted for nitrate determination as described above. About 50 mg of seeds were used.

RESULTS

Requirement for Endogenous Nitrate

To determine whether the requirement of germination for nitrate was absolute, the nitrate contents of seeds from five different seed lots were measured. Nitrate contents were measured in the dry seeds and after 48 h of preincubation in water at 15°C. Seeds were irradiated after 2 and 48 h, respectively, and germinated at 24°C in the dark. A linear relationship was obtained between dry seed nitrate levels and the probit of germination after 2 h at 15°C (Fig. 1). This suggests that nitrate levels are log-normally distributed over the collection of seed lots used here, around a value for half-maximal germination. After 48 h at 15°C nitrate levels were considerably lower, due to leaching of nitrate into the medium. However, a similar linear relationship was maintained. At nitrate levels below 100 nmol g^{-1}, germination percentage was close to zero while the germination response saturated at nitrate levels around 10,000 nmol g^{-1}.

Of a seed lot with a nitrate content of 1900 nmol g^{-1}, the distribution of nitrate over different (dry) seed parts was determined (Table I). The relative nitrate content was highest in the axes plus radicle part. This part contributed only 10% to the total weight of the seed. In an absolute sense, the seed coats, including the thin endosperm layer, contained most nitrate: approximately half of the total amount. The large amount of nitrate that leached out during the first few hours

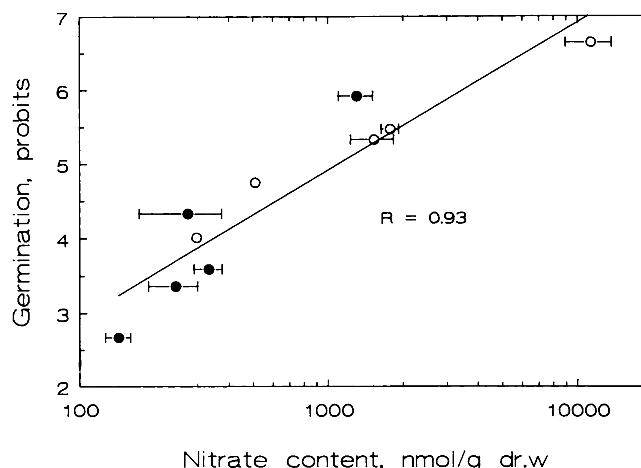

Figure 1. Plot of nitrate content of five different seed lots against germination in probits. Seeds were incubated at 15°C for 2 (○) or 48 h (●), irradiated, and germinated at 24°C. Nitrate contents were measured in dry seeds (○) or in seeds rinsed with distilled water after 48 h (●). R, correlation coefficient.

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Table I. *Nitrate Content of Dry Seed Parts*
A seed lot with a total nitrate content of 1900 nmol g^{-1} was used.

	Percent weight of total	Percent nitrate of total	Nitrate content
			nmol g^{-1}
Seed coats	30	49	3098
Cotyledons	60	34	1078
Axes + radicles	10	19	3653

of imbibition (see below) probably originated for the greater part from the seed coat.

Leaching of Nitrate

During dark-incubation at 15°C in water, the germination capacity declined to zero in approximately 120 h, while in 25 mM KNO_3 the germination response started to decrease after 120 h with a slower rate than in water (5). The decrease of the germination response in water was well correlated with the decrease in nitrate content (Fig. 2). It should be noted that seeds lost most of their nitrate content during the first 24 h of imbibition. In the present case the nitrate level dropped within 24 h from approximately 1800 nmol g^{-1} in dry seeds to about 400 nmol g^{-1} after 24 h. This fast leaching process was probably due to nitrate that was very loosely bound to structures on or close to the surface of the tissues surrounding the seeds.

Nitrate-Response Curves

As suggested before (5), the decreasing response to 25 mM KNO_3 after 120 h at 15°C might be the result of decreasing Pfr-receptor levels. If this is the case, this process should already be observable at periods shorter than 120 h when subsaturating nitrate concentrations are used.

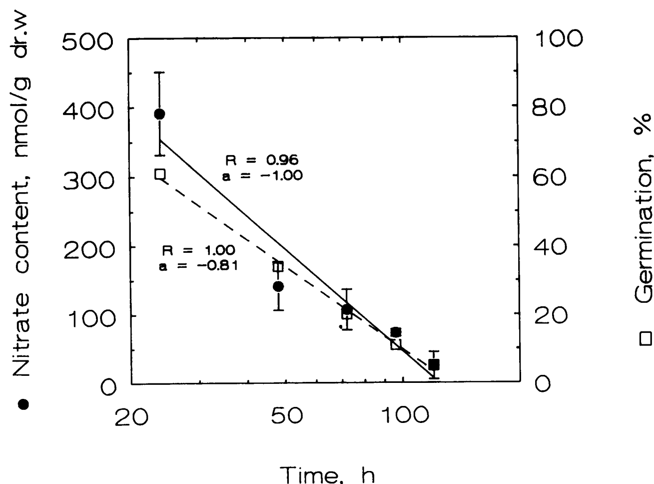

Figure 2. Plot of nitrate content (●) and R-induced germination at 24°C (□) in water against the logarithm of preincubation time at 15°C. R, correlation coefficient; a, slope of regression line, relative to slope of decay of nitrate level (a = −1.00). Germination data are adapted from (5).

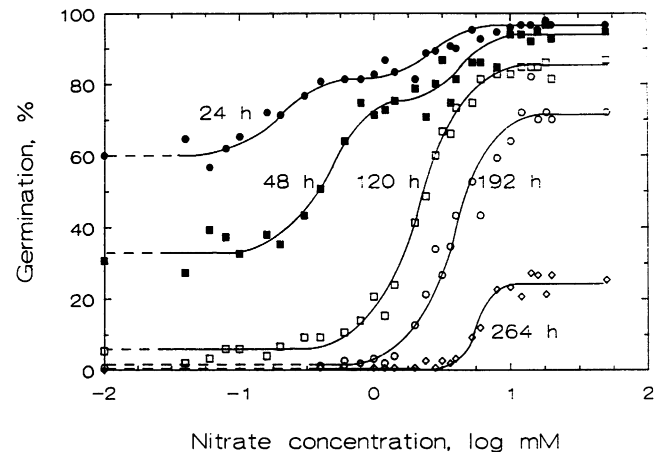

Figure 3. R-induced germination at 24°C of seeds preincubated at 15°C for 24 (●), 48 (■), 120 (□), 192 (○), and 264 h (◇) in a range of nitrate concentrations. Curves were calculated from population parameters. Values at 10^{-2} mM are similar to values for germination in water.

Nitrate-response curves were obtained at several intervals during 264 h of preincubation at 15°C (Fig. 3). The most remarkable aspect of the set of nitrate-response curves is the biphasic character of the 24 and 48 h curves. The VLNR occurred between 0.05 and 1 mM exogenous nitrate, while the LNR occurred between 1 and 10 mM nitrate. From the curve parameters (Table II) it can be concluded that both the VLNR and the LNR curves shifted to the right at increasing incubation periods since the log-fluence value for half-maximal germination increased significantly. The shift was parallel because, with exception of the 264 h curves, B remained constant.

In order to use the curve parameters as interaction parameters, we attempted to simulate the observed curves with the following equation from receptor-occupancy theory for a simple bimolecular cooperative interaction:

$$R = R_{max}[NO_3]^n/([NO_3]^n + [NO_3]^n_{0.5}) \quad (1)$$

where R is the response, R_{max} is the maximum response, n is the cooperativity coefficient, $[NO_3]$ is the nitrate concentration of the medium in mM, and $[NO_3]_{0.5}$ is the nitrate concentration for half-maximal response in mM.

The derivation of this equation was discussed previously (5). The parameters of Equation 1, R_{max}, $[NO_3]_{0.5}$, and n, were substituted by the parameters of the observed curves, R^+, 10^m, and B, respectively, where m is the log-fluence value for half-maximal germination. In the case of the biphasic curves (24 and 48 h) the VLNR and LNR were treated as separate curves. Furthermore, contrary to the fluence-response curves (5), the simulated curves were added to the minimum responses of the observed curves. This was done because the relationship between exogenous and endogenous nitrate levels was too complicated (see below) to be included in the calculations. Figure 4 shows that Equation 1, apart from the fluence-response curves, can also be applied to the nitrate-response curves. With the exception of the B values, all simulated curve parameters did not differ significantly from the observed ones (Table III).

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The Biphasic Nitrate-Response Curves

To test whether a relationship existed between the biphasic response and the uptake pattern of nitrate from the medium, nitrate levels in the medium and in the seeds were measured after preincubation for 48 h at 15°C in a range of nitrate concentrations (Fig. 5). For comparison the 48 and 192 h germination curves are included in Figure 5. When the levels of nitrate were based on nitrate extracted from the seeds the curve started to increase from a basic level of approximately 190 nmol g^{-1} at an exogenous nitrate concentration of about 1 mM. A sharp rise to at least 10,000 nmol g^{-1} at 50 mM exogenous nitrate followed. This value is about six times the original dry seed value of 1,800 nmol g^{-1}. This rise coincided with the LNR (see 192 h germination curve and the upper part of the 48 h curve). At exogenous nitrate levels below 1 mM the extractable amount of nitrate did not increase. Nevertheless, the amount of nitrate in the medium decreased. Recalculation of this decrease to levels taken up on a dry weight basis resulted in an uptake that coincided with the VLNR (see lower part of the 48 h germination curve). Apparently, the amount of nitrate taken up in the VLNR range was so strongly bound that the extraction procedure was not powerful enough. However, extraction with high salt concentrations, detergents, or extraction at elevated temperatures did not enhance the amount of extractable nitrate (data not shown).

To know whether the disappearance of the VLNR between 48 and 120 h at 15°C could be the result of a declining binding capacity for nitrate, nitrate uptake was measured after 48 and 120 h from solutions containing 0.10, 0.40, and 1.00 mM KNO_3. In addition, the influence of a R-pulse on the binding of nitrate was examined. In the 48 h preincubation treatment seeds were irradiated with R 8 h before the end of the preincubation. From Table IV it can be concluded that nitrate uptake in the VLNR range occurred in both the presence and the absence of the VLNR and in both the dark and after a pulse of R. Combined with the values for extractable amounts of nitrate at 48 and 120 h (Fig. 2) it is clear that also in the absence of the VLNR the amount of nitrate taken up in the VLNR range was bound.

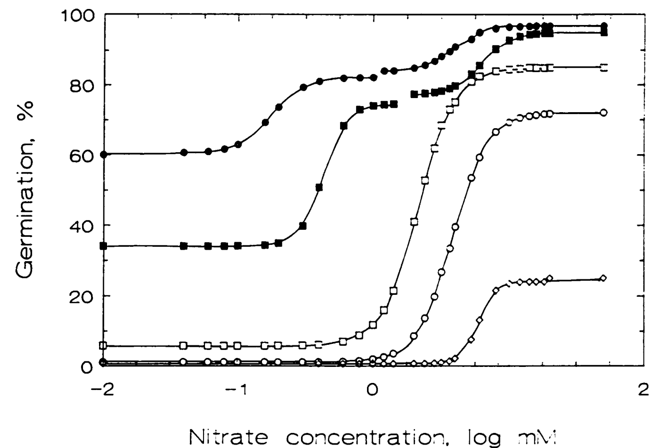

Figure 4. Simulations of the curves of Figure 3 by Equation 1. Curves are calculated from population parameters. Symbols as in Figure 3.

DISCUSSION

Role of Endogenous Nitrate

The present results clearly show that endogenous nitrate is a limiting factor in the light-induced germination in water of *S. officinale* seeds. Seeds that contained less than approximately 100 nmol g^{-1} nitrate did not germinate, either in light or in darkness (Fig. 1). The log-dose probit relationship between nitrate levels and germination response is indicative of a log-normal distribution of endogenous nitrate contents around a level required for 50% germination in water. This distribution was independent of the year of harvest and the length of the preincubation period at 15°C (Fig. 1).

The decrease of the germination in water after increasing preincubation periods was very well correlated with the decreasing endogenous nitrate level (Fig. 2). The decrease in nitrate content was due to leaching into the medium and not to nitrate reduction (8). Similar slopes in the semilogarithmic plot of the decrease of germination and nitrate levels suggest a direct relationship. It may now be argued that the loss of the dormancy-breaking agent nitrate induced secondary dor-

Table II. *Parameters of observed nitrate-response curves after several preincubation periods*

Parameters were calculated by means of weighted linear regression analysis in a log-dose probit diagram. R^-, minimal response; R^+, response range; m, log nitrate concentration (mM) for half-maximal response; B, slope of log-dose probit line.

h at 15°C	R^-	SD(R^-)	R^+	SD(R^+)	m	SD(m)	B	SD(B)
	%		%		mM			
24[a]	60.50	1.14	21.61	1.04	−0.75	0.04	3.63	0.62
48[a]	34.01	1.10	40.56	1.63	−0.37	0.03	5.04	0.96
24[b]	83.81	1.48	12.91	1.42	0.58	0.11	3.92	2.07
48[b]	77.06	0.74	17.82	1.44	0.79	0.08	4.12	0.99
120	5.77	0.46	79.14	0.75	0.33	0.01	3.30	0.17
192	1.15	0.48	71.96	1.65	0.58	0.02	3.33	0.23
264	0.73	0.13	24.05	1.64	0.77	0.02	6.98	0.72

[a] VLNR. [b] LNR.

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Table III. *Parameters and their* SD*(s) of nitrate response curves of Figure 3 simulated with Equation 1*

Parameters were calculated by means of weighted linear regression analysis in a log-dose probit diagram. Values for R_{max}, n, and log nitrate concentration for half-maximal response (m) were taken or calculated from Table II.

h at 15°C	R^-	SD(R^-)	R^+	SD(R^+)	m	SD(m)	B	SD(B)
	%				mM			
24[a]	60.59	1.16	21.51	0.24	−0.75	0.09	4.80	0.06
48[a]	34.03	1.13	40.67	0.10	−0.36	0.01	6.27	0.07
24[b]	85.05	1.68	11.55	1.33	0.60	0.02	6.30	1.48
48[b]	77.21	0.76	17.68	0.13	0.80	0.01	5.46	0.06
120	5.84	0.48	78.99	0.24	0.33	0.02	4.34	0.04
192	1.19	0.50	70.76	0.32	0.58	0.02	4.27	0.07
264	0.73	0.14	23.38	0.14	0.77	0.01	8.70	0.08

[a] VLNR. [b] LNR.

mancy. However, in the 1985 seed lot preincubations longer than 120 h reduced germination in supraoptimal nitrate concentrations (5). Because leaching of nitrate into a medium containing 25 mM KNO_3 is not very likely, this decline may be the result of a process other than leaching.

Following a similar line of argumentation as for the phytochrome-receptor interaction (5) it may be concluded that the declining response in high nitrate concentrations is the result of the decreasing number of nitrate receptors. This process may be superimposed on a process in which nitrate (the agonist) is the limiting factor, provided the number of receptors is not limiting.

The Nitrate Response

That spare receptors are present may be concluded from the nitrate responses after increasing preincubation periods (Fig. 3). These responses generally followed the same pattern as the fluence responses (5). The nitrate-response curves shifted along the x axis to the right, while the maximal response started to decrease rapidly after about 120 h (Fig. 3; Table II). Since the nitrate responses could be simulated by Equation 1, it is assumed that binding of nitrate to its receptor is a simple bimolecular interaction in which the receptor possesses a certain degree of cooperativity ($B > 0.576$). If a receptor reserve is present, a similar maximum response may be generated at a reduced total number of receptors, but at higher levels of nitrate, resulting in a right-hand shift (10). Therefore, it may be concluded that in addition to a reserve of Pfr receptors, a reserve of nitrate-receptors also exists. From the present results and those of the fluence-response experiments (5) it is difficult to make a clear distinction between receptors that are specific for Pfr or for nitrate. We may speculate that Pfr and nitrate bind to the same receptor. This is supported by the correlation between the values for half-maximal response of nitrate and Pfr/total level of phytochrome (Fig. 6), indicating similar decreases in receptor reserves.

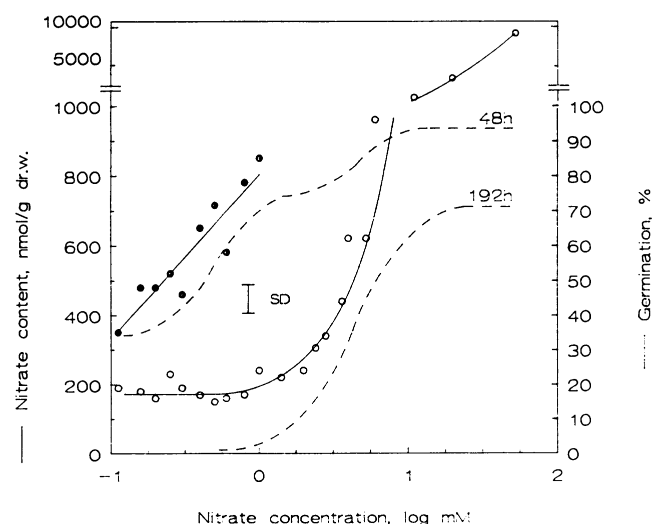

Figure 5. Uptake of nitrate after 48-h incubation at 15°C. Nitrate uptake was calculated from levels of seed extracts (○) or from the decrease of nitrate levels in the medium (●). For comparison, nitrate-response curves after 48 and 192 h preincubation from Figure 3 are shown (- - - -). SD, maximum standard deviation of the nitrate contents.

The Biphasic Character of the Nitrate Response

Surprisingly, the nitrate-response curves after 24 and 48 h of preincubation were biphasic. To our knowledge this is the

Table IV. *Uptake at 15 °C of nitrate from a medium containing 0.10, 0.40, or 1.00 mM KNO_3 after 48 or 120 h*

Uptake of nitrate was calculated from the decreased level in the medium. The light treatment in the 48 h incubation consisted of a *R* pulse after 40 h. SE are in parentheses.

$[NO_3^-]$ in Medium	NO_3^- Taken up after: 48 h		
	Dark	Light	120 h, dark
mM		nmol g^{-1}	
0.10	170 (28)	165 (16)	167 (20)
0.40	348 (29)	332 (17)	422 (65)
1.00	541[a]	589 (21)	584 (42)

[a] Single measurement.

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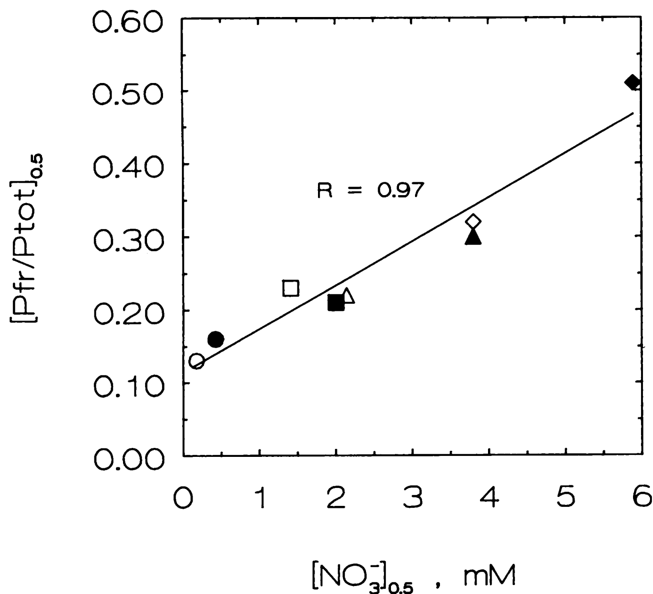

Figure 6. Values for half-maximal response for nitrate and Pfr/total level of phytochrome (P_{tot}). Values of nitrate concentration for half-maximal response ($[NO_3]_{0.5}$) were calculated from log nitrate concentration (mM) (Table II). Values for $[Pfr/P_{tot}]_{0.5}$ were calculated from fluence values for half-maximal response as described previously (ref. 5, Table I). Points, two values calculated from fluence- and nitrate-response curves after 24 (○), 48 (●), 72 (□), 96 (■),120 (△), 144 (▲), 192 (◇), and 264 (◆) h preincubation at 15°C. R, correlation coefficient.

first time a biphasic response is found for a germination response induced by nitrate or any other growth regulator other than light. This stresses the importance of applying sufficient detail to dose-response experiments. Since multiphasic patterns for the isothermic uptake of ions by root cells are well known (14), we studied the uptake of nitrate by the seeds (Fig. 4). The VLNR response was correlated with the uptake of a nitrate fraction that was not extractable from the seeds, whereas the LNR coincided with a sharp rise in the amount of extractable nitrate. Clearly, the binding characteristics of the receptor are not limiting for the VLNR. For the LNR, however, higher amounts of "free" endogenous nitrate seemed to be required to generate a response. One explanation could be that the LNR is generated by a different nitrate receptor. However, the slopes of the VLNR and the LNR did not differ significantly. This is an indication for similar receptors. Therefore, it may be argued that the nitrate receptor may occur in two conformations: a high affinity and a low affinity state. The disappearance of the VLNR after prolonged incubation periods was not the result of an inhibition of uptake, neither was the uptake influenced by phytochrome (Table IV). Therefore, reduction of the VLNR may be the result of a transition of receptors from the high to the low affinity state. However, also the level of endogenous nitrate plays a role at these low exogenous nitrate concentrations. Lowering of the endogenous level will reduce the magnitude of the VLNR but will not generate a shift of the curve.

In conclusion, the present results and those described in the previous article (5) favor a model in which induction of dormancy is a receptor-regulated process. Both the number of phytochrome receptors and the level of activation of the receptors by nitrate may be limiting for the final response. The number of phytochrome receptors present is the net result of synthesis and degradation, and is presumably under temperature control. For many species, including *S. officinale* (6), it has been shown that increasing temperatures enhance the rate of breaking and induction of dormancy. We may hypothesize that, at least for light-sensitive seeds, this is a reflection of the synthesis and degradation of phytochrome receptors. In this study we have shown that the requirement for nitrate is absolute. The results support the earlier suggestion that nitrate functions as a "co-factor" for phytochrome action (7). Optimal nitrate levels may create a certain number of active phytochrome receptors, including a fraction of spare receptors. The temperature dependent degradation decreases the receptor reserve up to the level that is required for maximal germination. Continued incubation will then result in a decreasing response, even in optimal light and nitrate conditions (Fig. 7). If the nitrate level is sub-optimal, the active phytochrome receptor reserve will be smaller or zero. In the latter case the endogenous nitrate level will be the limiting factor for the response. It explains the correlation between leaching of nitrate and germination in water (Fig. 2).

It has been suggested before that loss of light sensitivity upon dark incubation in *Chenopodium album* (12), *Rumex crispus* (3), and *Arabidopsis thaliana* (1), was the result of declining levels of the Pfr reaction partner X. However, *R. crispus, C. album,* and *A. thaliana* are also responsive to nitrate (7, 17).

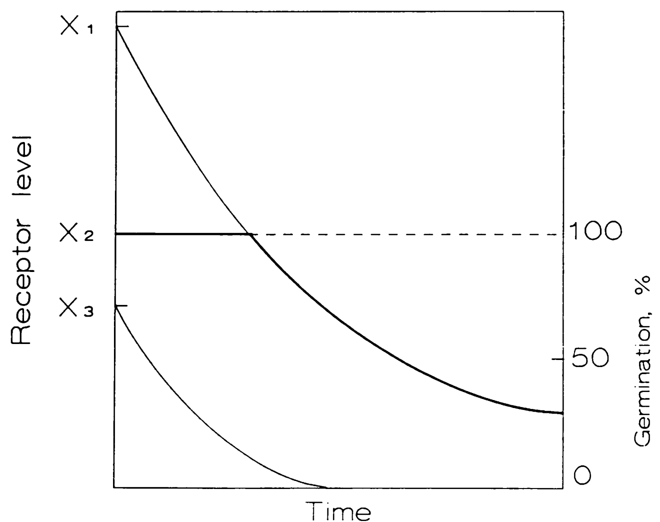

Figure 7. Change in receptor levels during induction of secondary dormancy. Receptor levels are related to germination response through the equation, response = k_e[X] (see ref. 5), where X_1 is the receptor level in the presence of supra-optimal nitrate concentration. Decrease of X_1 is temperature-dependent. X_2 is the receptor level required for maximal germination, hence $X_1 - X_2$ = receptor reserve. Bold line, germination pattern in high nitrate concentration (see ref. 5, Fig. 1). X_3 is the receptor level present in seeds as a result of endogenous nitrate. In water the decrease of X_3 is directly related to leakage of nitrate (Fig. 2).

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As stated before (5), the present model does not necessarily reflect the actual induction of germination by light and nitrate. However, the model based on Clark's equation has been successfully applied to a number of drug-receptor interactions and, in later stages, proven to be valid for the actual ligand-receptor binding and initiation of the transduction chain leading to the final response (10). The present study may provide a context for future research at the molecular level.

This study shows that formulations from the receptor-occupancy theory can also be used to describe the changing responses to light and nitrate of *S. officinale* seeds during induction of secondary dormancy. The induction of secondary dormancy in seeds of *S. officinale* depends on two Pfr reaction partners, nitrate and X. Thus, loss of light sensitivity during prolonged dark incubation may be regulated by two processes: (a) by leaching of a germination stimulating component; and (b) by a "true" inactivation or loss of the phytochrome receptor. Since numerous species are known to be responsive to the combination of light and nitrate the results presented here may reflect a general mechanism.

The mechanism of the combined action of Pfr and nitrate remains to be solved. In animal cells it is known that anions may alter receptor properties to make them accessible for ligands (11). This is an interesting option for the action of nitrate in light-induced germination, since it has been shown that nitrate may be active in the unreduced state (8).

ACKNOWLEDGMENTS

The author thanks Prof. C. M. Karssen for critical reading of the manuscript.

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