Short Communication

Biphasic Fluence Response Curves for Induction of Seed Germination

Richard E. Kendrick* and John W. Cone
Plant Physiology Research, Agricultural University, Generaal Foulkesweg 72, 6703 BW Wageningen, The Netherlands

ABSTRACT

Fluence-response curves for the induction of seed germination after 24 hours pretreatment at 35°C of Rumex obtusifolius and Arabidopsis thaliana show two phases of response: (a) a very low fluence-response (10^-4 - 10^-1 micromoles per square meter) and (b) a low fluence-response (1 - 10^1 micromoles per square meter).

A seed population of Rumex obtusifolius contains dark germinating seeds and light requiring seeds (11). Some light-requiring seeds can be induced to germinate in darkness by a 10-min 35°C pulse given to seeds otherwise maintained at 25°C. Do these seeds germinate because of increased sensitivity of the whole population to the endogenous far-red absorbing form of phytochrome? Recently, those seeds still requiring light after 35°C treatment have been shown not to have increased sensitivity to Pfr, arguing against this possibility (12). However, results with Pfr-depleted lettuce seeds (2, 3, 10, 13) and other responses (9) show biphasic response, a VLFR,^2 (10^-4 - 10^-1 μmol m^-2) and a LFR (10^-3 - 10 μmol m^-2). Therefore, 10 min 35°C could switch a proportion of seeds from the LFR to the VLFR state, resulting in the Pfr requirement being satisfied by the endogenous Pfr level. Here, biphasic fluence-response curves for the light induction of germination are demonstrated for R. obtusifolius and Arabidopsis thaliana.

MATERIALS AND METHODS

Approximately 100 seeds were sown in each of 4 Petri dishes on moistened filter paper. After sowing Rumex obtusifolius seeds were treated for 24 h at 35°C, irradiated and incubated for 3 d at 25°C before monitoring germination. After sowing Arabidopsis thaliana seeds were treated for 8 d at 2°C, 24 h at 35°C, irradiated and incubated for 4 d at 20°C before monitoring germination. All manipulations were carried out in absolute darkness. Results represent means ± se of two pooled independent experiments.

Irradiation was with 660 nm produced by filtering the light from a quartz-iodide projector lamp through an interference filter (Balzers B40, Liechtenstein) about 10 nm bandwidth at 660 nm.

RESULTS AND DISCUSSION

Biphasic fluence response curves for the light induction of germination have been observed by appropriate pretreatment of lettuce seed batches that exhibit high dark germination (2, 3, 10, 13). Since the sensitivity of VLFR seeds is so great, 0.01% of the total phytochrome as Pfr being sufficient to saturate the response, a prerequisite for their observation is to deplete the seeds of their endogenous Pfr. Blaauw-Jansen et al. (2, 3, 10) achieved this by a 24-h 37°C treatment, presumably working through stimulation of Pfr destruction and/or dark reversion to Pr. Since these seed batches initially germinate in darkness it is impossible to say that the 37°C treatment converts seeds from the LFR to the VLFR state. VanDerWoude (13) depleted the Pfr of a lettuce seed batch by short far red preirradiation and 24 h in darkness at 20°C. He clearly demonstrated induction of sensitivity by a terminal high or low temperature treatment. Even treatment with alcohol induced sensitivity and he explains the results on the basis of a dimeric phytochrome membrane receptor model of phytochrome action. He proposes that the VLFR state seeds respond to heterologous dimers (Pfr-Pfr), whereas in the LFR state seeds respond only to homologous dimers (Pfr-Pfr).

To visualize a biphasic fluence-response curve, two criteria must be satisfied: (a) depletion of the endogenous Pfr level and (b) the induction of the VLFR state. In our previous work (11, 12) with R. obtusifolius the short (10-60 min) 35°C treatment satisfies only one of these: the induction of the VLFR state. For depletion of the endogenous Pfr, a longer period at 35°C is required. In the experiment reported in Figure 1, R. obtusifolius seeds were incubated for 24 h from the time of sowing at 35°C. Such a treatment results in a low dark germination on transfer to 25°C. The fluence-response curve for induction of germination of these seeds clearly exhibits two phases of response. Since the VLFR seeds are so sensitive to Pfr, care must be taken not to expose the seeds to even the dim green safe light used for photomorphogenesis research, since this itself satisfies their Pfr requirement. Figure 1 also demonstrates that biphasic fluence response curves can be obtained with Arabidopsis thaliana by a similar 35°C treatment. Clearly, such a fluence response behavior is not restricted to lettuce and may indeed be characteristic of all phytochrome controlled seed germination. The constancy of the
light, germinate in darkness because of their high Pfr content or because the seed population consists of VLFR seeds (7). Prolonged far red is most effective in inhibiting germination of such seeds, but its precise working mechanism is not known. Does it remove Pfr, as it gradually becomes hydrated during imbibition or as it is formed from intermediates of phototransformation that were trapped upon dehydration (6)? These possibilities seem unlikely on the basis of measurements of water content (8). An alternative proposal can now be suggested, that far red works through a phytochrome cycling process (1) which switches seeds from the VLFR to the LFR state. There is some support for this standpoint in the literature with lettuce (10), where far red fluence-response curves for VLFR seeds show an optimum curve. High fluences result in low germination and induction of LFR characteristics. It is possible that dark germinating species, such as Amaranthus caudatus (7), have seed populations composed of a high proportion of VLFR seeds and that prolonged far red inhibits germination by induction of the LFR state.

Acknowledgments—We thank Dr. M. Koornneef for supplying the Arabidopsis seeds; G. H. Heerings and P. A. P. M. Jaspers for technical assistance.

LITERATURE CITED


Fig. 1. Fluence-response curves for induction of seed germination of (a), Rumex obtusifolius; (b), Arabidopsis thaliana; % germination plotted against logarithm of fluence of 660 nm. The irradiation time was kept constant at 20 s. Rumex seeds (a) were pretreated for 24 h at 35°C to induce high light sensitivity in the population and reduce the endogenous Pfr level, irradiated and incubated for 3 d at 25°C before monitoring for germination. Arabidopsis seeds (b) were sown as above, pretreated for 8 d at 2°C, 24 h at 35°C; irradiated and incubated for 4 d at 20°C before monitoring for germination. D, dark.

Response regions of the VLFR and LFR seeds with those also observed for photocatalysis of growth, Chl, and anthocyanin synthesis is noteworthy and suggests a common underlying control system (2).

What is the possible significance of a biphasic fluence-response in the natural environment? It has been shown that seeds buried near the soil surface are capable of germinating if they have favorable conditions (4). If light-requiring seeds only responded to fluences in the LFR, seeds buried more than a few millimeters would not be able to germinate (5). A biphasic fluence-response enables a broader range of fluence to be used as an indication of the proximity of the soil surface.

An intriguing question which can now be asked is, do seeds of those species called dark germinators, which are inhibited by