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Light Regulation of the Growth Response in Corn Root Gravitropism[1]

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ABSTRACT

Roots of Merit variety corn (*Zea mays* L.) require red light for orthogravitropic curvature. Experiments were undertaken to identify the step in the pathway from gravity perception to asymmetric growth on which light may act. Red light was effective in inducing gravitropism whether it was supplied concomitant with or as long as 30 minutes after the gravity stimulus (GS). The presentation time was the same whether the GS was supplied in red light or in darkness. Red light given before the GS slightly enhanced the rate of curvature but had little effect on the lag time or on the final curvature. This enhancement was expanded by a delay between the red light pulse and the GS. These results indicate that gravity perception and at least the initial transduction steps proceed in the dark. Light may regulate the final growth (motor) phase of gravitropism. The time required for full expression of the light enhancement of curvature is consistent with its involvement in some light-stimulated biosynthetic event.

Gravitropism in roots of many varieties of corn is enhanced by, or in some cases requires, light (6, 12, 14). Light has also long been known to inhibit growth of a wide variety of plant roots (13, 15, 19). The light requirement common to these two phenomena has been accounted for in a comprehensive model (18) in which it is proposed that light inhibits root growth and, through this effect, enhances or induces the asymmetry of growth rates that results in curvature.

The root cap serves as the sensor both for gravitropism and for the red-light regulation of growth. The latter effect is thought to involve the biosynthesis of the inhibitor, ABA (21). Wilkins and Wain (21) suggested that the root cap has a dual role, serving as the site of light sensing as well as of gravity sensing.

The capability of some roots to express diagravitropic curvature in the dark indicates that some initial steps of gravitropism proceed in the absence of light (10). Perhaps, then, the light enhancement or induction of gravitropism is unrelated to the initial steps of gravitropism and the light effect may occur instead at some later step. It may play a role in the final growth response phase of gravitropism.

The enhancement of gravitropism by red light offers an opportunity to analyze the signal transduction pathway in plant gravitropism. The study reported herein was undertaken to determine with greater certainty the light-regulated step in gravitropism of Merit corn, a variety that requires red light for normal orthogravitropic curvature.

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MATERIALS AND METHODS

Plant Material and Growth

Caryopses of corn (*Zea mays* L. cv Merit, Asgrow Seed Co., Kalamazoo, MI) were sown in rolls of damp germination paper standing in 1 L beakers with sufficient tap water to ensure a humid environment. The caryopses were oriented so that the radicles emerged and grew in a downward orientation. The beakers were incubated in the dark at 25 ± 1°C for 40 to 46 h.

Experimental

Under a dim green safelight (General Electric 25 W incandescent green bulb, shielded with a second green glass filter; approximately 95% of the surface area of the filter was covered with black tape), seedlings with straight roots approximately 10 to 20 mm long were selected. Eight to 10 seedlings were arranged between plexiglass slabs lined with damp cheesecloth so that the roots protruded approximately 1 cm beyond the plexiglass. The plexiglass assembly was secured in a plastic box that was closed to maintain an environment of high humidity. The roots were exposed to the safelight for approximately 2 min during the preliminary manipulation. The box was equilibrated at least 90 min in the dark with the roots at 180° orientation from the upright vertical. All angles are relative to the upright vertical considered to be 0°. The desired gravistimulus was given by orienting the entire box assembly. All experiments were done at 25°C.

Red light was supplied by two fluorescent red bulbs (approximately 2 $\mu E\ m^{-2}\ s^{-1}$). Clinostat treatments were applied with a horizontal clinostat at 2 rpm. When red light was applied during clinostating, the fluence was about 10% of the standard light treatment, but both levels, given continuously, should have fully saturated the light requirement of Merit corn roots (6, and consistent with our experience). White light was supplied with ambient room fluorescent lighting. At the end of the experiments, curvature was measured by enlarging the root image with a video camera, and measuring the angle of curvature of the image between the original straight orientation and the root tip.

RESULTS

In this study, we have examined the effect of red light given before, during, and after the gravity signal to probe the sequence of events involved in gravitropism in Merit corn roots. We use the terminology of Feldman (3), and consider gravity perception to be related to the very early physical consequences of a change in the orientation of an organ with respect to gravity. Only the response to a 90° stimulus was considered, as these roots require light for the development of positive curvature in response to this stimulus (6, 10).

The behavior of the seedling roots given a continuous gravity signal in the dark is presented as a time course of curvature in Figure 1A. About 10° of curvature accumulated

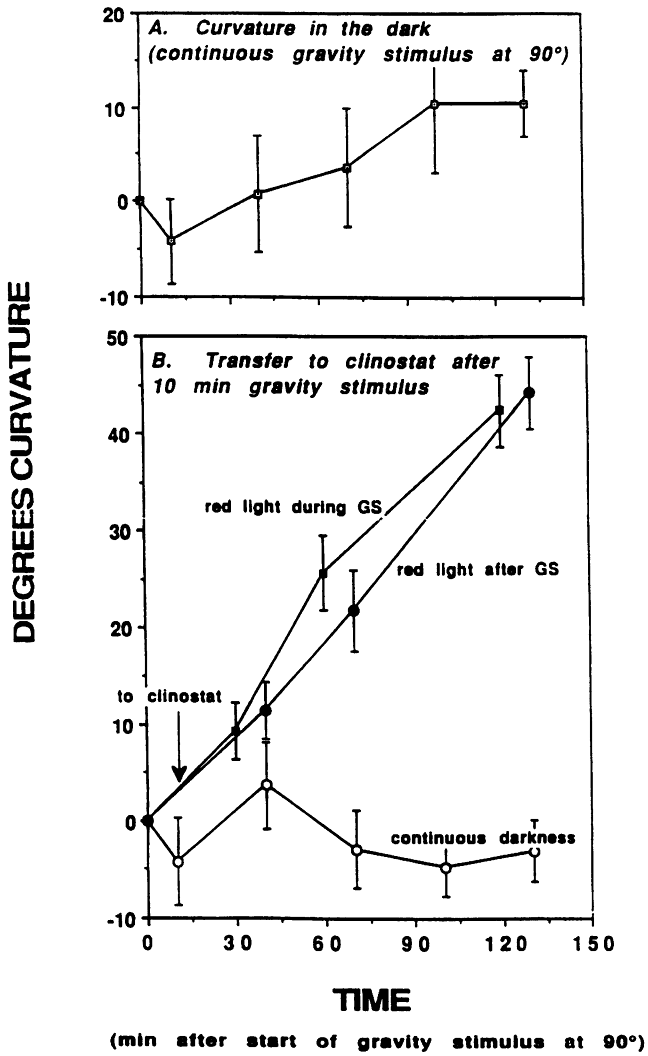

Figure 1. A, Time course of gravitropic curvature of Merit corn roots in complete darkness. Seedlings were given a continuous 90° gravity stimulus. B, Time course of curvature of seedling roots transferred to a clinostat after 10 min gravity stimulus (GS). The open circles represent an experiment done in complete darkness. The closed circles represent an experiment in which red light was supplied after transfer to a clinostat. The square symbols represent an experiment in which red light was given at the start of the gravity stimulus as well as during clinostat treatment. Data represent the mean values of single typical experiments; error bars represent SE of the mean (n = 8–10).

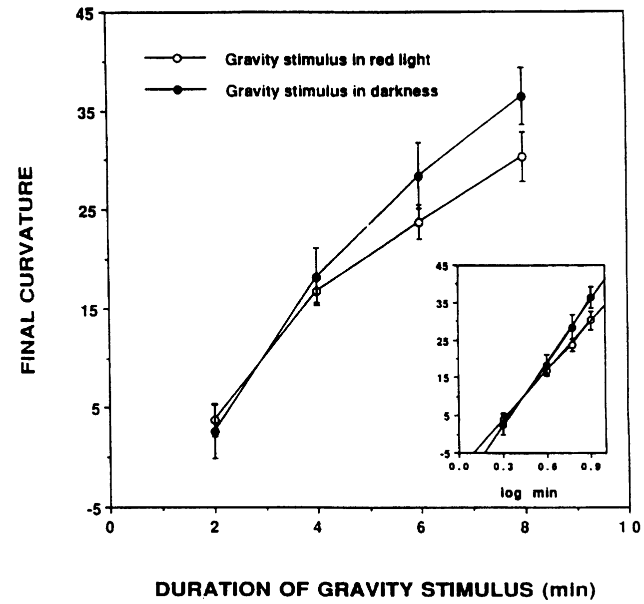

Figure 2. Gravitropic curvature following brief periods of gravity stimulus at 90° given in the dark or in red light. Seedlings were transferred to a clinostat in red light after the gravity stimulus. Curvature was measured 90 min after the start of the red light treatment. Data for dark treatment represent the mean values of two experiments; data for the light treatment represent the mean values of three experiments. Error bars represent SE of the mean (n = 18–26).

in 2 h of orientation at 90° in the dark. When the seedlings were oriented at 90° for 10 min only and then transferred to a clinostat, all in darkness, there was no detectable curvature (Fig. 1B). However, when red light was applied immediately after the seedlings had been given 10 min of gravity stimulus in the dark and transferred to the clinostat (within 0–20 s), the roots developed 45° of curvature in 2 h (Fig. 1B). The time course and the final amount of curvature detected were essentially the same whether the red light was given concomitant with or after the gravity stimulus (Fig. 1B). Clearly, the roots responded to a gravistimulus supplied in the dark.

The next experiment was designed to determine whether red light during the gravistimulus affected the presentation time, the minimum duration of gravity stimulus necessary for detectable curvature (16). The presentation times for a 90° gravity stimulus given in the dark and in red light were measured and compared. The data are presented in Figure 2. Presentation times were extrapolated from log plots of these data (Fig. 2, inset). The x axis intercept of lines fitted (r^2 = 0.998) to these curves is considered an estimate of the presentation time (9). The values derived from these plots were 1.8 min for seedlings given a gravity stimulus in the dark *versus* 1.6 min for those given a gravity stimulus in red light. Thus, the presentation times were not significantly altered by red light during gravistimulation.

An experiment was designed in an attempt to distinguish between red light effects on gravity perception and on subsequent signal transduction. The duration of the "memory" of a brief gravity signal given in the dark was determined by delaying the application of red light for various intervals up to 30 min after withdrawal of the gravity stimulus, as shown in Figure 3. The promotion of curvature by red light was almost the same regardless of the timing of the red light application, up to a 30-min delay. This result indicates that some transductive effect persisted long after the stimulus had been withdrawn, when it seems likely that gravity-sensing systems such as statoliths (1, 2) or a hydrostatic pressure gradient (17) would have lost their gravity-induced orientation.

An earlier finding showed that red light given before the gravity signal enhances gravitropism of corn roots of cv LG11, another variety that requires red light for gravicurvature (20). Based on this finding, the onset and development of curvature were compared in seedlings given a 30-s pulse of red light, followed either by an immediate gravity signal or by a signal delayed by 30 min in darkness. The data are presented in Figure 4. In each case, curvature was detected 15 to 20 min after the gravity stimulus was supplied. Once it had begun, curvature proceeded at a faster rate in the light-pretreated roots, but both treatments had developed the same curvature by 2 h (Fig. 4). In this experiment, the early "wrong way" curvature described by Hild and Hertel (8) can be detected, although we do not consistently observe this behavior. This experiment confirms the enhancement of curvature development after red light pretreatment (20).

To determine the optimum time for pretreatment with red

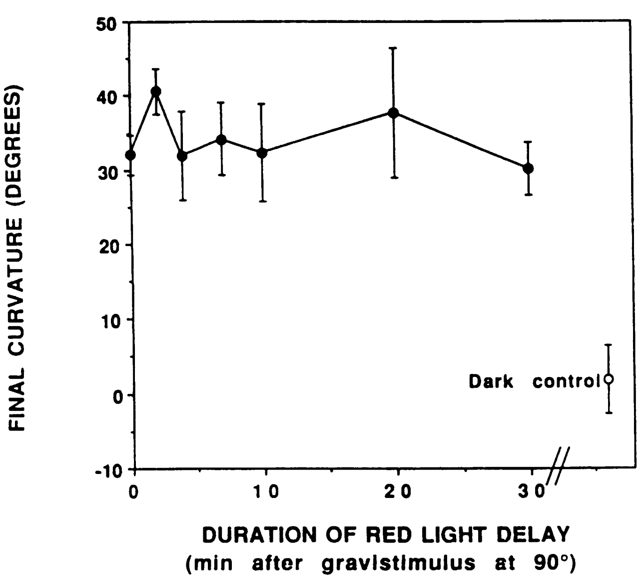

Figure 3. Gravitropic curvature following a 10-min gravity stimulus at 90° given in the dark. The seedlings were transferred to a clinostat immediately after the gravity stimulus and given red light at the indicated times after transfer to a clinostat. The curvature was measured 90 min after the red light was supplied. The dark control was measured 90 min after transfer to a clinostat and was wrapped in aluminum foil to exclude light. Data represent the mean of three experiments. Error bars represent the SE of the mean of the experiments (n = 26).

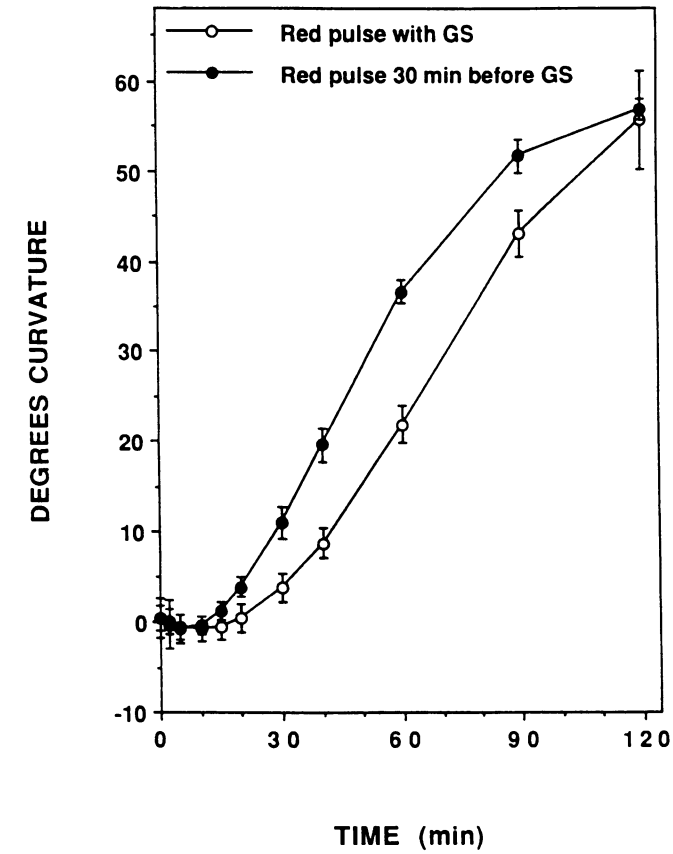

Figure 4. Time course of curvature of roots given a continuous 90° gravity stimulus in white light. Seedlings were treated with a 30-s red light pulse, either concomitant with or 30 min before the gravity signal. Curvature was measured consecutively with the same seedlings. Data represent the mean curve generated from three experiments (n = 15–16). Error bars represent SE of the mean of the experiments.

light before gravistimulation, the pulse of red light was followed by various times in darkness before the gravity signal. Curvature was measured after 40 min of gravity stimulus at 90° in white light. The data from these experiments, presented in Figure 5, indicate that periods of approximately 30 min or more after the red light pulse were maximally promotive of subsequent curvature.

DISCUSSION

This study was undertaken to determine at what point red light may act in the gravity signal transduction pathway of corn roots. A reasonable deduction, based on evidence that gravity perception is separate from the light effect (10, 21), is that red light acts in the final step, *i.e.* the growth response. Our rationale in testing this deduction is that if the growth response is the light-requiring step, it should be possible to show its independence from gravity perception and signal transduction. In this report, the term gravity perception is used to refer to the very early physical consequences of gravistimulus.

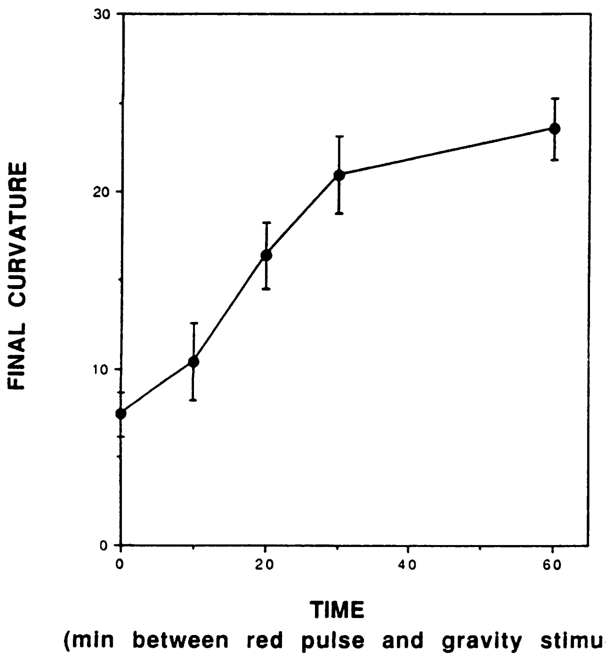

Figure 5. Gravitropic curvature of Merit corn roots as effected by different periods of delay of the gravity signal after a red light pulse. Seedlings were held in the dark for the indicated time at 180° orientation after the 30-s red light pulse. Final curvature was taken as the difference between initial curvature and that measured 40 min after gravity stimulation at 90° in white light. Data represent the mean of a single, typical experiment with 8 to 10 seedlings at each station. Error bars represent SE of the mean.

In the first set of experiments, we demonstrated that Merit corn roots responded with essentially identical curvature and the same presentation time whether the red light was given after or concomitant with the gravity signal (Figs. 1B and 2), consistent with our assumption that gravity perception does not require red light. Application of red light at various times after gravistimulation in the dark indicated that a "memory" of the signal persisted for at least 30 min, a long enough time to assume that the physical consequences of gravity would have been negated. This memory effect might logically be attributed to the persistence of some transduction events after the gravity signal was ended. Thus, we conclude that at least the initial transduction steps, as well as perception, proceeded in the dark. These results are consistent with the hypothesis that red light acts late in gravitropism.

In the second part of this study, we exploited an earlier finding that red light supplied before a gravity signal enhances subsequent curvature (20). In this experiment (Fig. 4), red light pretreatment enhanced primarily the rate of curvature, and neither the time of curvature onset nor the final curvature. An effect of red light on transduction processes should have been expected to hasten the onset of curvature. Thus, the similar times of curvature onset are also consistent with our conclusion that the primary effect of light was either on late transduction processes or on the growth response. The length of time needed for maximal enhancement of curvature was about 30 min (Fig. 5), which is consistent with red light induction of a biosynthetic step(s). The 30-min period for optimal enhancement of curvature by red light agrees well with the time course of red light-stimulated protein and mRNA synthesis in Merit roots (4, 7).

The results reported here are consistent with the following model: light enhances biosynthesis of some constituent(s) (5, 11) that may be necessary for growth regulation in corn roots. In the presence of a gravity signal, perception and early stages of transduction occur regardless of the presence of this compound(s), whereas expression of the curvature is amplified by its accumulation. Collectively, the results of these experiments indicate that red light acts late in gravitropism; given the known effect of light on growth regulation in corn roots (13, 20), it may regulate the final, growth phase.

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