

Effect of Light on Ethylene Production and Hypocotyl Growth of Soybean Seedlings¹

Received for publication August 24, 1977 and in revised form November 28, 1977

CYRUS SAMIMY

Department of Biology, College of Science, Ferdowsi University, Mashad, Iran

ABSTRACT

The apical 1-cm hypocotyl of dark-grown 'Clark' soybean (*Glycine max* [L.] Merr.) seedlings produced ethylene at rates of 7 to 11 nanoliters per hour per gram when attached to the cotyledons. Such physiologically active rates occurred prior to the deceleration of hypocotyl elongation caused by the temperature of 25 C.

Daily exposure of the etiolated seedlings to red light promoted hypocotyl elongation and prevented its lateral swelling. Red light treatment also caused a 45% decrease in ethylene production. Far red irradiation following the red treatment reversed the red effects, suggesting that the ethylene intervenes as a regulator in the phytochrome control of 'Clark' soybean hypocotyl growth at 25 C.

It has been shown (5, 10) that hypocotyl elongation of certain soybean cultivars, e.g. 'Clark,' is inhibited and the hypocotyl swells when grown at 25 C in darkness. At 20 and 30 C, the hypocotyl grows normally as other cultivars do, e.g. 'Mandarin,' at all three temperatures. We have previously presented (10) evidence to support the hypothesis that enhanced endogenous ethylene production at 25 C is responsible for both the inhibition and the swelling. The location of this ethylene synthesis was not determined.

Goeschl *et al.* (3) and Burg and Burg (1) have shown that the production of ethylene by etiolated pea epicotyls is confined to the plumule and plumular hook portion. Goeschl *et al.* (3) have suggested that this ethylene intervenes as a regulator in the phytochrome control of plumular expansion. Burg and Burg (1) showed that red light causes plumular hook opening by decreasing the rate of ethylene production. Kang *et al.* (7) have also reported that red light-induced opening of excised bean hypocotyl hooks can be prevented by application of ethylene, and that red light reduces the production of ethylene by the hypocotyl hook segments.

In the following work I report: (a) a more accurate time of the ethylene production; (b) the essentiality of cotyledons for the production of an inhibitory amount of ethylene by Clark soybean hypocotyls; (c) an indication that phytochrome is involved in regulating the hypocotyl growth of soybean seedlings by changing the rate of their ethylene production.

MATERIALS AND METHODS

Plant Material. Seeds of soybean (*Glycine max* [L.] Merr.), cultivars Mandarin and Clark, were obtained from the Botany Department of Iowa State University. Seeds were sterilized in 10% Whitex for 10 min, rinsed, and aligned between two sheets of

germination paper (6 × 14 cm) with the embryos upward. Sheets were rolled into a cylinder, which was stood in 50 ml of distilled H₂O in a 1,000-ml glass jar. Seedlings were grown in darkness at 25 C. All of the manipulations were conducted under a dim green light, consisting of a 25-w incandescent lamp filtered through two layers of green Cinemoid and one layer of blue cellophane. The data for the hypocotyl length measurements were taken from our previous report (10). Prior to every experiment ungerminated seeds, seeds with broken cotyledons, seed coats, and inverted seedlings were discarded.

Ethylene Determination. In measuring endogenous ethylene evolution of the seedlings, five or six seedlings, still wrapped in paper, were transferred to a 210-ml glass cylinder (3.4 i.d. and 23 cm long) and sealed. Each treatment consisted of three glass cylinders. After closure for 5 hr, 1-ml samples were withdrawn with a Hamilton gas-tight syringe. Ethylene was measured in a Varian 2400 gas chromatograph equipped with a hydrogen flame ionization detector and a stainless column (213 × 0.32 cm) packed with activated alumina.

Localization of Ethylene Production. Immediately after each 5-day-old seedling was cut into specific segments (Fig. 1) they were allowed to float in 1% sucrose - 50 mM KH₂PO₄ solution for 1 to 4 hr (experiment I) and 9 to 12 hr (experiment II) prior to being transferred to appropriate containers and sealed for ethylene measurements. Excised portions of the seedlings were transferred to 39-ml flasks (except for the roots which were placed in 57-ml centrifuge tubes containing a filter paper soaked with the sucrose-phosphate solution) and sealed. After closure for 5 hr, 1-ml samples were withdrawn for gas analysis.

Light Experiments. The red light source consisted of two 20-w, Toshiba white fluorescent bulbs filtered through one layer of 3-mm red Plexiglas and two layers of red cellophane. The far red light source consisted of one 500-w lamp filtered through 5 cm of water, one layer of 3-mm blue Plexiglas and one layer of ruby Cinemoid. Seedlings were illuminated with either red or far red 2.5 days after planting for 4 min at a distance of 50 and 25 cm, respectively. The light treatments were repeated every 24 hr, up to 9 days. Ethylene evolution of the light-treated seedlings was measured 6 days after planting, when the rate of ethylene production by dark-grown seedlings was very high.

RESULTS

Time Sequence of Ethylene Production and Hypocotyl Growth. Hypocotyl length of Mandarin increased at higher rates than Clark when grown at 25 C (Fig. 2). Conversely, the ethylene production by Clark seedlings increased at considerable higher rates than Mandarin at this same temperature. Six and 7-day-old Clark seedlings produced ethylene almost at similar rates, but Mandarin seedlings produced ethylene at low increasing rates up to 7 days when the experiment was terminated.

Localization of Ethylene Production. In experiment I each segment of the seedling produced ethylene at a higher rate than

¹ This research was supported by a Ferdowsi University research grant.

the corresponding segment in experiment II (Table I). The reverse was true for the intact cotyledons + apical 1-cm hypocotyl segments. In experiment I, roots produced the highest ethylene but in experiment II their rates of ethylene production subsided considerably (Table I). In a separate experiment (data not reported) it was found that after about 9 hr the level of ethylene production by roots subsided and remained constant up to 15 hr when the experiment was terminated. In experiment II the apical 1-cm hypocotyl portion produced the highest ethylene. In both experiments when cotyledons remained attached to the apical 1-cm hypocotyls, the rates of ethylene production were two to five times higher than when they were separated.

Effects of Light. Red light increased the hypocotyl lengths of 6- and 9-day-old Clark seedlings by 38.5 and 20.5%, respectively (Table II). Conversely, it decreased their hypocotyl thickness by 26.7 and 21.7%, respectively (Table II).

Far red irradiation following the red light reversed the red effects on hypocotyl length and thickness (Table II). Its reversal on the hypocotyl length of 9-day-old seedlings was almost complete.

Red light reduced the ethylene production of 6-day-old seedlings and far red reversed its effect, partially.

The effects of far red alone were not studied.

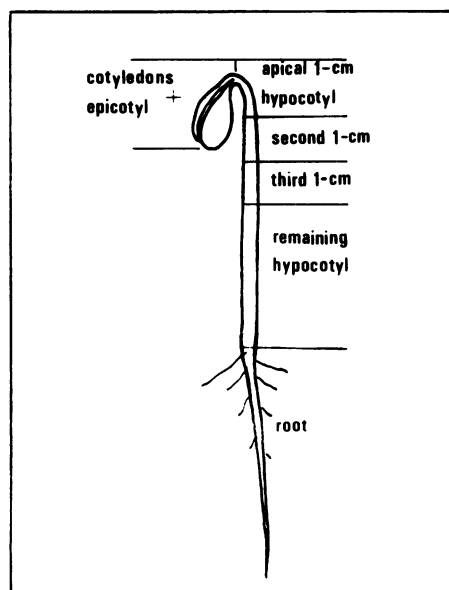


FIG. 1. Drawing depicting various segments of an etiolated soybean seedling whose ethylene production is shown in Table I.

Table I. Localization of ethylene production in 5-day-old Clark seedlings, grown at 25 C in darkness.

In determining the rate of ethylene production (based on the initial fresh wt), the excised portion of seedlings were allowed to float in 1% sucrose-50mM KH_2PO_4 solution for 1-4 (Experiment I) and 9-12 hrs (Experiment II) before being transferred to appropriate containers and sealed.

Excised Portion of Seedling ¹	Experiment I		Experiment II	
	nl/hr/gm	nl/hr/segment	nl/hr/gm	nl/hr/segment
Cotyledons + epicotyl (9-10)	0.33	0.12	0.26	0.09
Apical 1-cm hypocotyl (17-25)	2.51	0.09	0.74	0.03
Second 1-cm (21-25)	1.53	0.06	0.48	--
Third 1-cm (21-25)	1.42	0.05	0.37	--
Remaining hypocotyl (10-11)	0.73	0.15	0.46	--
Roots (5-6)	7.55	0.96	0.50	--
	Total 1.43/seedling			
Intact cotyledons + epicotyl + apical 1-cm hypocotyl (9-10)	0.95	0.40	1.42	0.53
Cotyledons + epicotyl + excised apical 1-cm hypocotyl	0.50	0.21	0.29	0.12

¹Each value is the mean of either 3 or 4 replications. The number of segments in each replication is shown in parentheses.

Table II. Far red reversal of red light-induced changes in ethylene production and hypocotyl growth of Clark seedlings grown at 25 C.

	Dark	Red	Red: far red
6-day old seedlings			
Ethylene production (nl/hr/g) ¹	2.71	1.49	1.98
Hypocotyl length (cm) ²	9.10	12.60	11.40
LSD (10%) = 1.20			
Hypocotyl fresh wt per unit length (mg/cm) LSD (5%) = 4.67	47.90	35.10	39.90
9-day old seedlings ³			
Hypocotyl length (cm)	12.20	14.70	12.50
Hypocotyl fresh wt per unit length (mg/cm)	45.10	35.30	41.80

¹Each value is the mean of 3 replications, consisting of 5 seedlings each.

²Each value is the mean of 15 seedlings.

³Each value for the dark, red and red: far red treatment is the mean of 56, 44, and 35 seedlings, respectively.

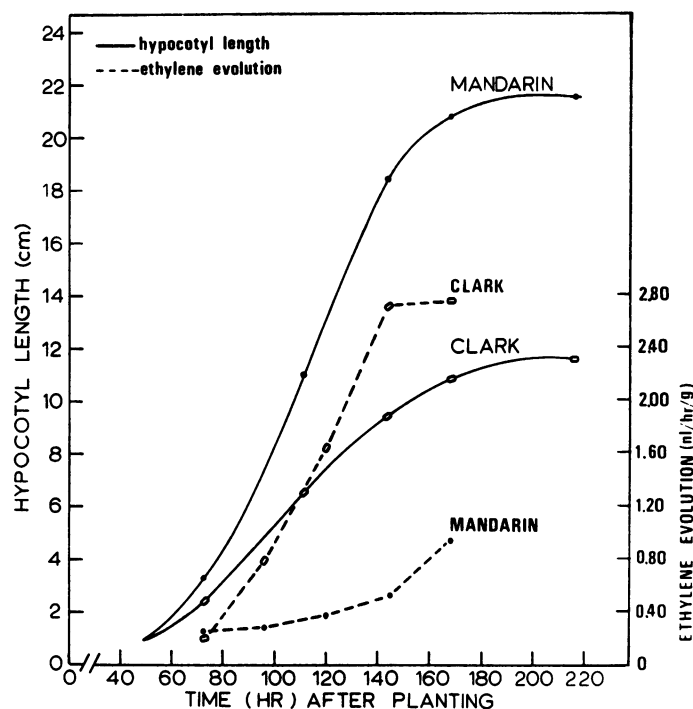


FIG. 2. Hypocotyl elongation and time sequence of ethylene production of soybean seedlings grown in darkness, at 25 C. Rates of ethylene production are based on the total fresh wt of seedlings.

DISCUSSION

In experiment I (Table I) the total ethylene produced by a 5-day-old seedling is almost the same regardless of whether the seedling is excised (1.43 nl/hr) or intact (1.39 nl/hr). This shows that 1 to 4 hr after excision ethylene evolution in response to wounding is negligible.

When cotyledons remain attached to the apical 1-cm hypocotyl segments the ethylene production is substantially higher than when they are separated (Table I). This synergistic effect can be explained on the basis of continuous translocation of food materials, including ethylene or auxin precursors, from cotyledons to the hypocotyl. Burris and Knittle (2) have shown that removal of 50% of soybean cotyledons partially reverses the 25 C inhibition of hypocotyl elongation. Results of Grover and Purves (6) have also indicated that IAA translocated from the cucumber cotyledons causes ethylene production in the hypocotyl. By subtracting the ethylene production of excised cotyledons from that of the intact cotyledons + apical 1-cm hypocotyl segment and dividing the results by average fresh wt (0.04 g) of each apical 1-cm hypocotyl segment, the resulting rates of ethylene production of apical hypocotyl in experiments I and II are 7 and 11 nl/hr·g, respectively. These rates of ethylene production, which are well within the physiological active range (3, 4, 8), occur prior to the deceleration of hypocotyl elongation at 5 days (Fig. 1).

At 25 C, etiolated seedlings of Clark acquire several conspicuous features not observed in Mandarin (10). Two of these, short and thick hypocotyl, are shown to be affected by red and far red lights. Stimulation and reduction of hypocotyl elongation and thickness, respectively, by red light and far red reversal of red light effects, strongly suggest that phytochrome is involved. Morris (9) also found that at 25 C red light inhibited hypocotyl swelling of Clark and enhanced its length but far red reversed only the former and

not the latter effect of the ref light. Far red reversal effects on hypocotyl thickness and hypocotyl length of 6-day-old seedlings were not complete. This may be due to the overlap of Pr absorption spectrum with the source of far red light (3).

If the morphological features of Clark hypocotyl at 25 C are caused by endogenous ethylene, then it too must respond to the action of phytochrome. The results show (Table II) that red light decreased the ethylene production rate of 6-day-old seedlings to the level lower than the 5-day-old (Fig. 2). Conversely, the far red, following the red light, increased the rate of the ethylene production. Hence, it can be concluded that phytochrome control of Clark hypocotyl growth is mediated by ethylene.

LITERATURE CITED

1. BURG SP, EA BURG 1968 Auxin stimulated ethylene formation: its relationship to auxin inhibited growth, root geotropism and other plant processes. *Biochemistry and Physiology of Plant Growth Substances*. Runge Press, Ottawa Canada, pp 1275-1294
2. BURRIS JS, KH KNITTLE 1975 Partial reversal of temperature-dependent inhibition of soybean hypocotyl elongation by cotyledon excision. *Crop Sci* 15: 461-462
3. GOESCHL JD, HK PRATT, BA BONNER 1967 An effect of light on the production of ethylene and the growth of the plumular portion of etiolated pea seedlings. *Plant Physiol* 42: 1077-1080
4. GOESCHL JD, L RAPPAPORT, HK PRATT 1966 Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. *Plant Physiol* 41: 877-884
5. GRABE DF, RB METZER 1969 Temperature-induced inhibition of soybean hypocotyl elongation and seedling emergence. *Crop Sci* 9: 331-333
6. GROVER S, WK PURVES 1976 Cobalt and plant development. Interaction with ethylene in hypocotyl growth. *Plant Physiol* 57: 886-889
7. KANG BG, CS YOKUM, SP BURG, PM RAY 1967 Ethylene and carbon dioxide: mediation of hypocotyl hook-opening response. *Science* 156: 958-959
8. LIPE JA, PW MORGAN 1973 Ethylene, a regulator of young fruit abscission. *Plant Physiol* 51: 949-953
9. MORRIS JE 1974 Effects of red and far red light on temperature-dependent seedling development in soybean. MS thesis. Iowa State University, Ames Iowa
10. SAMIMY C, CE LAMOTTE 1976 Anomalous temperature-dependence of seedling development in some soybean (*Glycine max* [L.] Merr.) cultivars: role of ethylene. *Plant Physiol* 58: 786-789