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Dual Effect of Light on the Gibberellin- and Nitrate-Stimulated Seed Germination of *Sisymbrium officinale* and *Arabidopsis thaliana*

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ABSTRACT

Red light (R) has a dual effect on the seed germination of the two related species *Arabidopsis thaliana* and *Sisymbrium officinale*. The two species provide different means to separate the light-effects. In *S. officinale*, stimulation of germination by R depends on the simultaneous presence of nitrate (light-effect I). The effect of both factors is completely blocked by tetcyclacis, an inhibitor of gibberellin (GA)-biosynthesis. Addition of a mixture of gibberellins A_4 and A_7 (GA_{4+7}) antagonizes the inhibition. In the absence of nitrate, R shifts germination to lower GA-requirement (light-effect II). In *A. thaliana* a similar second light-effect is seen on the GA-requirement of GA-deficient *ga-1* mutant seeds. R stimulates germination of wild type seeds in water (light-effect I). For both species, light-effect I shows a fluence threshold value of approximately 10^{-5} moles per square meter, which is independent of the nitrate concentration. Increasing nitrate concentrations narrow the fluence-range required for maximal germination whereby the product of nitrate concentration and fluence value determines the germination level, indicating a multiplicative interaction between R and nitrate. Fluence-response curves for light-effect II are similar for both species. Germination occurs in the range of 10^{-6} to 10^{-2} moles per square meter fluence. The maximal level of germination is determined by the level of dark-germination and light-effect II. Increasing GA_{4+7} concentrations induce a shift to lower fluence values. It is shown that in the second effect the co-action of R and exogenous GA_{4+7} is clearly additive. It is concluded that light-effect I induces a chain of events leading to GA biosynthesis. Light-effect II seems to enhance the sensitivity of the seeds to GAs.

Seed germination of a large number of species is promoted by Pfr. However, most light-requiring seeds do germinate in the dark when exogenous GAs[1] are applied (25). This by-pass of light requirement has led to the suggestion that Pfr plays an essential role in the biosynthesis of GAs. Indeed, it has been observed that in some light-requiring species exposure to R increases endogenous levels of GAs, as measured by bioassay (15, 24). In several species light also enhances the stimulation of germination by exogenous GAs whereby the effects of light and GA are mostly additive. Therefore it was suggested that Pfr also increases the sensitivity of the seeds to GAs (3, 26). Previous studies with seeds of *Sisymbrium officinale* (11) and *Arabidopsis thaliana* (12), two closely related wild species, presented arguments for a dual light-effect on GA-biosynthesis and the sensitivity to GAs. Both species provided different means to separate the light effects. Seed germination of *S. officinale* depends on the simultaneous availability of Pfr and nitrate. The effect of both factors can be completely blocked by tetcyclacis, an inhibitor of GA-biosynthesis (19). Addition of GA_{4+7} antagonized the inhibition. Besides its effect on GA-biosynthesis in higher plants (20) where tetcyclacis may inhibit the oxidative reactions from *ent*-kaurene to *ent*-kaurenoic acid (10), inhibition of sterol synthesis has been reported (18). However, sterol synthesis is mainly connected with cell-division while it is widely accepted that during the first stages of germination cell-elongation is the principal growth process. Since nitrate could not stimulate dark germination in *Sisymbrium* it was concluded that Pfr was the essential factor for the initiation of GA-biosynthesis (light-effect I) (11). In the absence of nitrate, light shifted germination to a lower GA-requirement than in darkness (light-effect II). Tetcyclacis did not inhibit this second light-effect.

In *A. thaliana* dwarf mutants have been isolated that are absolutely dependent on GAs for germination (16). The mutants (gene symbol *ga-1*) lack endogenous GAs. This was demonstrated by fractionation of acidic fractions of entire plants by HPLC and subsequent testing of all the fractions in the *d-5* corn bioassay. No GA-like activity was found in the *ga-1* mutant while in the wild type several zones with GA-like activity were present (JAD Zeevaart, personal communication). The *ga-1* mutant has a strongly reduced *ent*-kaurene synthesizing capacity (2). Feeding studies have demonstrated that the *ga-1* block is prior to *ent*-kaurene (JAD Zeevaart, personal communication). Irradiation with R shifts the requirement for GAs of *ga-1* seeds to a lower level (light-effect II) (12). Wild type seeds only require GA in darkness, light induces germination in water (light-effect I).

It is the aim of the present study to further characterize the light effects in seeds of both species in the absence and presence of GA-biosynthesis. Fluence response experiments and subsequent analysis of the dose-response relations with the aid of models for different types of co-action (17, 23) will be used to answer the questions as to the nature of the light effects.

MATERIAL AND METHODS

Seeds. Ripe seeds of *S. officinale* were collected from wild plants growing in natural habitats in the vicinity of Wageningen. A batch collected in 1984 was used. Seeds were cleaned and stored dry at 2°C in the dark. Under these conditions no changes in dormancy were observed during the experimental period.

Seeds of *A. thaliana* were provided by Dr. M. Koornneef (Department of Genetics, Agricultural University Wageningen, The Netherlands). In this study seeds of wild type and of the

[1] Abbreviations: GA, gibberellin; GA_{4+7}, mixture of gibberellins A_4 and A_7; (V)LF, (very) low fluence; R, red light; LDP, log dose-probit.

GA-deficient *ga-1* (NG5) mutant were used. The seeds were stored dry at room temperature.

Germination Conditions. Triplicates of 50 or 50 to 100 seeds of *S. officinale* and *A. thaliana*, respectively, were sown in 5 cm Petri dishes on one layer of filter paper (Schleicher and Schüll No. 595) and moistened with 1.5 ml of distilled water or the test solution. At the start of each experiment primary dormancy of the seeds was broken by dark incubation for 40 h at 15°C for the *Sisymbrium* seeds and 7 d at 10°C for the *Arabidopsis* seeds. After this period seeds were kept at 24°C for 1 h in the dark, were irradiated and then returned to 24°C in the dark. Germination of the *Sisymbrium* seeds was counted after 3 d, and germination of the *Arabidopsis* seeds after 5 d at 24°C.

Broadband red light (620–700 nm) was obtained from six red fluorescent tubes (Philips TL 20W/15) filtered by 3 mm red Plexiglas (Red 501, Röhm & Haas, Darmstadt, FRG). Fluence at seed level was 1.5×10^{-3} mol·m^{-2}. Fluence-response curves were obtained by irradiating the seeds with a custom-build projector with a 250 W quarts-iodine lamp (Philips) equipped with a narrow waveband interference filter of 660 nm (B40; Balzers, Liechtenstein) with approximately 10 nm bandwidth at 50% of the transmission maximum. The fluence rate was varied by inserting neutral glass filters (NG; Schott u Gen., Mainz, FRG) behind the interference filter. The fluence rate was calculated from the transmission characteristics of the neutral filters. During irradiation of the seeds the lids of the Petri dishes were removed. Irradiation time was 20 s except when fluence values higher than 1.06×10^{-3} mol·m^{-2} were required. In those cases the fluence rate was varied by prolonging the irradiation time to maximally 5 min. Reciprocity was not affected at these irradiation times (HWM Hilhorst, unpublished results). Every experiment was repeated at least once with qualitatively similar results. Sowing and all further handling of the seeds were performed in dim green light obtained by filtering one green fluorescent tube (Philips TL 40W/17) with two layers of yellow (No. 46) and two layers of blue (No. 62) Cinemoid filters (Strand Electric, London, U.K.). Dark controls were conducted in absolute darkness and germination results were always similar to those obtained by seeds that were manipulated in dim green light and irradiated with red light of the lowest fluence values used (10^{-10} mol·m^{-2}).

Test Solutions. All compounds were dissolved in distilled water. Tetcyclacis was dissolved in a small volume of acetone and then mixed with a large volume of distilled water. The solution was stirred until no further increase of the absorbance at 240 nm could be observed. The solution was filtered and kept in the dark at room temperature. Final concentration of a saturated solution was approximately 10^{-4} M. The following substances were used: 5-(4-chlorophenyl)-3,4,9,10-pentaazatetracyclo-5,4,1,$0^{2,6}$,$0^{8,11}$ dodeca-3,9-diene (tetcyclacis = NDA = BAS 106W, LAB 102 883); GA_{4+7}, a mixture of gibberellins A4 and A7 (ICI, Yalding, U.K.); KNO_3 of highest available purity (BDH, Poole, U.K.).

Calculations. For the analysis of interaction types it is necessary to compare the subpopulations that react to the light stimulus. Germination is a quantal or 'all-or-none' response. For every individual seed of a population there is a level of intensity (tolerance) of the stimulus below which germination does not occur. This tolerance varies from seed to seed and may be assumed to be normally distributed around the logarithm of a mean level of Pfr required for 50% germination (μ) and with standard deviation σ (7). Response expressed in percentage germination results in a cumulative normal distribution which can be linearized with germination in terms of probits (8) to a log-dose probit (LDP) relation:

$$\text{probit } y = 5 + (\ln[\text{Pfr}] - \mu/\sigma)$$

where μ is a function of those factors which affect the Pfr tolerance in individual seeds and σ is a function of changes in the range of Pfr requirement of the seed population. In this work it is assumed that the Pfr requirement of the individual seeds of the seed population is normally distributed around a mean level of Pfr required for half-maximal germination, instead of 50% germination (21). With this approach it is possible to compare dose-response relationships of Pfr and the applied growth regulators independent of the response of zero dose (zero response) and the maximal response at the experimental conditions (maximal response), leaving a nongerminating fraction). Also the population parameters can be calculated and from the weighted regression line (LDP line) the sigmoid curve of germination *versus* log fluence can be generated which fits the original data points (5, 6). The data obtained from the fluence-response experiments were subjected to probit analysis with the aid of a computer program developed by De Petter *et al.* (6). The population parameters and the log dose-probit line were calculated and subsequently the best fitting sigmoid curve through the data points was produced. The automated calculation procedure included variance calculations with standard formulae.

RESULTS

Germination under Non-GA-Producing Conditions. Following a dormancy breaking preincubation both *ga-1* seeds of *Arabidopsis* and *Sisymbrium* seeds deprived of nitrate failed to germinate in water, neither in darkness nor after a saturating dose of R (Fig. 1, A and B). There was an absolute dependency on the presence of exogenous GA_{4+7}. In the dark the reaction to a range of GA_{4+7} concentrations was similar. A saturating dose of R induced the seeds to germinate at lower GA_{4+7} levels: In the *ga-1* mutant of *Arabidopsis* to about one-third (Fig. 1A) and in *Sisymbrium* to one-tenth of the GA_{4+7} concentration required in the dark (Fig. 1B). In the suboptimal segment of the dose-response curves this shift was parallel in both species. This indicates an additive effect of R to the GA_{4+7} response.

Germination under GA-Producing Conditions. It was shown before that wild-type *Arabidopsis* seeds germinated readily in water after saturating R, whereas the response of *Sisymbrium* seeds to R was dependent on nitrate (11, 12). The reaction of *Sisymbrium* seeds to a range of KNO_3 concentrations is shown in Figure 1C. It is seen that light hardly had a stimulative effect without KNO_3 and KNO_3 was ineffective in the dark. It should be noted that active nitrate concentrations were 1000 times higher than the active GA_{4+7} concentrations. In *ga-1 Arabidopsis* seeds also the combination of saturating R and KNO_3 could not replace the requirement for application of GA_{4+7} (data not shown).

Fluence-Response Curves under GA-Producing Conditions. In order to quantify the effect of R on germination under GA-producing conditions fluence-response curves were made for the germination of *Sisymbrium* seeds in different concentrations of nitrate (Fig. 2) and of wild type *Arabidopsis* seeds in water and nitrate (Fig. 3).

Sisymbrium. In water, germination of *Sisymbrium* seeds was only slightly stimulated by R (Fig. 2). At the nitrate concentrations used germination was stimulated by R at fluences higher than 10^{-5} mol·m^{-2} (Fig. 2). This threshold value was the same for all concentrations and below this value no germination occurred. The slope of the LDP line increased significantly with the nitrate concentration (Table I). Addition of tetcyclacis to 5 mM nitrate shifted the fluence-response curve to higher fluence values and reduced maximal germination levels (Fig. 2). The slopes of the LDP lines were similar to the slope of the curve of 1 mM nitrate (Table I) and this is a lower value than could be expected for 5 mM nitrate. Although maximal germination was strongly decreased at increasing concentration of tetcyclacis, both LDP lines were similar. A good correlation was found between the product of fluence and nitrate concentration, expressed as the sum of the logarithms, and the probit of the corresponding

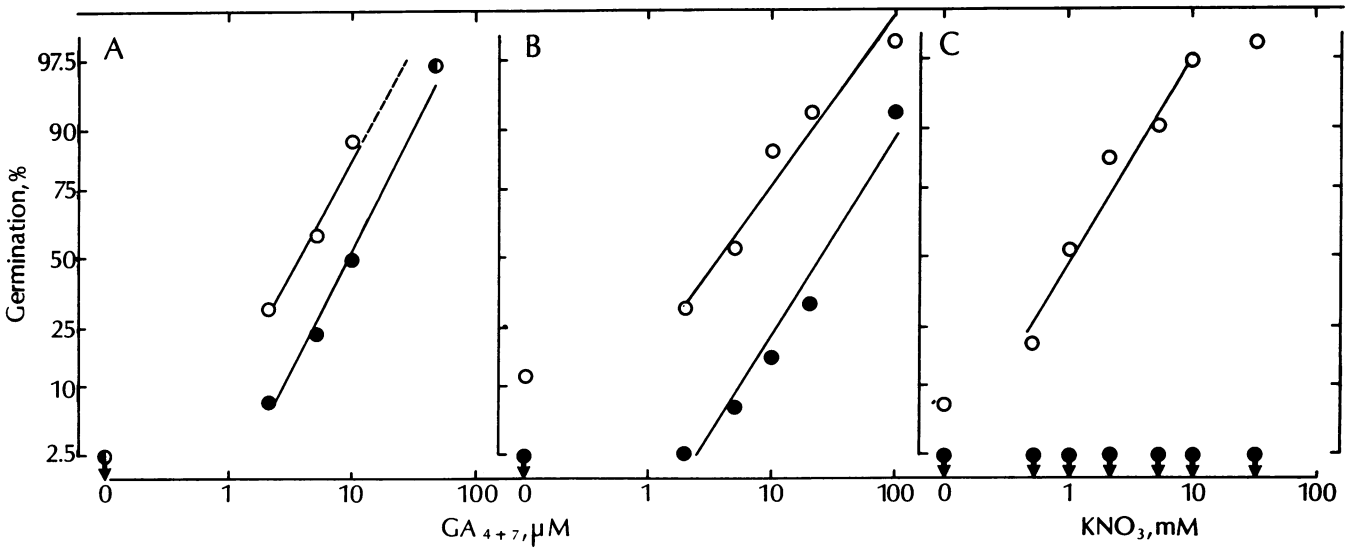

FIG. 1. Effect of a 10 min R irradiation on the germination at 24°C of seeds of the *ga-1* mutant of *A. thaliana* (A) and *S. officinale* (B, C) in a range of concentrations of GA_{4+7} (A, B) or nitrate (C). (○), R; (●), dark; (●→), no germination.

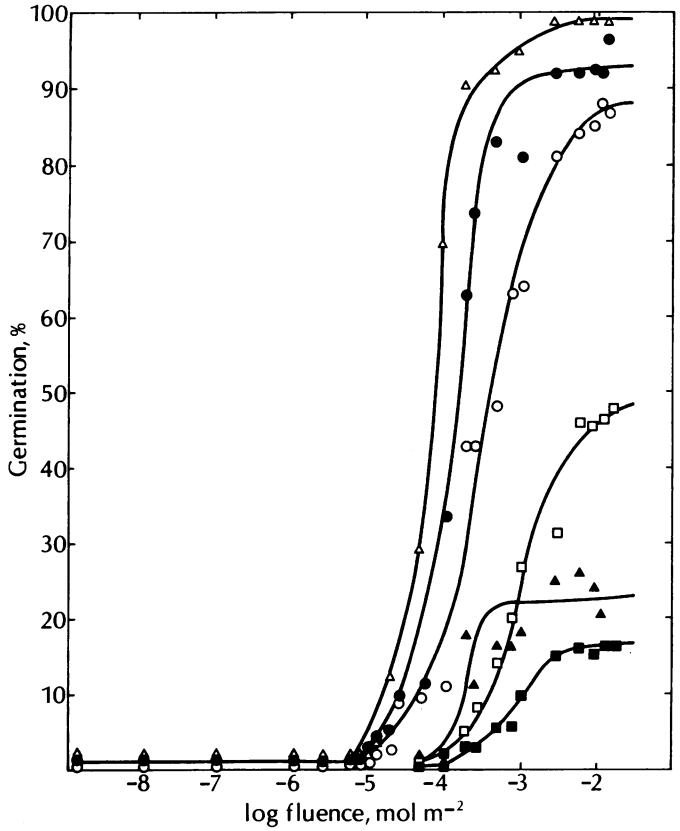

FIG. 2. Fluence response curves for the germination at 24°C of seeds of *S. officinale* in water (▲), 1 (○), 2 (●), 32 (△) mM KNO_3 and 5 mM KNO_3 with 5 (□) or 10 (■) μM tetcyclacis. Curves are calculated from the population parameters.

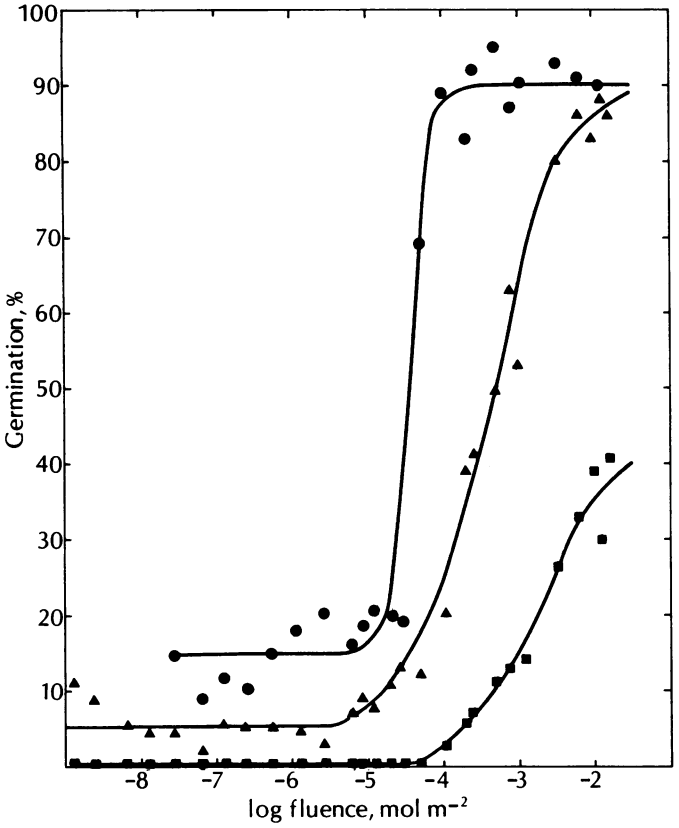

FIG. 3. Fluence-response curves for the germination at 24°C of wild type seeds of *A. thaliana* in water (▲), 10 mM KNO_3 (●) and 2μM tetcyclacis in water (■). Curves are calculated from population parameters.

germination response (Fig. 4A), indicating a multiplicative interaction between Pfr and nitrate. The data from the 32 mM nitrate fluence-response curve were not included since this concentration was highly saturating.

Arabidopsis. Germination of wild type *Arabidopsis* seeds did not depend on nitrate. Nearly all seeds germinated in water at saturating fluence values (Fig. 3). R stimulated in the range of 10^{-5} to 10^{-2} mol·m^{-2}. Addition of 10 mM KNO_3 narrowed the fluence range to 10^{-5} to 10^{-4}, but the fluence threshold did not change. Dark germination (fluences $< 10^{-5}$ mol·m^{-2}) was some-

Table I. *Population Parameters and Standard Deviations(s) Obtained after Weighted Regression of the Data Points of Figures 2, 3, 5, and 6*

Species	Medium	Concentration	R⁻[a]	s(R⁻)	R⁺[b]	s(R⁺)	m[c]	s(m)	B	s(B)
		mol·L^{-1}	%	%	%	%	*log mol · m*$^{-2}$			
S. officinale	H_2O		0.55	0.13	21.47	1.27	−3.75	0.04	4.99	0.26
(Fig. 2)	KNO_3	1*10^{-3}	1.70	0.59	86.07	1.77	−3.45	0.04	1.47	0.10
	KNO_3	2*10^{-3}	1.49	0.32	89.41	1.05	−3.88	0.03	2.07	0.19
	KNO_3	3.2*10^{-3}	1.20	0.19	96.12	0.40	−4.14	0.02	3.00	0.16
	KNO_3	5*10^{-3}								
	+Tetcy-	5*10^{-6}	0		50.53	2.19	−2.94	0.05	1.44	0.11
	clacis	1*10^{-5}	0		16.98	1.02	−3.03	0.06	1.53	0.20
(Fig. 5)	GA_{4+7}	2*10^{-6}	2.34	0.23	28.64	1.99	−3.31	0.10	1.19	0.14
	GA_{4+7}	5*10^{-6}	5.81	0.56	52.74	3.48	−3.56	0.12	0.96	0.12
	GA_{4+7}	1*10^{-5}	18.67	1.43	70.96	1.90	−4.57	0.08	0.91	0.09
	GA_{4+7}	2*10^{-5}	35.76	1.33	57.26	1.14	−4.62	0.06	1.27	0.12
A. thaliana	H_2O		5.62	0.44	84.62	2.20	−3.36	0.05	1.21	0.08
Wild type	KNO_3	1*10^{-2}	15.65	0.81	73.72	1.10	−4.40	0.02	4.66	0.56
(Fig. 3)	Tetcy-	2*10^{-6}	0.40	0.10	45.33	5.93	−2.66	0.15	1.16	0.01
	clacis									
A. thaliana	GA_{4+7}	2*10^{-6}	7.56	0.43	21.50	2.33	−3.43	0.17	1.00	0.20
ga-1 (Fig. 6)	GA_{4+7}	5*10^{-6}	20.27	0.87	40.27	6.39	−4.34	0.25	1.01	0.25
	GA_{4+7}	1*10^{-5}	49.45	1.53	41.59	5.98	−4.46	0.26	0.83	0.18

[a] R⁻, minimum response. [b] R⁺, response range. [c] m, dose for half-maximal response. [d] B; slope = $1/\sigma$.

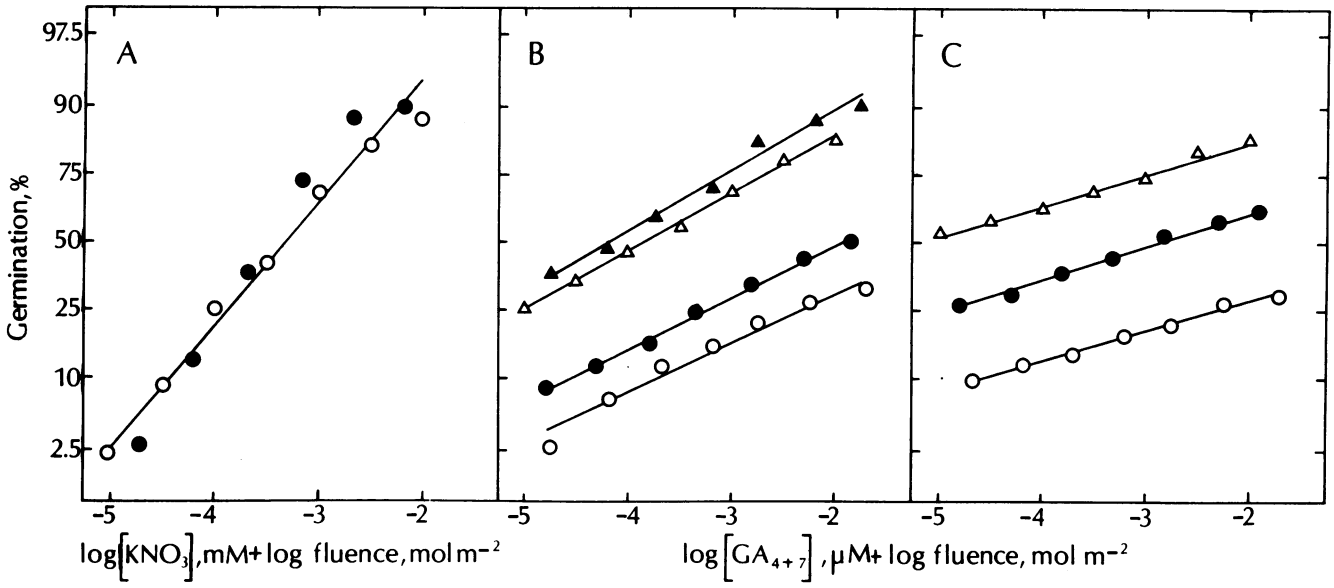

FIG. 4. Plot of germination against the sum of logarithm of nitrate (A) or GA_{4+7} (B, C) concentration and fluence of *S. officinale* (A, B, data obtained from Figs. 2 and 5) and the *ga-1* mutant of *A. thaliana* (C, data obtained from Fig. 6). A, 1, (○) and 2 (●) mM KNO_3; B, C, 2 (○), 5 (●), 10 (△), and 20 (▲) µM GA_{4+7}.

what increased by the addition of nitrate (Fig. 3). The LDP line for the nitrate stimulated germination was much steeper than the line for the germination in water (Table I). Addition of tetcyclacis caused a shift to higher fluence values (Fig. 3), which was parallel (Table I). Moreover, the dark germination was lowered to zero and maximal germination was reduced.

The results described here show remarkable similarities between the two species with respect to response range, threshold values of fluences, slopes of LDP lines, and effects of tetcyclacis.

Fluence-Response Curves under Non-GA-Producing Conditions. Fluence-response curves for the GA_{4+7}-stimulated germination of *Sisymbrium* seeds (Fig. 5) shared all the characteristics of the curves of the *ga-1* mutant of *Arabidopsis* (Fig. 6). In both species the R-sensitive subpopulations germinated in the fluence range of 10^{-7} to 10^{-2} mol · m^{-2} and showed a shift to higher fluence values at nonsaturating GA_{4+7}-concentrations. This shift was parallel as can be deduced from the slopes of the LDP lines (Table I). In both species a GA_{4+7}-dependent germination response was shown in the VLF range (<10^{-6} mol·m^{-2}). This response remained at a constant level until fluence values were reached in the LF range (10^{-6} to 10^{-2} mol·m^{-2}). In both species the germination response range (Table I, R⁺) of the two highest GA_{4+7} concentrations was of the same magnitude. Maximum level of germination, therefore, depended on the initial

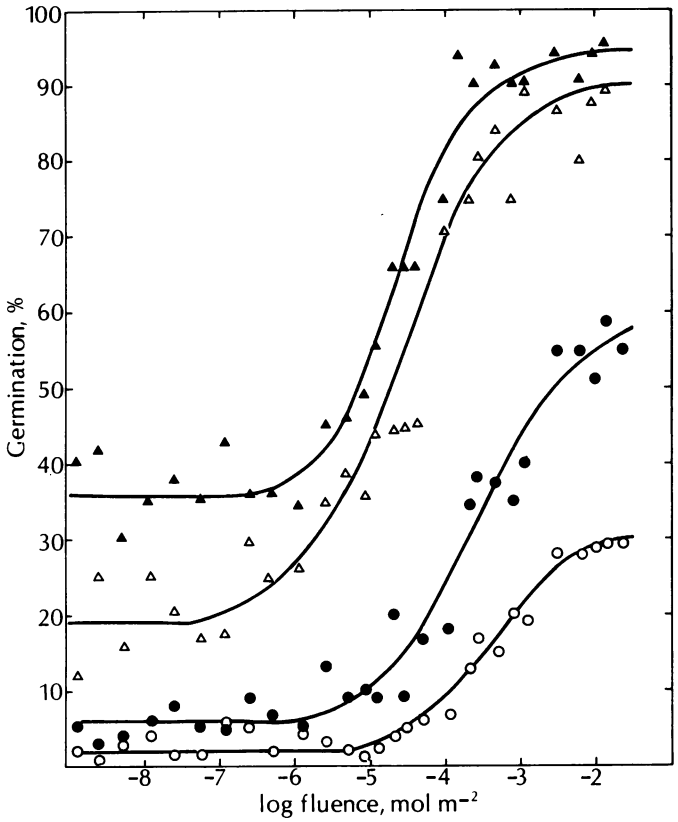

FIG. 5. Fluence-response curves for the germination at 24°C of *Sisymbrium* seeds in 2 (○), 5 (●), 10 (△), and 20 (▲) μM GA_{4+7}. Curves are calculated from population parameters.

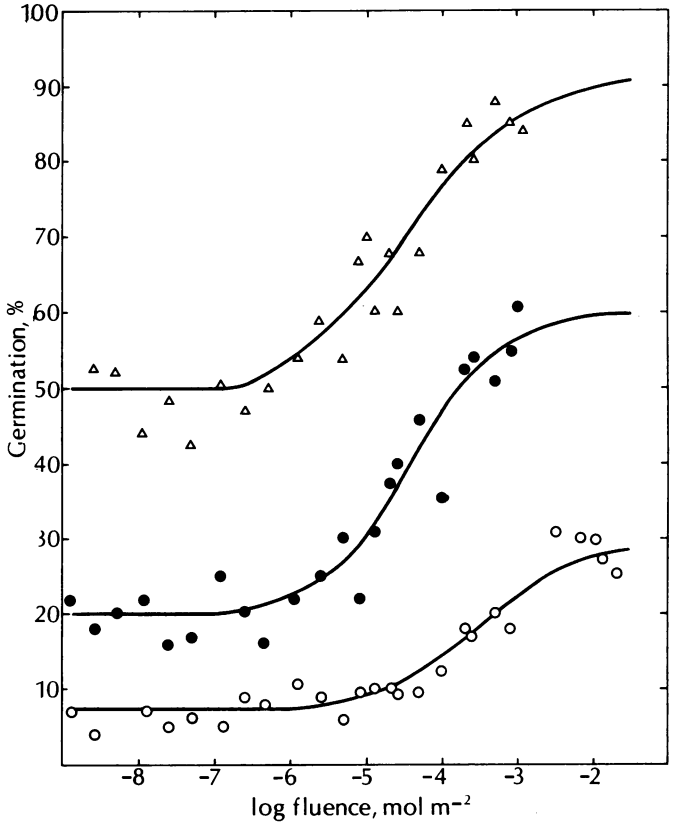

FIG. 6. Fluence-response curves for the germination at 24°C of the *ga-1* mutant of *A. thaliana* in 2 (○), 5 (●), and 10 (△)μM GA_{4+7}. Curves calculated from population parameters.

'dark' germination. In contrast with the fluence-response curves of the nitrate stimulated germination of *Sisymbrium* seeds (Fig. 4A) the correlation lines of the product of fluence and GA_{4+7} concentration and the germination response did not coincide (Fig. 4, B and C), suggesting an additive type of co-action of Pfr and GA_{4+7}.

DISCUSSION

From the present results it can be argued that the effect of light on seed germination is indeed regulated by two different light-reactions. The use of two related species with their specific requirements for germination reinforces the arguments since the results were remarkably similar. Light-effect I was expressed under conditions where GAs could be synthesized and germination did not depend on application of GA_{4+7}. These conditions were met in nitrate-incubated *Sisymbrium* seeds and in wild type *Arabidopsis* seeds. Light-effect II was demonstrated under conditions at which GA-synthesis was blocked and germination was absolutely dependent on exogenous GA_{4+7}. These conditions were realized by depriving *Sisymbrium* seeds of nitrate and by using the *ga-1* mutant of *Arabidopsis*. Both light-effects and their interrelationship will be discussed in detail.

Light-Effect I. The promotive action of nitrate on seed germination has been the subject of a number of studies (22, 28). Besides several hypotheses on its possible role in dormancy mechanisms (1, 22) there have been speculations on the action of nitrate on the Pfr-stimulated germination (5). However, there is no agreement as to its general mechanism of action. The present results strengthen our previous hypothesis that nitrate- and Pfr-action are closely connected (11). The nitrate concentration modulated the fluences required for half-maximal germination of both *Arabidopsis* and *Sisymbrium* seeds (Figs. 2, 3; Table I). A plot of the logarithmic sum of fluence value and nitrate concentration against the germination response of *Sisymbrium* seeds (Fig. 4A) shows that a certain response can be generated by more than one combination of nitrate and Pfr concentrations. The response is a function of the product of both factors and by definition this represents a multiplicative interaction between both stimulators. This type of interaction expresses the action of both factors at different points of the same pathway of reactions (23). In both species light-effect I was expressed essentially in the LF range. Nitrate had no effect on the threshold value of approximately 10^{-5} mol m^{-2}. Similar values were reported for *Kalanchöe blossfeldiana* (5) and *Rumex obtusifolius* (14). The results indicate that light-effect I may reflect a general mechanism for the light-stimulated germination. In addition, other factors, like nitrate, may be essential or nonessential to this stimulation. For both species it can be argued that light-effect I plays an essential role in GA-biosynthesis. Direct evidence for this hypothesis is difficult to obtain, but we have shown that in both species germination in the dark was absolutely dependent on the presence of exogenous GA_{4+7}. Moreover, the *ga-1* mutant of *Arabidopsis*, which lacks the ability to synthesize GAs, germinated after R only when exogenous GA_{4+7} was present (Fig. 1A). In a previous paper (11) we demonstrated that the R induced germination of *Sisymbrium* seeds could be inhibited by tetcyclacis. The effect of tetcyclacis could be fully reversed by exogenous GA_{4+7}. In the present study both tetcyclacis under GA-producing conditions (Figs. 2, 3) and exogenous GA_{4+7} under non-GA-producing conditions (Figs. 5, 6) shifted the fluence-response curves to higher fluence values and reduced maximal germination. Tetcyclacis may therefore indeed modulate the lev-

els of endogenous GAs. Tetcyclacis also opposed the effect of nitrate (Fig. 2; Table I) possibly because, in this case, nitrate is not the limiting factor but the synthesis of GAs. The low number of wild type *Arabidopsis* seeds that germinated in water in the VLF range (Fig. 3) was also reduced by tetcyclacis, indicating that a small fraction of these seeds is capable of synthesizing GAs with very low levels of Pfr.

Light-Effect II. Gibberellins have been found to substitute for light in the seed germination of several species (25). However, it is difficult to determine whether this substitution is complete. Very low levels of (preexisting) Pfr may still play a role. Seeds of *Sisymbrium* and the *ga-1* mutant of *Arabidopsis* showed dark germination which depended on the concentration of exogenous GA_{4+7} (Fig. 1, A and B). The level of dark germination had no effect on the slopes of the LDP lines (compare data on R^- and B in response to GA_{4+7} in Table I). Dramatic changing of the slopes of fluence response curves caused by preexisting Pfr has been reported for wild type *Arabidopsis* seeds (4). Since the germination level in the VLF range remained fairly constant with varying fluence in our case (Figs. 5, 6) we conclude that the dark response to exogenous GA_{4+7} is not limited by Pfr. In both species, limiting GA_{4+7} concentrations shifted *m* to higher fluence values (Table I). These results indicate that the expression of light-effect II depends on the GA_{4+7} concentration. An interaction between GA_{4+7} and Pfr does not occur. This can be concluded from the plot of the logarithmic sum of GA_{4+7} concentration and fluence against germination response (Fig. 4, B and C) which results in parallel lines for different GA_{4+7} concentrations. This is a good argument for an independent co-action of the two factors (23).

Light-Effects I and II. The present results make clear that the two light-effects are of a different nature with respect to their respective interactions with nitrate and GAs. Previous observations on *S. officinale* have shown that both light-effects differ in more respects (11). Upon incubation at temperatures that favor secondary dormancy light-effect I gradually disappeared while seeds remained responsive to light-effect II. Moreover, the escape time for far red reversion of light-effect I was approximately 8 h and of light-effect II approximately 1 h (HWM Hilhorst, unpublished results). Thus it can be argued that light-effect I is the limiting factor for the light-stimulated germination. If it is assumed that the germination response is proportional to the number of active GA-receptor complexes and that the formation of such complexes primarily depends on the availability of GAs and active receptor sites, we may hypothesize that light-effect I acts through the production of GAs and light-effect II through the production of active receptor sites. It should be noted that the concept of sensitivity not only comprises the formation of active GA-receptor complexes but also includes phenomena like affinity, response capacity, and uptake efficiency (9). The physiological relevance of light-effect I may be its role in linking the reception of favorable light conditions with germination through GA-biosynthesis. The expression of this light-effect may be regulated by other environmental factors such as nitrate, probably depending on the specific environmental requirements of the plant that grows from the seed. The physiological importance of light-effect II is less clear. It may occur before light-effect I but will not come to expression in the absence of the first light-effect. As both light-effects are initiated in the same fluence-range it may be questioned why the receptor activation is under Pfr control. As yet we have not been able to find natural conditions under which this process is limiting for germination. The present study makes clear that seed germination of *S. officinale* and *A. thaliana* is under control of both the level of endogenous GAs and the sensitivity to GAs. Both controls are regulated by Pfr. We believe that our results may add arguments to the recent discussion on the hormonal regulation of growth and development (27). Instead of a choice between control by changing hormone levels and changing sensitivity to hormones a dual control by light of both mechanisms would be favored.

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