

Phytochrome Action in *Oryza sativa* L.

IV. RED AND FAR RED REVERSIBLE EFFECT ON THE PRODUCTION OF ETHYLENE IN EXCISED COLEOPTILES¹

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ABSTRACT

Excised apical segments of etiolated rice (*Oryza sativa* L.) coleoptiles produced ethylene. Increasing the number of cut sites per coleoptile increased the rate of ethylene formation. Ethylene produced by an etiolated-intact seedling in the dark was about a half of that by the excised coleoptile segment. Red light of low energy as well as of continuous irradiation inhibited the production of ethylene. The inhibition by a low energy dose of red light was partly relieved, if the red light was followed immediately by a small dose of far red light. The effect of red and far red light was repeatedly reversible, indicating that ethylene production was regulated by a phytochrome system. If the exposure to far red light was preceded by a period of darkness, this photoreversibility disappeared; 50% of the initial reversibility was lost within 5 hours. Applied ethylene (10 microliters per liter) significantly promoted the growth of intact coleoptiles of either totally etiolated or red light-treated seedlings, but had no effect on the excised apical segment of coleoptile.

In etiolated rice plants, the growth of intact coleoptiles was inhibited by an exposure to red light, while that of excised segments in a buffer was promoted by irradiation with red light, and both effects were reversible if the red irradiation was immediately followed by irradiation with far red light (12). In intact coleoptiles, polar auxin transport was found as one of the factors in the system mediating between the photoreceptor phytochrome and the growing zone (3).

The possible roles of ethylene in auxin-induced growth phenomena in various plants were discussed (14), and it was suggested that either auxin-induced production of ethylene or exogenously applied ethylene can be involved in the growth and development of seedling plants (4, 10). In addition, the production of ethylene was significantly promoted when plant tissues were injured in various ways such as mechanical, chemical, or biological injury (8, 15, 16).

Therefore, the present study with etiolated rice seedlings was

designed to determine whether ethylene formation can be modified by cutting the coleoptile, to examine any red and far red reversible effect on ethylene production in coleoptiles, and, if possible, to find a correlation between phytochrome-mediated growth responses and ethylene effects.

MATERIAL AND METHODS

Plant Material and Light Sources. Seeds of *Oryza sativa* L., cv. Aichi-Asahi, were used throughout the present study. The procedures for the culture of totally etiolated seedlings and the sources of red, far red, and dim green safelight were already described (12, 13). The energy of incident light was determined by a radiometer (Yellow Springs Instrument Co., Inc., Model 65). In most experiments, forty 12-mm apical segments without primary leaves were placed in test tubes (14 ml in volume). The tubes were sealed with a silicon stopper and incubated in the dark at 27 to 28 C for 24 hr following light treatment.

Measurement of Ethylene. After an appropriate period of incubation, 5-ml gas samples in test tubes containing coleoptile segments were withdrawn by a syringe injected through a silicone stopper. To facilitate the operation, 5 ml of ethylene-free air was first injected into the tube, and the atmosphere in the tube was mixed by several up and down movements of the syringe plunger. Ethylene in the 5-ml gas sample was measured by a hydrogen flame gas chromatograph (Shimadzu GC-1C) equipped with an activated alumina column run at 80 C. Other conditions were described previously (9).

RESULTS

Effects of Light and Injury on Ethylene Production. Since the rate of ethylene production has been shown to be altered by the physical stress (6), which might arise from the handling of rice seedlings during experiments, the rates of ethylene production by intact etiolated seedlings were measured. Two hundred seedlings were grown on 0.4% agar (1 cm in thickness) in an aluminum chamber (7 × 12 × 4 cm³) for 5 days in darkness. The chamber was sealed and 24 hr later the amount of ethylene in the atmosphere was measured (Table I). Similar rates of ethylene production for a 24-hr period after the 6th day were observed, and by that time coleoptiles grew to the top of the chamber and, in some plants, the primary leaf penetrated the coleoptiles. Table I presents data which show that ethylene production by intact seedlings was about one-half of that produced by a coleoptile segment, and that ethylene production by sectioned coleoptiles was inhibited by continuous irradiation at either wavelength.

Effect of Cutting on Ethylene Production. Since the rate of

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Table I. *Effects of Cutting and Light on Ethylene Production by Etiolated Rice Coleoptiles*

Coleoptiles were subjected to a continuous irradiation for 24 hr at 28 C. Intensity of red light was 800 ergs cm⁻²·sec⁻¹, far red, about 18 kiloergs cm⁻²·sec⁻¹. Data are mean values of assays with standard errors.

Material	Light Treatment	Ethylene Production <i>pl/segment·24 hr</i>
Intact seedling	Dark	587 ± 120
12-mm apical coleoptile segments	Dark	1070 ± 63
	Red	501 ± 10
	Far red	627 ± 22

Table II. *Relationship between Length of Coleoptile Segments and the Amount of Ethylene Production in Darkness*

Segments were obtained from 6-day-old etiolated rice seedlings having about 20-mm-long coleoptiles. Data are mean values of assays with standard errors.

Length of Apical Segment <i>mm</i>	Ethylene Production	
	<i>pl/segment·24 hr</i>	<i>pl/mm of segment</i>
3	136 ± 10	45.2
8	660 ± 52	82.5
12	973 ± 63	81.0

Table III. *Effect of Cutting on the Rate of Ethylene Production of Rice Coleoptile in Total Darkness*

Twelve-millimeter apical segments of rice coleoptile were used.

No. of Pieces per Segment	No. of Cut Surfaces per Segment	Ethylene Production ¹
		<i>pl/segment·24 hr</i>
1	1	950
2	3	1370
3	5	1630

¹ Mean of three experiments.

ethylene production by a coleoptile segment was approximately twice that produced by an intact seedling, an attempt was made to determine the relationship, if any, between coleoptile length and the rate of ethylene formation. Table II presents the rate of ethylene production of 3, 8, and 12 mm long apical segments. The results show that the longer coleoptiles produced more ethylene. When the amount of ethylene produced was calculated per unit length, the production of the apical portion was one-half that of lower portions, but there was no significant difference between the values for 8-mm and 12-mm segments.

Further experiments were carried out to determine the relationship between the degree of injury and ethylene production. Twelve-millimeter apical coleoptiles from 6-day-old etiolated seedlings were cut into two or three pieces of equal lengths, and ethylene production during a 24 hr period was measured (Table III). When the number of cut surfaces was increased by cutting the coleoptile into smaller pieces, the amount of ethylene produced per 12-mm segment increased, but it was not proportional to the number of sites of injury.

Effect of Low Intensity Red Light on Ethylene Production of Coleoptile Segments. Table IV shows the relationship be-

tween the amount of ethylene produced and the hours after excision. The 12-mm rice coleoptile segments were irradiated with red light (5 min, 800 ergs cm⁻²·sec⁻¹). After irradiation the tubes were stoppered and incubated for 24 hr. The results in Table IV show that a short red irradiation inhibited ethylene production from the segments, and that the treatment 2 hr after the excision was most inhibitory.

Figure 1 presents a time-course of ethylene production by apical 12-mm coleoptile segments kept in the dark, illuminated with red light, or given a 5 min red irradiation at the 2nd hr after excision, followed by darkness. The 5 min red light irradiation inhibited ethylene production, and the inhibition persisted for 10 hr. Continuous red irradiation was necessary for the maximum inhibition. Thus, a small amount of low intensity red light appears to be enough to inhibit of ethylene evolution from coleoptile segments.

Far Red Reversal of the Red Light-induced Inhibition. Since red light inhibited ethylene production, experiments were performed to test the idea that the process was under the control of phytochrome and could be reversed by far red light.

Figure 2 shows that a 5-min treatment of far red light (18 kiloergs cm⁻²·sec⁻¹) reduced the rate of ethylene production to 87% of the dark control, and that of far red light could reverse the inhibition by red light to the level of the production following far red light treatment, but did not restore ethylene production to the level of the dark control. The reversibility gradually became incomplete, as the number of red far red cycles increased.

Effect of Intervening Darkness on the Reversibility of the Red Light-induced Response by Far Red Light. The escape

Table IV. *Relation between the Irradiation Time of Red Light after Excision and the Rate of Ethylene Production*

Red irradiation was 800 ergs cm⁻²·sec⁻¹ for 5 min.

Time of Red Light Irradiation <i>hr after excision</i>	Ethylene Production ¹ <i>pl/segment·24 hr</i>
1	569
2	362
4	486
8	494
Dark	985

¹ Mean of three experiments.

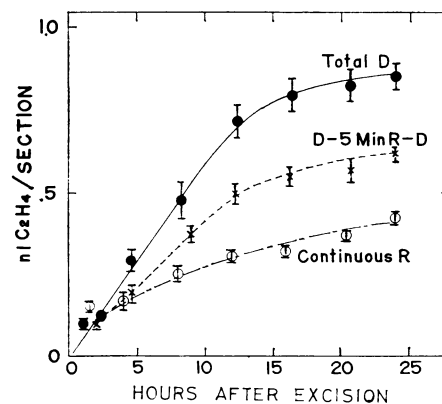


FIG. 1. Ethylene production by apical 12-mm rice coleoptile segments under total darkness, continuous irradiation of red light, and 5 min of red light at 2nd hr. Vertical bar indicated standard error of mean.

reaction of photoreversibility was measured by exposing coleoptile segments to 5 min of red light ($800 \text{ ergs cm}^{-2} \cdot \text{sec}^{-1}$) followed by darkness at 27 to 28 C for various periods of time before exposure to far red light. The total amount of ethylene produced by the segments so treated was measured 24 hr after the red light irradiation. The results presented in Figure 3 show that the photoreversibility of ethylene production was completely lost within 24 hr following red exposure. A 50% loss of the photoreversibility was obtained within 5 hr.

It was concluded that ethylene production by rice coleoptile segments is regulated in part by phytochrome.

Effect of Applied Ethylene on Growth of Rice Coleoptiles.

To examine the effect of ethylene on growth of intact coleoptiles, 5-day-old totally etiolated seedlings grown on 0.4% agar medium were placed in an atmosphere with or without $10 \mu\text{l/l}$ ethylene for 20 hr at $24 \pm 1 \text{ C}$ in the dark. Some dishes in each treatment were exposed to red light for 5 min just before the ethylene treatment.

Apical 8-mm coleoptile segments from 5-day-old etiolated seedlings were distributed into 50-ml flasks containing 3 ml of pH 7 20 mM potassium phosphate buffer. Ethylene was added to the gas phase by injection with a syringe through the silicone rubber stopper to yield a final concentration of $10 \mu\text{l/l}$. The flasks were then incubated for 20 hr at 27 to 28 C in the dark. Lengths of coleoptiles were measured immediately before and after the incubation. Table V shows that exogenously applied ethylene promoted intact coleoptile growth with or without red light irradiation. In excised coleoptiles, however, neither growth promotion nor inhibition by ethylene application was observed.

DISCUSSION

Increased ethylene production following injury has been reported earlier (2, 9, 15). Slicing rice coleoptiles also increased ethylene formation, although the injury had less of an effect on ethylene production by the apical portion of the coleoptiles than the basal portion. This may be related to the fact that ethylene production of rice coleoptiles did not increase when

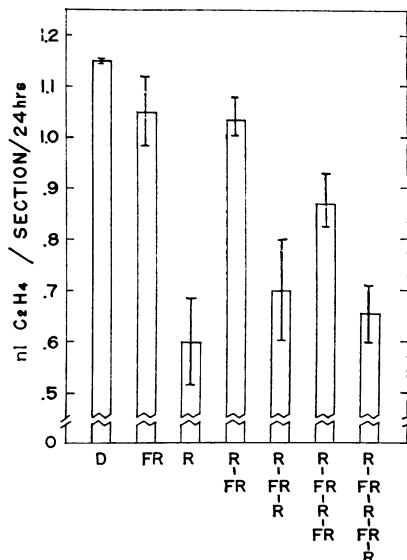


FIG. 2. Far red reversal of red light-induced inhibition of ethylene production by 12 mm rice coleoptile segments. D: dark control; FR: 5 min irradiation of far red light; R: 5 min irradiation of red light.

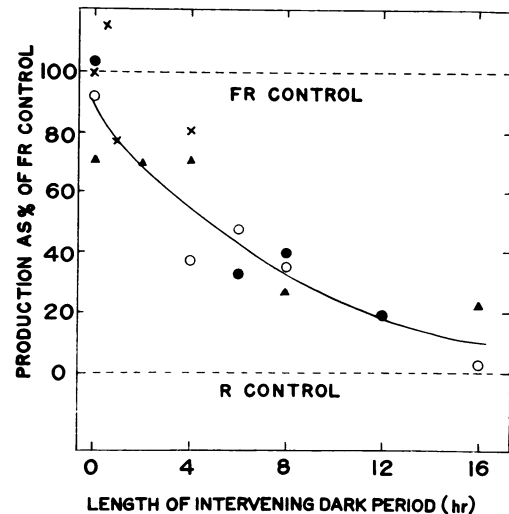


FIG. 3. Effects of intervening darkness on the reversibility between the red and far red light. Ethylene production was determined with apical 12 mm rice coleoptile segments. The different symbols represent values from different experiments.

Table V. Effect of Ethylene on the Growth of Intact and Excised Rice Coleoptiles with or without Red Light Irradiation

Red irradiation was $800 \text{ ergs cm}^{-2} \cdot \text{sec}^{-1}$ for 3 min at 24 C. Rice was treated with $10 \mu\text{l/liter}$ of ethylene. Data are mean values of assays with standard errors.

Material	Treatment		Coleoptile Length	
	Red light	Ethylene	Initial	Final
Intact coleoptile	-	-	12.7 ± 1.3	<i>mm</i> 17.8 ± 1.7
	-	+		22.8 ± 1.9
	+	-		15.1 ± 1.2
	+	+		18.7 ± 1.4
Sectioned coleoptile	-	-	7.9 ± 0.3	8.9 ± 0.4
	-	+		8.7 ± 0.2

their tips had reached to the top of the incubation chamber, while etiolated pea seedlings do when they are under stress (6).

About half of the ethylene produced by rice coleoptile segments was controlled by the red far red reversible reaction. However, it was unclear whether phytochrome controlled the rate of endogenous ethylene production or the injury regulated system. A 50% loss of the photoreversibility of phytochrome-mediated ethylene formation from rice segments was observed at 5 hr, which was slower than the rate of disappearance of optically detectable Pfr in the segments and faster than the rate of escape from red and far red control of coleoptile growth. Namely, a 50% of Pfr decay in the dark was determined spectrophotometrically with rice coleoptile segments to be about 90 min after a red exposure at 27 C (13), while a 50% loss of the initial photoreversibility growth response was found within 9 hr in the dark at the same temperature (12).

A good correlation has been reported to exist between the phytochrome-dependent decrease in ethylene production and an increased plumular expansion in etiolated pea seedlings (5). Another phytochrome-mediated response, the inhibition of flowering of *Xanthium* by red light during the 16-hr dark induction period, could be mimicked by ethylene application

(1). Growth promotion by ethylene of intact rice coleoptiles has been reported by Ku *et al.* (11) with a cultivar of a dwarf type. The cultivar used in the present study was a normal type, and it is interesting to note that rate of ethylene production by the dwarf type (1 nl/seedling·day) was about twice that of the normal type in the present study (0.59 nl/seedling·day). Excised coleoptiles, however, must be in a completely different situation, because the growth increment of excised segments was much smaller than that of intact coleoptiles and the growth promotion by exogenous ethylene was not apparent in excised segments. The insensitivity of excised coleoptiles to ethylene may be related to level or physiological state of endogenous auxin, since ethylene was recently found to stimulate growth of excised rice coleoptiles in the presence of auxin (7).

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