Actions of Gibberellic Acid and Phytochrome on the Germination of Grand Rapids Lettuce Seeds[1]

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ABSTRACT

Red light and gibberellic acid were about equally effective in promoting germination of Grand Rapids lettuce (*Lactuca sativa* L.) seeds. With initial far red light treatment more than 80% remained dormant in subsequent dark storage. After 2 days of dark storage, red light effectively promoted germination, while gibberellic acid action was weak. With between 2 and 10 days of dark storage, gibberellic acid had little effect, while promotion by red light decreased slowly and finally disappeared. After 10 days of dark storage, both gibberellic acid and red light were required for germination. The dark storage treatment interferes with phytochrome-independent germination processes and cannot be overcome by added gibberellic acid. However, storage may also decrease the effectiveness of endogenous gibberellins. Phytochrome-dependent germination seems to require only low levels of endogenous gibberellin activity or the addition of gibberellic acid. Gibberellins and red light appear to act on germination by regulation of sequential sites of a branched-looped pathway.

A recurring problem in light-mediated seed germination studies centers on interactions of R^2 and gibberellins in promoting germination. Some considerations have included R stimulation of endogenous gibberellin production or activity (11, 14). Ikuma and Thimann (8, 9) have concluded that instead of being a product of R-promoted processes, gibberellins initiate one of the chemical reactions resulting from the primary light reaction. Negbi *et al.* (12), suggested that two phytochrome (or phytochrome-like) pigments mediate germination in these seeds; one site presumably regulates the production of "substrate" required for germination, while another and later site can act in conjunction with gibberellins or other promoters to stimulate germination. Haber and Tolbert (5) and others (2, 3) present evidence that R or FR cannot influence levels of gibberellin or other hormones. Synergy in germination promotion by the FR-absorbing form of phytochrome and added gibberellins, reported by most workers (1–4) tends to preclude a possibility that gibberellins merely mimic the effect of R. That there is little agreement on the mechanisms by which gibberellins and R influence germination suggests that their interactions are poorly understood.

We have examined aspects of gibberellin-phytochrome interactions and conclude that these agents exert their influence on the germination of Grand Rapids lettuce seeds by regulation of sequential sites on a branched-looped germination pathway.

MATERIALS AND METHODS

All experiments were carried out at 20 C with three replicates of 33 Grand Rapids lettuce seeds (*Lactuca sativa* L.), 1970 harvest, obtained from Buckerfields Ltd., Vancouver, B. C. These seeds have been stored at −20 C since their acquisition and normally exhibit strong light sensitivity. In some experiments, seeds were given an initial 1-min irradiation with FR (730 nm, 60,000 ergs $cm^{-2} sec^{-1}$) at about 0.5 hr after the onset of imbibition; in others no initial light treatment was given. Seeds were, in some cases, irradiated with R (660 nm, 60,000 ergs $cm^{-2} sec^{-1}$) either initially, immediately after FR, or some multiple of 2 days after the onset of imbibition. When 0.5 mM GA (Sigma, St. Louis, Mo.) was given, it was either present from the onset of imbibition or seeds were transferred from distilled water to fresh GA solution at some multiple of 48 hr after the beginning of imbibition. For clarity all time intervals from day 0 till the final treatments with R, GA, or both will be referred to as dark storage, and the post-treatment interval (usually 48 hr) until germination was scored, as the germination test. After each 2-day DS interval, any seeds observed to have germinated were removed and discarded; subsequent germination percentages are those of the remaining seeds. Inspection of Figures 1, 2, and 3 shows that few seeds germinated after the first 2 days of storage unless further treatment was given. Except as noted here, all experiments and irradiations were carried out according to the methods of Hsiao and Vidaver (6). Standard error is shown in the figures.

RESULTS AND DISCUSSION

Figure 1 shows germination percentages determined 48 hr following DS for seeds receiving initial FR or no light after 0 to 10 days of storage. Closed symbols show germination percentages of unstored seeds after 24 hr of imbibition for comparison. It is clear that whether or not initial FR was given, very few seeds germinated after 48 hr of dark storage. As expected, without storage germination by seeds not given FR was considerably higher than in seeds receiving FR. The stored seeds, as will be shown, are completely viable, but without further treatment remain in a state of secondary dormancy (12).

Figure 2 shows the influence of added GA and R on dark-stored seeds receiving initial FR irradiations. A slight promotion of germination occurs as a response to the hormone when given during the first few days. By day 4, however, GA has little effect. Also shown in this figure is the more pronounced promotive effect of R. Despite the greater effectiveness of R

than of GA in breaking the dormancy imposed by dark storage, it, too, eventually becomes ineffectual after about 10 days. At any time up to and including the 10 days used here a combined treatment with GA and R can break the dormancy resulting from dark storage.

Figure 3 shows germination responses of dark stored seeds not receiving initial FR. While the initial germination (day 0) of these seeds is considerably higher than in seeds irradiated with FR (Fig. 2), those seeds not germinating become dormant as quickly as the irradiated seeds. Subsequent germination tests indicate that R and GA influence the dark seeds similarly and to about the same extent as FR irradiated seeds.

R and GA need not be given at the same time in order to induce high germination percentages. Examples not presented in the figures show that seeds given R at day 8 followed with transfer to GA at day 10 germinated 87%, while those given the inverse treatment produced 82%. Merely giving multiple R treatments in the absence of GA or second transfers to fresh GA without R did nothing to promote germination however.

Four distinct phases of dormancy can be seen in seeds given storage treatments. (a) Initially, R and GA are about equally effective in promoting germination. (b) After 2 days of dark storage, R retains most of its effectiveness, but the promotive action of GA is weak. (c) With between 2 and 8 days, storage GA has little to no effect, but the R effect decreases relatively slowly. (d) After 8 days of storage, both agents are required to induce germination, but this is near maximal, as it is when both treatments are given at anytime during dark storage.

The well known fact that added GA will overcome the influence of FR in retarding germination applies here only to seeds stored for 2 days or less. The capacity of FR-irradiated seeds to germinate with added GA appears to be a manifestation of processes leading to germination which are independent of the phytochrome system but require minimum gibberellin levels for their activity. Storage appears to cause irreversible blockage of this dark germination pathway which cannot be overcome by added GA.

The R response, which disappears much less rapidly than the

FIG. 2. Germination percentages of seeds 48 hr following 0 to 10 days of dark storage given initial FR. One the day shown, seeds were transferred to GA solution (FR → GA), given R (FR → R), or transferred to GA and given R (FR → R → GA). Other conditions are as shown in Fig. 1.

FIG. 3. Germination percentages of seeds 48 hr following 0 to 10 days of dark storage receiving no initial light (D). On the day shown seeds were transferred to GA solution (D → GA), given R (D → R), or transferred to GA and given R (D → R + GA). Other conditions are as shown in Fig. 1.
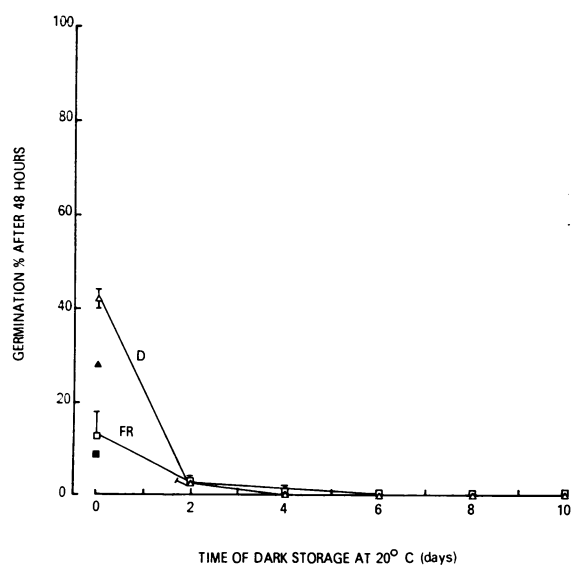

FIG. 1. Germination percentages of seeds 48 hr following 0 to 10 days of DS, given initial FR or no light (D). Seeds which germinated during storage were discarded and percentages shown are those of the remaining seeds. Solid symbols indicate germination percentages after 24 hr of unstored seeds with FR or no light.
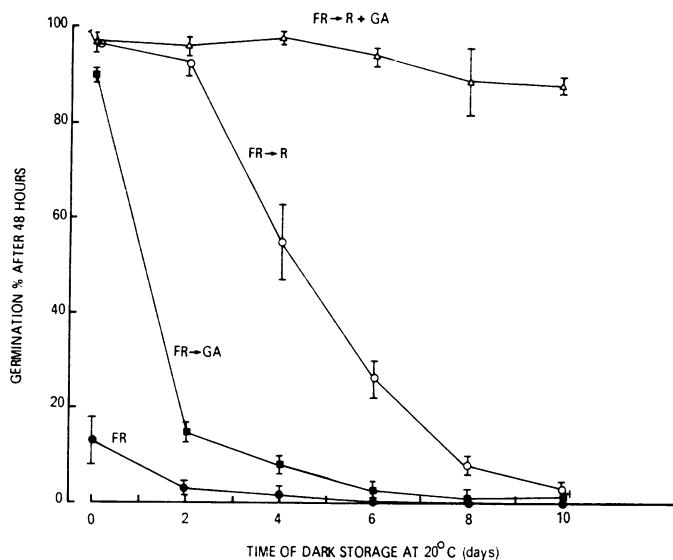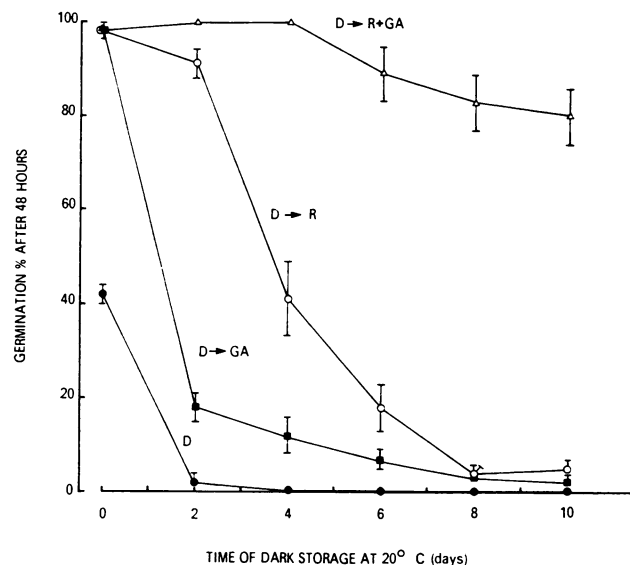
dark GA-mediated one, obviously represents the promotive functioning of the phytochrome system. Retardation of germination, apparent for the first two days of storage is of course attributable to low levels of Pfr. Loss of capacity to respond to R with storage again suggests an endogenous dark process which reduces the effectiveness of Pfr in promoting germination. In this case, however, dormancy is completely broken when GA and R are both given. Phytochrome-mediated germination promotion is now dependent on exogenous GA for its expression.

At first consideration it might appear as though GA mediates two independent processes, either of which can lead to germina-

tion in these seeds (10, 13). The first of these requires no light, but is rapidly inhibited by dark storage, the second, which is certainly mediated by the phytochrome system, becomes ineffective relatively slowly with dark storage. Possibly two distinct pathways are initially capable of supporting germination in these seeds, especially so since the conditions under which they function are so clearly different. Sites of regulation by the agents used here, could in some, if not all, cases be at the level of intermediate metabolism. The main differences between these pathways appear to be the lability of the dark one to storage and its requirement for exogenous GA in these seeds. Since the phytochrome-dependent pathway also requires added GA after several days, it may be concluded that the effect of extended storage is to reduce the effectiveness of endogenous gibberellin to levels below which the phytochrome-mediated pathway is able to support germination.

These results with fully imbibed seeds (DS) contrast with those obtained by Hsiao and Vidaver (7) using dark-moist storage treatments (storage in a water saturated atmosphere) where the partially hydrated seeds remain highly responsive to R for at least 28 days. Presumably, endogenous gibberellin activity persists in low water content seeds but is slowly lost in the fully imbibed seeds. If loss of gibberellin activity depends on respiration or other enzymatic processes, water sufficient to activate the enzymes involved is essential. The differences in results with the two kinds of treatments may reflect low and relatively higher metabolic levels.

Our findings may be summarized by a scheme which is a modification of one proposed by Negbi et al. (12), and partially formulated previously by Bewley et al. (1).

A. Light-sensitive pathway

B. Nonlight-sensitive pathway
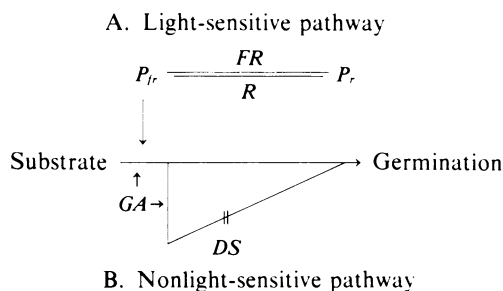
Where A requires low levels of GA and is reversibly mediated by the phytochrome system, B requires high levels of gibberellin, no light, and is irreversibly labile to DS treatment.

Secondary dormancy in lettuce seeds, according to this scheme, is a consequence of DS treatment effecting blockage of the nonlight-sensitive germination pathway.

Our evidence for this model is admittedly by no means conclusive; any of numerous alternative explanations may be correct. Nevertheless, variations in germination responses of

different lots of Grand Rapids lettuce seeds, so well known to all who have worked with them, could be explained by means of the model shown here. Seeds with high dark germination percentages and which respond weakly to FR would have both high endogenous gibberellin activity levels and an effective dark germination pathway. Seeds which are highly responsive to R and FR might express minimal gibberellin activity, but if promotable by GA, should have a functioning dark pathway as well as the phytochrome-dependent system. Intermediate types of responses would result from variations in the relative activities of the dark germination pathway and endogenous gibberellins. The phytochrome-mediated pathway seems to be remarkably unaffected by the treatments used here. Consequently, the effects of previous treatment, such as harvesting conditions or processing procedures of the distributor, appear not to be on the phytochrome system but rather on endogenous gibberellin activity levels and nonlight-mediated germination pathways.

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