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Function of Light in the Light-induced Geotropic Response in *Zea* Roots

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ABSTRACT

The light doses just above the threshold energy value for inducing the geotropic responsiveness in the roots of *Zea mays* L., cv. Golden Cross Bantam 70, caused a drastic rise in the NADPH level and a drop in the NADP level in 2-millimeter root tips. Some reducing agents lowered the threshold energy value up to about one-third of the control. From these results, we deduce that light may exert two functions in the geotropic response of *Zea* primary roots, one being the photochemical transformation of a photoreceptor and the other being the induction of a reduction state in the tissue.

Light makes available a critical component in the geotropic response mechanism of roots of some *Zea mays* cultivars (7, 9–12). We have recently demonstrated (11) that there is a threshold light energy for the induction of geotropic response in *Zea* primary roots because the response to light is governed by the "all-or-none law." This suggested that the response is initiated by the photochemical transformation of a photoreceptor, the induction of this response by light satisfying the Bunsen-Roscoe reciprocity law. However, the perception mechanism of light and the amplifying mechanism for light stimulus are not known at present.

As the amplifying mechanism for radiation signal, various investigators have suggested, in various photomorphogeneses, the metabolism related to nicotinamide nucleotide coenzymes (2, 3, 5, 6). Klein and Edsall (4) found that reducing agents interacted synergistically with low irradiances of red light. These findings gave us the suggestion that changes of the oxidation-reduction state by light could serve to amplify the light signal in the light-induced geotropic response in *Zea* primary roots.

Here, attention is focused on the changes of oxidation-reduction state (especially the changes in the levels of nicotinamide nucleotides) after a brief red irradiation and also on the effects of reducing agents on the induction of the light-induced geotropic response in *Zea* primary roots.

MATERIALS AND METHODS

Plant Material. Caryopses of *Zea mays* L., cv. Golden Cross Bantam 70 (Sakata Seed Co., Yokohama, Japan), were imbibed for 36 h in a Petri dish in the dark in running tap water which covered the grains and were then planted, with the embryo upward, on 0.4% solidified agar in containers covered with a nylon sheet. The primary roots were allowed to elongate horizontally on aluminum foil, which was placed on the agar to prevent the primary roots from getting wet in water. They were grown at 26 C in the dark, except that, during transfer and other operations, they were handled in weak green light, transmittance 490 to 580 nm with a peak at 530 nm, obtained from a 20-w "cool-green" fluorescent lamp (Mitsubishi Electric Co., Tokyo, Japan: SL-20SG) filtered through six sheets of green cellophane. The seedlings were exposed to this light for not more than 5 min, and even 1 h exposure did not establish georesponsiveness. Caryopses with primary roots 1.5 to 2.0 cm in length, obtained about 30 h after the transfer, were used for the experiments.

Geotropic Stimulation and Light Source. The procedures for gravity and light treatments were the same as described in Suzuki and Fujii (11). The primary roots of the seedlings were horizontally oriented by pinning the grains on a styrofoam plate, exposed to light, and then allowed to bend for 4 h in a moist chamber. Geotropic responsiveness was recorded by making shadow photographs of the roots. The per cent of roots exhibiting curvature and the mean curvature of the roots which did curve were used as parameters. Each result represents the response of at least 30 roots. Standard errors of the mean have also been calculated. Red light at 649 nm was obtained from a tungsten lamp (300 w: Kondo Electrical Industrial Co., Tokyo) through 30 mm water, an interference filter (Toshiba Glass Co., Shizuoka, Japan: KL-65; peak, 649 nm; half-band width, 11.0 nm), and a glass filter (Toshiba Glass Co.: V-R 62) for reducing scattered light. The radiant energies were measured with a Kipp-Zonen (Delft, Netherlands) compensated thermopile CA-1 and a direct-current microvoltmeter (Toa Electronics, Tokyo: PM-16A).

Treatment with Reducing Agents. One-mm tips of the primary roots were dipped for 15 min into 20 mM KH_2PO_4-Na_2HPO_4 buffer (pH 5.8) containing reducing agents at various concentrations and immediately wiped free of these agents with filter paper. Ascorbate, DTT, glutathione (reduced form), and sodium hydrosulfite were used as reducing agents.

Measurement of Nicotinamide Nucleotides. One hundred and fifty 2-mm-long apical segments of primary roots were excised and used as the material. The segments were exposed to various energies of light at 649 nm, immediately frozen in the dark with liquid N_2, and ground finely in a chilled mortar. The homogenates were boiled for 2 min either in 2 ml 0.1 N KOH to destroy NADP or in 2 ml 0.1 N HCl to destroy NADPH. The homogenates then were rapidly transferred to an ice bath. Each homogenate was neutralized with 0.1 N KOH or 0.1 N HCl, respectively, and then adjusted to pH 7.6 with 1.0 ml 0.2 M Tris-HCl buffer. The homogenates then were centrifuged at 8,000*g* for 15 min at 4 C. The supernatant fractions were used for the assay of nicotinamide nucleotides by cyclic enzymic systems, as reported by Yamamoto (13). The assay system for NADP and NADPH was 1.2 ml 0.2 M Tris-HCl (pH 7.6), 0.1 ml 0.2 M $MgCl_2$, 0.2 ml 1.2 mM 2,6-dichlorophenol, 0.3 ml NADPH diaphorase, 0.1 ml isocitrate, 0.7 ml H_2O, and 1.0 ml of the sample. The concentrations of NADP and NADPH were calculated from A_{610}. NADPH diaphorase was

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extracted from spinach chloroplasts by the method of Avron and Jagendorf (1) and purified up to and including step 4. The preparation contained 1.6 mg protein/ml.

RESULTS

Effect of Light on Levels of NADP and NADPH. Two-mm root tips, including the root cap which is the site of light perception in primary maize roots (8), were excised from 1.5- to 2.0-cm *Zea* primary roots and exposed to 1.97 × 10^{-10} E cm^{-2} s^{-1} red light at 649 nm for various periods of time, and the amount of nicotinamide nucleotides was determined (Fig. 1). A preliminary experiment showed that the minimum exposure time for the induction of georesponsiveness in *Zea* primary roots was 15 s in this light intensity and that the minimum light energy (the threshold energy value) was 2.96 × 10^{-9} E cm^{-2}. Figure 1 shows that the light doses just above the threshold energy value caused a drastic rise in the NADPH level and a drop in the NADP level. The data calculated from Figure 1 indicate that the ratio of NADPH/NADP rose sharply immediately after the irradiation with light doses above the threshold energy value (Fig. 2).

Effect of Reducing Agents on Light-Induction of Geotropic Response. *Zea* primary roots pretreated with 20 mM KH_2PO_4-Na_2HPO_4 buffer (pH 5.8) with or without 1 μM ascorbate for 15 min were exposed to 1.97 × 10^{-10} E cm^{-2} s^{-1} red light at 649 nm for various periods of time and then horizontally oriented in darkness. After 4 h, geotropic responsiveness was recorded (Fig. 3). The minimum exposure time for inducing the response was shortened by the pretreatment with ascorbate up to about one-third of the control, the threshold energy value being 8.86 × 10^{-10} E cm^{-2}. Significant changes in the degree of curvature of roots that do undergo curvature could not be observed, regardless of the presence or absence of ascorbate, the mean value being about 45°. These results indicate that the effect of red light for the induction of geotropic responsiveness in *Zea* primary roots can be partially substituted by adding exogenous ascorbate.

The next experiments were conducted to examine the effect of ascorbate concentrations on the induction of geotropic responsiveness. *Zea* primary roots pretreated for 15 min with ascorbate at various concentrations ranging from 10 μM to 1 pM were exposed to 1.97 × 10^{-10} E cm^{-2} s^{-1} red light for 9 s; this amount of energy is slightly above the threshold energy value for inducing the georesponsiveness of *Zea* primary roots pretreated with 1 μM ascorbate (Fig. 3). The results, obtained after 4 h geostimulation, are shown in Figure 4. The percentage of curved roots gradually increased with increasing concentration of ascorbate and reached the maximum effect at 1 μM ascorbate, whereas the actual response (the degree of curvature) does not seem to differ at any concentration of ascorbate. These results confirm that ascorbate can lower the threshold energy but cannot completely substitute for red light for inducing the geotropic responsiveness.

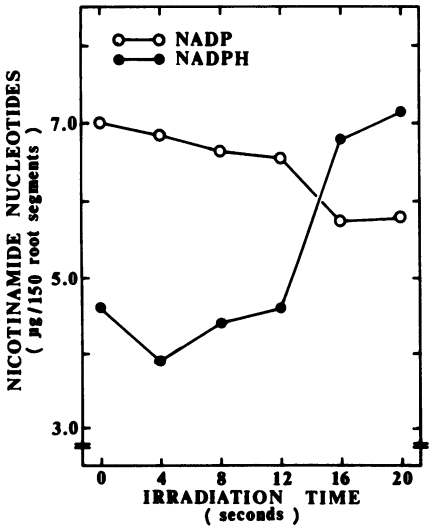

FIG. 1. Effect of light on the level of NADPH and NADP in *Zea* primary roots. Two-mm root tips excised from the primary roots were exposed to 1.97 × 10^{-10} E cm^{-2} s^{-1} red light at 649 nm for various lengths of time.

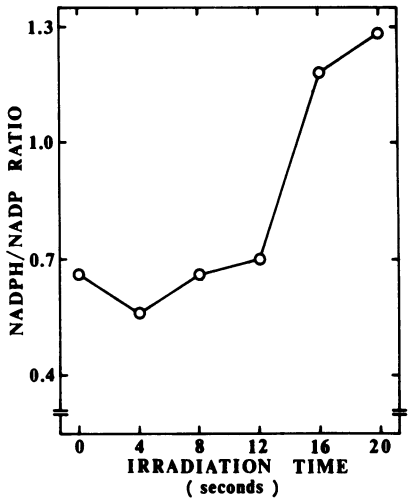

FIG. 2. Effect of light on NADPH/NADP ratio in 2-mm root tips of *Zea*. Ratio was recalculated from Figure 1.

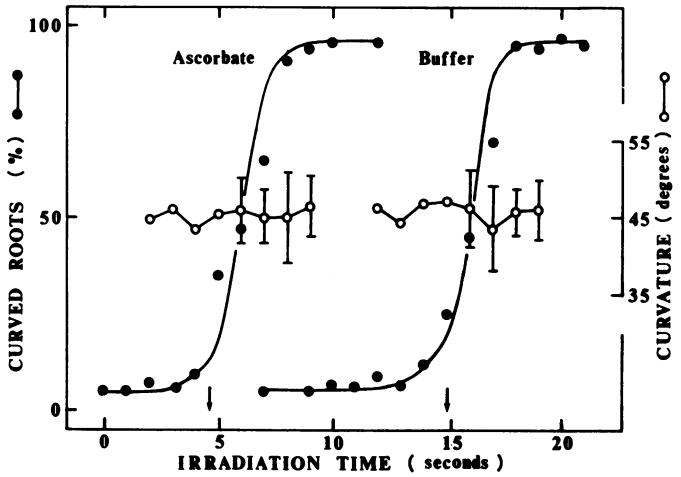

FIG. 3. Effect of ascorbate on the geotropic responsiveness in *Zea* primary roots. The primary roots pretreated for 15 min with 20 mM KH_2PO_4-Na_2HPO_4 buffer with or without 1 μM ascorbate were exposed to 1.97 × 10^{-10} E cm^{-2} s^{-1} red light at 649 nm for various lengths of time. Results were determined after keeping roots for 4 h in a horizontal position. Arrows indicate the minimum exposure time which was determined by the method previously described (11). The points indicating degree curvature are means of those roots which did curve. Bracket is the standard error of the mean.

Other reducing agents, DTT, glutathione, and sodium hydrosulfite, also lowered the threshold energy value up to one-third of the control value at the optimum concentration of each, but did not change the mean angle of cuvature. Data in Table I show the threshold energy values for inducing the geotropic response in the roots pretreated with these reducing agents. The threshold energy values were very similar for all reducing agents used.

In another experiment, the primary roots were exposed to 1.97 × 10^{-10} E cm^{-2} s^{-1} red light for 9 s, and then treated with 1 μM ascorbate for 15 min after various dark periods. After 4 h geo-

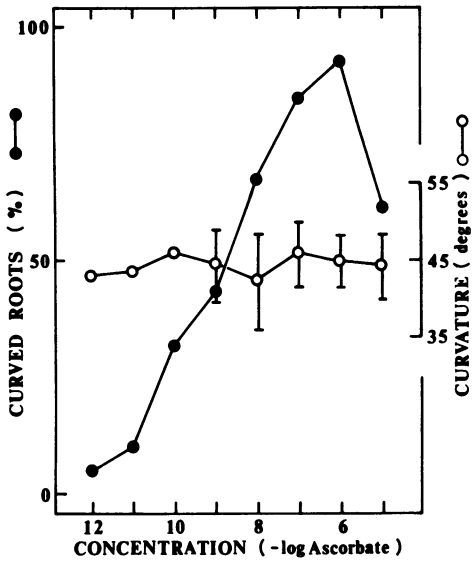

FIG. 4. Effects of ascorbate concentration on the geotropic responsiveness in *Zea* primary roots. The primary roots pretreated with ascorbate at various concentrations were exposed for 9 s to 1.97×10^{-10} E cm^{-2} s^{-1} red light at 649 nm. Results were determined after keeping them for 4 h in a horizontal position. The points indicating degree curvature are means of those roots which did curve. Bracket is the standard error of the mean.

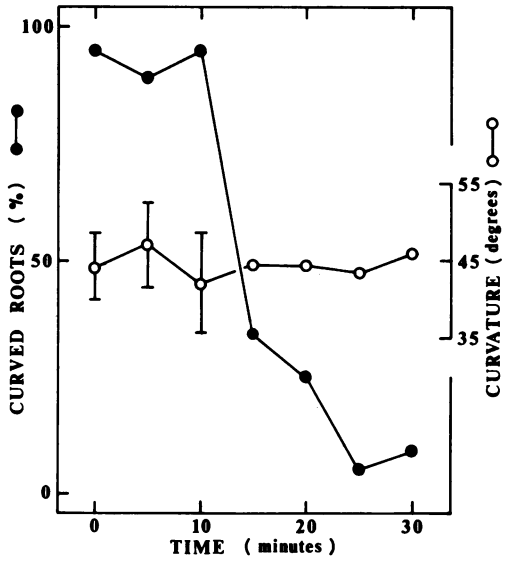

FIG. 5. Timing of ascorbate treatment in the induction of geotropic responsiveness in *Zea* primary roots. The primary roots exposed for 9 s to 1.97×10^{-10} E cm^{-2} s^{-1} red light at 649 nm were treated with 1 μM ascorbate for 15 min after the various dark periods. Results were determined after keeping the roots for 4 h in a horizontal position. The points indicating degree curvature are means of those roots which did curve. Bracket is the standard error of the mean.

Table I. *Effect of Some Reducing Agents on Threshold Energy Value*

The primary roots pretreated for 15 min with 20 mM KH_2PO_4-Na_2HPO_4 buffer including various reducing agents were exposed to 1.97×10^{-10} E cm^{-2} s^{-1} red light at 649 nm for various lengths of time. The threshold energy value was calculated by the method perviously described (11).

Reducing Agent	Threshold Energy Value
	$\times 10^{-10}$ E cm^{-2}
None	29.6
Ascorbate, 1 μM	8.86
DTT, 1 mM	8.26
Glutathione, 0.1 mM	8.48
Sodium Hydrosulfite, 10 nM	8.48

stimulation, the geotropic responsiveness was recorded. Ascorbate was effective in inducing the response only when it was applied within 10 min after the light irradiation, and its effect was gradually decreased with increasing length of the inserted dark period between light and ascorbate treatments (Fig. 5). These results, together with the results in Table I and Figure 3, imply that reducing agents would exert their effects on the amplifying mechanism for light stimulus but not on the perception mechanism of light, resulting in the stimulation of geotropic responsiveness of *Zea* primary roots.

DISCUSSION

It now seems likely that an explanation of the light effect on the geotropic response in the primary roots of some *Zea mays* cultivars should be sought in initiation or enhancement of production of the growth inhibitor(s) in the root cap, which is widely believed to control the geotropic curvature. At present we have no information which could lead to a better understanding of the mechanism of the action of light on the geotropic responsiveness in *Zea* primary roots.

Here, we have shown clearly that red light increases NADPH levels with respect to NADP levels in *Zea* primary roots, and the effect can be partially bypassed by adding exogenous reducing agents (Table I; Figs. 1 and 3). These results suggest that light somehow increases the reducing capacity of the cells by increasing NADPH, that the increase in reducing power is someplace along the chain of events leading to induction of the geotropic response, and that addition of external reductant eliminates at least the need for endogenously generated reductant to function in the induction of georesponsiveness in *Zea* primary roots. However, exogenous reducing agents could not completely substitute for the light effect. This suggests that the induction of geotropic response in *Zea* primary roots is initiated by a dual function of light, one being the photochemical transformation of a photoreceptor and the other being the induction of a reduction state in the tissue. The actual threshold energy value necessary for the photochemical transformation of a photoreceptor would be about 8.5×10^{-10} E cm^{-2}, but not 2.96×10^{-9} E cm^{-2}, and the processes following the photochemical transformation of a photoreceptor would progress only under the proposed reduction state. If this is so, the geotropic response in roots irradiated with the threshold energy value necessary for the photochemical transformation of a photoreceptor would not be induced without adding exogenous reducing agents after the irradiation. In fact, the reducing agents were not effective when they were not applied up to 10 min after the light irradiation (Fig. 5). The fact that the increase in the NADPH/NADP ratio was caused by irradiation with light doses above the apparent threshold energy value (2.96×10^{-9} E cm^{-2}) supports the possibility mentioned above.

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