The fluence-response curves for the effect of two red pulses separated by 24 hours on the germination of Kalanchoë blossfeldiana Poelln. cv Vesuv seeds, incubated on gibberellic acid (GA₃) are biphasic for suboptimal concentrations. The response in the low fluence range corresponds with a classical red/far-red reversible phytochrome mediated reaction. GA₃ induces an additional response in the very low fluence range, which is also phytochrome mediated. The sensitivity to phytochrome-far-red absorbing form (Pfr), however, is increased about 20,000-fold, so that even far-red fluences become saturating. Both in the very low and low fluence response range, the maximal responses induced by saturating fluences are modulated by the GA₃ concentration. GA₃ having no direct influence on the phytochrome phototransformations, alters the Pfr requirement and determines the responding seed population fraction in the very low and low fluence range. The effect of GA₃ appears to be on the transduction chain of the phytochrome signal.

The germination of Kalanchoë seeds is light-requiring. Incubated on water or KNO₃ solution, germination requires several daily light pulses given from the first day on after sowing, to prevent induction of secondary dormancy (8, 20). The seed population responds in the LF² range (3×10⁻² to 3×10⁻¹ mol/m², R) and reversion of a promoting light pulse by FR is complete (8, 11). The KNO₃ concentration determines the responding population fraction in the LF range (8).

In the presence of GA₃, germination can be induced with a single light pulse. This irradiation was given on d 7 after sowing, the moment of highest response in the presence of GA₃ (6). The seed population responds in the VLF range (10⁻³ to 10⁻⁴ mol/m², R) and a single FR pulse is inductive (8). For a single R or FR pulse, the GA₃ concentration determines the responding population fraction in the VLF range, in an identical way (8). Both in the presence and absence of GA₃, preliminary action spectra point to phytochrome as the photoreceptor (7). GA₃ increases the physiological activity of Pfr so that even FR light becomes saturating for germination (8, 13). For the VLF as well as for the LF response, the apparent efficiency of phytochrome phototransformations, reflected in the slopes of the LDP lines, are nearly the same (8). When two low fluence R or FR pulses, separated by 24 h, are given to seeds incubated on a range of GA₃ concentrations, the effect of R is higher than that of FR in the 0.1 to 0.01 nmol concentration range (6). These results suggest the possibility of a LF response, additional to the VLF response, which is induced by two R but not by two FR irradiations at suboptimal GA₃ concentrations.

Fluence-response curves for two R or FR pulses are presented, allowing the quantification and correlation of both the VLF and the LF response types in the same experimental conditions. We also studied the relation between exogenously applied GA₃ and the maximal germination induced with VLF of R and FR, saturating the VLF response and with low R fluences, saturating the LF response. Probit analysis is used to calculate the population parameters (see "Materials and Methods"). From the LDP equations and from the zero response and the maximal response, the sigmoid curves fitting the experimental data are generated. The phytochrome initiated dose-response kinetics and the seed population parameters are discussed.

Induction of a VLF response, resulting in biphasic fluence-response curves was obtained for Lactuca seeds with ethanol (26, 27) and, for Lactuca, Rumex, and Arabidopsis seeds, with temperature treatments (3, 4, 19, 24, 26, 27). Besides the effect of GA₃ on Kalanchoë seed germination, induction of a VLF response by a plant growth substance (IAA and several classes of synthetic auxins) was only demonstrated for the R-stimulated growth of etiolated Avena coleoptile sections (22, 23).

MATERIALS AND METHODS

Kalanchoë blossfeldiana Poelln. cv Vesuv seeds were purchased from Daehnfeldt, Odense, Denmark and stored dry at -20°C. About 100 seeds were sown under very dim green light (maximum fluence was 4×10⁻⁸ mol/m²) in 4 cm Petri dishes on one Whatman glass microfiber filter GF/A, moistened with 1.0 ml water for control experiments or 1.0 ml GA₃ solution (0.002-2 nmol) containing 0.8 ml GA₃ solution was used. The very dim green light, used during sowing, had no effect on dark germination or on the germination induced by a subsequent experimental irradiation. Dishes were wrapped in black cloth and kept in darkness at 20 ± 0.5°C and 95% RH. All subsequent
manipulations, except for the explicitly described experimental irradiations and germination monitoring, were carried out in complete darkness.

All irradiations were given with a dark interval of 24 h on d 7 and 8 after sowing and were performed at 20 ± 1.0°C. Final germination, based on at least six batches of about 100 seeds, was counted 5 d after the last irradiation. Broad-band R was supplied by red fluorescent lamps (TL 15, Philips, The Netherlands), combined with a red filter (Röhm and Haas nr. 501). Narrow-band R and FR were obtained by filtering the light of a Volpi Intralux 250 H (250 W) light source (Switzerland) through Balzers B-20 interference filters (660 and 730 nm, half-bandwidth 12 and 15 nm). A Balzers B-40 interference filter (723 nm, half-bandwidth 29 nm) was used for reversion experiments (Fig. 3). Different fluence rates were obtained by inserting neutral density filters or varying the distance and were measured with an International Light spectroradiometer system (IL600 and IL700, MA). Irradiation times were automated with a Compur electronic m-1 shutter (Germany). A number of overlapping fluences, obtained with different fluence rates (1 × 10^{-6}, 9 × 10^{-6}, 3–5 × 10^{-6}, and 1 × 10^{-5} mol/m^{2}·s for R; 2–3 × 10^{-6}, 4–10 × 10^{-6}, 1 × 10^{-5} mol/m^{2}·s for FR) and exposure times between 0.01 and 1,000 s, indicated the validity of the reciprocity law in the 10^{-8} to 10^{-2} mol/m^{2}·s range for FR and in the 10^{-6} to 5 × 10^{-6} mol/m^{2}·s range for R. At a R fluence of 2 × 10^{-6} mol/m^{2}, reciprocity still holds for fluence rates between 5.6 × 10^{-6} and 1.6 × 10^{-2} mol/m^{2}·s and exposure times between 125 and 3,226 s. Exposure times between 0.01 and 1,000 s were used for fluence-response experiments.

**Calculation of Dose-Response Curves.** As germination is a quantal (all-or-none) response, monophasic dose-response relations are sigmoid in a response versus log D plot. Assuming that there is a logarithmic normal distribution of D requirement in the responding seed population fraction around a mean level of D required for half-maximal response, the sigmoid dose-response curves can be linearized by means of probit transformation of the germination response (9, 12). The equation of the straight line in the probit diagram is defined as the LDP equation

\[ Y = S + | \log(D) - m | B \]  

(1)

where \( Y \) is the probit of the germination percentage, \( m = \log \mu \) with \( \mu \) the mean of the distribution or dose for half-maximal response and the slope \( B = 1/\sigma \) with \( \sigma \) the standard deviation of \( \log D \) around \( \mu \). Similar statistical approaches have been previously employed (5, 10, 14, 25), but they assumed a logarithmic normal distribution of Pfr requirement of the individuals in the seed population around a mean level of Pfr required for 50% germination, instead of half-maximal germination used in this paper.

The equation of the best fitting curve to the experimental data is given by

\[ Y = K+ \Psi [(\log(D) - m) B] + K- \]  

(2)

where \( Y \) is the germination percentage at a given dose \( D \), \( K+ \) is the response range in %, \( K- \) is the zero response in %, and \( \Psi \) is the cumulative distribution function of the normalized normal distribution (\( \mu = 0 \) and \( \sigma = 1 \)).

The equation for the best fitting curve to biphasic dose-response data can be obtained assuming a heterogeneous population consisting of two populations with different promoter requirements and is given by

\[ Y = f_1Y_1 + f_2Y_2 \]  

(3)

with \( 0 < (f_1 + f_2) < 1 \), where \( Y \) is the response in % of the heterogeneous population, \( Y_1 \) and \( Y_2 \) are the responses of population 1 and 2, respectively, \( f_1 \) and \( f_2 \) are the proportions of the two responding populations (16). The latter are reflected by the respective response ranges (\( K+ \)).

**RESULTS**

The fluence-response curves for two R pulses, given to seeds imbibed on different GA_3 concentrations, are presented in Figure 1. The irradiations were given on d 7 and 8 after sowing, the period of highest response in the presence of GA_3 (6). In the suboptimal concentration range (0.01–0.1 mm) increasingly biphasic relations between log R fluences and germination are obtained. The first response component is promoted by VLF, in the 10^{-4} to 10^{-2} mol/m^{2} range, called the VLF response. The second response component occurs at LF in the 3 × 10^{-5} to 3 × 10^{-3} mol/m^{2} range, called the LF response (8). At 1 mm GA_3, nearly the entire seed population responds in the VLF range.

With two FR pulses, for different GA_3 concentrations, monophasic (increasing) relations between log FR fluences and germination are obtained (Fig. 2). However, in comparison with R, considerably more light is required to obtain the VLF response. No LF response is induced by FR fluences as high as 2 × 10^{-1} mol/m^{2}. Dark germination is very poor (generally below 5%). Seeds sown on water and given two R or FR irradiations on d 7 and 8 after sowing, did not germinate.

For R as well as for FR light, the maximal response of the VLF component, and consequently the slope of the curve, increases with increasing GA_3 concentrations. The maximal ger-

![FIG. 1. Fluence-response curves for germination of *Kalanchoë* seeds for two narrow-band R pulses on d 7 and 8. Each consisting of half of the total fluence indicated, on different GA_3 concentrations: 1 mm (+), 0.1 mm (•), 0.07 mm (○), 0.05 mm (■), 0.02 mm (Δ), 0.01 mm (□). All germination percentages are based on six batches of about 100 seeds. Calculated curves from population parameters (— — — —), indication of \( \mu \) for each fraction (— — — —). Dark germination from high to low GA_3 concentrations is 6.0, 1.5, 0.7, 0.0, and 0%, respectively.](https://www.plantphysiol.org/)

![FIG. 2. Fluence-response curves for germination of *Kalanchoë* seeds for two narrow-band FR pulses on d 7 and 8. Each consisting of half of the total fluence indicated, on different GA_3 concentrations: 1 mm (+), 0.1 mm (•), 0.07 mm (○), 0.05 mm (■), 0.02 mm (Δ), 0.01 mm (□). All germination percentages are based on six batches of about 100 seeds. Calculated curves from population parameters (— — — —), indication of \( \mu \) for each fraction (— — — —). Dark germination from high to low GA_3 concentrations is 3.2, 2.0, 0.9, 0.8, 0 and 0%, respectively.](https://www.plantphysiol.org/)
mination response as a consequence of VLF plus LF component (see second maximum in the curves of Fig. 1) also increases with the GA3 concentration. For each experiment of Figures 1 and 2, the population parameters of both VLF and LF component, are calculated by means of probit analysis. At all GA3 concentrations and for VLF and LF component, there is a linear relation between the logarithm of the total photon flux and the probit of the germination response. Despite individual deviations, due to consecutive experimental conditions, the slopes of all LDP lines for the LF as well as for the VLF response are very similar (data not shown). The fluence for half-maximal response (μ) indicates the median light requirement of the responding seed population. Three light-sensitivity (1/μ) ranges are observed. For the LF response the R fluences for half-maximal germination range from 3.9 \( \times 10^{-4} \) to 4 \( \times 10^{-3} \) mol/m². The VLF response can be saturated with R as well as with FR. The fluences for a half-maximal saturation of this VLF response with R range from 3.9 \( \times 10^{-4} \) to 8.2 \( \times 10^{-4} \) mol/m². About 200 times more light is needed to saturate the same response with FR. The VLF response requires about 20,000 times less R fluence than the LF response (Fig. 1). The limited increase in median light-requirement (also for the LF response) at decreasing GA3 concentrations (Figs. 1 and 2) is probably due to increasing induction of secondary dormancy (20). In the presence of 0.01 to 1 mm GA3, establishment by R or FR of very low amounts of Pfr, promotes the VLF response. Based on the formulas of Hartmann and Cohnen-Unser (15) and the photochemical cross-section values measured by Kelly and Lagarias (18), the calculated Pfr amounts established by one R pulse in the VLF range (10\(^{-9}\)–10\(^{-6}\) mol/m²) (8) range from 0.0002 to 0.2%. These values were calculated, taking into account wavelength-dependent screening and assuming that the level of preexisting Pfr is negligible as can be deduced from the very low dark germination. Considerably higher Pfr amounts are necessary to obtain the LF response (from about 2–3% Pfr to saturation).

The LF response, induced by 7.2 \( \times 10^{-4} \) mol/m² (total fluence) broad-band R, is reversed by FR to the level of maximum VLF response induced by saturating FR alone (Fig. 3).

To investigate the effect of exogenously applied GA3 on the maximal response as a consequence of the VLF response, the seeds were given very limited fluences of R or FR, saturating the VLF response. At all GA3 concentration levels two R or FR saturating pulses induce nearly identical maximal germination responses (Fig. 4). With increasing GA3 concentrations the maximal response increases to reach a maximum of 95% at 1 mm GA3.

We also investigated the effect of exogenously applied GA3 on the maximal response as a consequence of VLF plus LF component (see second maximum in the curves of Fig. 1) also increases with the GA3 concentration. For each experiment of Figures 1 and 2, the population parameters of both VLF and LF component, are calculated by means of probit analysis. At all GA3 concentrations and for VLF and LF component, there is a linear relation between the logarithm of the total photon flux and the probit of the germination response. Despite individual deviations, due to consecutive experimental conditions, the slopes of all LDP lines for the LF as well as for the VLF response are very similar (data not shown). The fluence for half-maximal response (μ) indicates the median light requirement of the responding seed population. Three light-sensitivity (1/μ) ranges are observed. For the LF response the R fluences for half-maximal germination range from 3.9 \( \times 10^{-4} \) to 4 \( \times 10^{-3} \) mol/m². The VLF response can be saturated with R as well as with FR. The fluences for a half-maximal saturation of this VLF response with R range from 3.9 \( \times 10^{-4} \) to 8.2 \( \times 10^{-4} \) mol/m². About 200 times more light is needed to saturate the same response with FR. The VLF response requires about 20,000 times less R fluence than the LF response (Fig. 1). The limited increase in median light-requirement (also for the LF response) at decreasing GA3 concentrations (Figs. 1 and 2) is probably due to increasing induction of secondary dormancy (20). In the presence of 0.01 to 1 mm GA3, establishment by R or FR of very low amounts of Pfr, promotes the VLF response. Based on the formulas of Hartmann and Cohnen-Unser (15) and the photochemical cross-section values measured by Kelly and Lagarias (18), the calculated Pfr amounts established by one R pulse in the VLF range (10\(^{-9}\)–10\(^{-6}\) mol/m²) (8) range from 0.0002 to 0.2%. These values were calculated, taking into account wavelength-dependent screening and assuming that the level of preexisting Pfr is negligible as can be deduced from the very low dark germination. Considerably higher Pfr amounts are necessary to obtain the LF response (from about 2–3% Pfr to saturation).

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The GA3 concentration determines the response population fraction in the VLF range of a seed population irradiated with a single light pulse (8), and also determines the responding population fraction in the VLF and LF range of a seed population irradiated with two separated light pulses (Fig. 4).

The sigmoid curves calculated from the equations (1, 2) for a homogeneously responding population, fit well the experimental data (Fig. 2; see also Ref. 8). Moreover, the biphasic curves calculated from the equation (3) for a heterogeneously responding population also fit well with the experimental data (Fig. 1). Consequently, probit analysis indicates two distinct responses; for both, the probit of the germination response is linear on a log fluence scale, indicating a logarithmic normal distribution of the response around a mean level of fluence required for half-maximal germination in the responding seed population fraction.

**Fig. 4.** Maximal germination of Kalanchoë seeds as a function of the external GA3 concentration for two irradiations on d 7 and 8. Calculated curves from population parameters (--), indication of μ (----). Pooled results from three experiments using different saturating VLF irradiations with narrow-band R: 4, 10 and 40 \( \times 10^{-4} \) mol/m² (+), pooled results from three experiments using different saturating LF irradiations with narrow-band FR: 2, 6.5 and 20 \( \times 10^{-4} \) mol/m² (x), pooled results from three experiments using different saturating LF irradiations with broad-band R: 3, 8 and 20 \( \times 10^{-4} \) mol/m² (○). All germination percentages are based on 3 \( \times \) 6 batches of about 100 seeds.

**DISCUSSION**

Four daily R irradiations, given from the first day on after sowing to seeds incubated on water or KNO₃ solution, induce a germination response in the LF range and no VLF responding population fraction is present. One irradiation given on d 7 after sowing, to seeds incubated on GA3, induces germination responses in the VLF range and no clearcut LF responding population fraction is present (8).

Two irradiations given to seeds incubated on GA3 induce a germination response both in the VLF and in the LF range. The population parameters (slopes and median light requirements), for both response types, are hardly affected by the various GA3 concentrations. The similarity of the slopes of the LDP lines for VLF and LF responses indicates that GA3 has no direct influence on the phytochrome-phototransformations. Both responses are Pfr initiated, the LF response is FR reversible, the VLF response is not.

The GA3 concentration determines the response population fraction in the VLF range of a seed population irradiated with a single light pulse (8), and also determines the responding population fraction in the VLF and LF range of a seed population irradiated with two separated light pulses (Fig. 4).

The sigmoid curves calculated from the equations (1, 2) for a homogeneously responding population, fit well the experimental data (Fig. 2; see also Ref. 8). Moreover, the biphasic curves calculated from the equation (3) for a heterogeneously responding population also fit well with the experimental data (Fig. 1). Consequently, probit analysis indicates two distinct responses; for both, the probit of the germination response is linear on a log fluence scale, indicating a logarithmic normal distribution of the response around a mean level of fluence required for half-maximal germination in the responding seed population fraction.
In the VLF range, this reflects a logarithmic normal distribution of the response around a mean level of Pfr-requirement for half-maximal germination. Probit analysis can also be applied for calculation of the sigmoid dose-response curves for the effect of different GA₃ concentrations on the response range (K⁺) indicating a cumulative distribution of the response as function of the log GA₃ concentration (Fig. 4). This suggests that the probability for activating a proportion of the population responding in the VLF or LF range is determined by the GA₃ concentration. Consequently, in *Kalanchoë* seeds, GA₃, the factor sensitizing the seeds, also affects the LF response, in contrast with the situation in sensitized lettuce seeds (27) or *Avena* coleoptile sections (23). As shown in Figure 4, however, the VLF response is promoted by GA₃ concentrations up to approximately 0.5 mm, whereas maximum LF response can be calculated (difference between total response and VLF response) and is already obtained with 0.03 to 0.04 mm GA₃.

Since it is very unlikely that GA₃, inducing the VLF response, could alter the total phytochrome pool by a factor of more than 10,000, it is probable that GA₃ changes the sensitivity of the seeds or by altering the transduction of the Pfr signal, as suggested by Shinkle and Briggs (22) or by activating another transduction chain with a much lower threshold as proposed by Sharma (21).

GA₃, inducing a VLF response, might activate one or more genes. The GA₃ concentration could determine the degree of activation reflected by the modulation of the responding population fraction. Gene activation by GA₃ was already shown for the production of α-amylase in barley aleurone layers (17). Alternatively, GA₃ might have an effect on membrane properties as was proposed for the sensitizing effect of temperature transitions and ethanol (26, 27).

Dark germination (maximum about 5%) is due to the presence of a very low level of native Pfr and takes place during the first 7 d of dark incubation. Based on the similarity of the population parameters μ and B (8), the effect of GA₃ on the dark responding population fraction is analogous with the effect of GA₃ on the VLF responding population fraction. This could indicate that, for a given Pfr concentration, independently of its origin (served in dry seeds or formed by light), the probability for inducing a response is directly proportional to the GA₃ concentration. Dark germination was never observed in the cv Feuerblute (13), indicating that GA₃ alone cannot induce germination. The relatively high dark germination of most seed species in the presence of GA₃'s, not or only partially reversible by FR, could be due to the induction of a VLF response by GA₃'s in seeds, containing more native Pfr than *Kalanchoë*.

Comparison of the results for two pulses with those for one (in the presence of GA₃) or four (in the absence of GA₃) pulses (8), suggests that the number of irradiations could, in first instance, determine the LF responding population fraction and could also determine, less drastically, the VLF responding population fraction. A clearcut LF response is only obtained after at least two irradiations. The length of the intervening dark period is of crucial importance for the development of responsivity to low fluence R pulses (data not shown).

In phytochrome-mediated seed germination, shifts from a LF response to a VLF plus LF response were obtained with high and low temperature treatments (1, 3, 4, 14, 19, 24, 26–28) and ethanol treatment (26, 27). R-stimulated growth or subapical coleoptile sections cut from etiolated oat seedlings shift from a LF response to a LF plus VLF response, or to a VLF response only, depending on the concentration of added IAA (22). The effect of the GA₃ concentration on *Kalanchoë* seed germination is comparable with the duration of prechilling (27) or the IAA concentration (22, 23). Fusisocin or low pH cannot induce a VLF response. GA₃→₃, however has a GA₃-like effect but at lower concentrations (6).

From two current models, accounting for the occurrence of VLF and LF induced phytochrome responses (2, 27), the Blaauw-Jansen model, based on phytochrome destruction, seems not compatible with our results. Indeed, the different characteristics of VLF response and LF response with regard to the number of irradiations required and to the range of GA₃ concentrations promoting them, indicate that they are two physiologically distinct responses. VLF and LF responses may be related to the respective formation of two active phytochrome dimer-receptor complexes, Pr:Pfr-X and Pfr:Pfr-X as proposed by VanDerWoude (26, 27). GA₃ sensitization of germination responses to VLF irradiations is consistent with this dimer model.

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