Planta (1982) 155:261–266

Planta

Light inhibition of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in leaves is mediated through carbon dioxide

Ching Huei Kao* and Shang Fa Yang
Department of Vegetable Crops, University of California, Davis, CA 95616, USA

Abstract. The mechanism of light-inhibited ethylene production in excised rice (*Oryza sativa* L.) and tobacco (*Nicotiana tabacum* L.) leaves was examined. In segments of rice leaves light substantially inhibited the endogenous ethylene production, but when CO_2 was added into the incubation flask, the rate of endogenous ethylene production in the light increased markedly, to a level which was even higher than that produced in the dark. Carbon dioxide, however, had no appreciable effect of leaf segments incubated in the dark. The endogenous level of 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene, was not significantly affected by light-dark or CO_2 treatment, indicating that dark treatment or CO_2 exerted its effect by promoting the conversion of ACC to ethylene. This conclusion was supported by the observations that the rate of conversion of exogenously applied ACC to ethylene was similarly inhibited by light, and this inhibition was relieved in the presence of CO_2. Similar results were obtained with tobacco leaf discs. The concentrations of CO_2 giving half-maximal activity was about 0.06%, which was only slightly above the ambient level of 0.03%. The modulation of ACC conversion to ethylene by CO_2 or light in detached leaves of both rice and tobacco was rapid and fully reversible, indicating that CO_2 regulates the activity, but not the synthesis, of the enzyme converting ACC to ethylene. Our results indicate that light inhibition of ethylene production in detached leaves is mediated through the internal level of CO_2, which directly modulates the activity of the enzyme converting ACC to ethylene.

Key words: 1-Aminocyclopropane-1-carboxylic acid – Carbon dioxide and C_2H_4 production – Ethylene production – Light and C_2H_4 production – *Nicotiana* – *Oryza*.

Introduction

Depending on the tissue involved, light can promote (Craker et al. 1973; Saltveit and Pharr 1980) or inhibit (Goeschl et al. 1967; Kang and Burg 1972; Samimy 1978) ethylene production. Carbon dioxide has also been reported to promote or inhibit ethylene production in various tissues (Aharoni and Lieberman 1979; Young et al. 1962). Recently Gepstein and Thimann (1980), de Laat et al. (1981) and Grodzinski et al. (1981) reported that light markedly inhibited the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene in plant tissues (Adams and Yang 1979), into ethylene by various leaf tissues. Gepstein and Thimann (1980) suggested that light exerted its inhibitory effect by oxidation of essential SH group(s) of the enzyme system converting ACC to ethylene. De Laat et al. (1981) proposed that light regulated both synthesis and activation of the ACC-converting enzyme in leaves. Most recently, Wright (1981) also found that light substantially inhibited the production of ethylene induced by water stress in detached wheat leaves and in leaves on the intact plant. He concluded that either the biosynthesis of ACC or the conversion of ACC to ethylene required an obligatory dark stage. In these studies the leaf tissues were incubated in enclosed containers under light

* *Permanent address:* Department of Agronomy, National Taiwan University, Taipei, Taiwan, Republic of China
Recipient of a Republic of China National Science Council Fellowship

Abbreviation: ACC = 1-aminocyclopropane-1-carboxylic acid

0032-0935/82/0155/0261/$01.20

or dark conditions, where the concentration of carbon dioxide decreased or increased because of CO_2 fixation by photosynthesis or CO_2 production by respiration. Since CO_2 is known to promote ethylene production rates in detached leaves (Aharoni and Lieberman 1979; Gepstein and Thimann 1981) and intact plants (Dhawn et al. 1981), the inhibition of ethylene production by light may result from a decrease in internal CO_2 concentration. In this communication we present data showing that light inhibition of ACC-dependent ethylene production in leaves is indeed mediated through CO_2.

Materials and methods

Plant materials. Rice (*Oryza sativa* L. cv. Taichung Native 1) seeds obtained from Taiwan were sterilized with sodium hypochlorite solution (0.5%) for 5 min, rinsed with deionized water, and soaked for 48 h in the dark at 30° C. Uniformly germinated seeds were then grown in vermiculite for 8–9 d at 30° C under continuous white light (80 μmol quanta m^{-2} s^{-1}). The seedlings were watered with half-concentration Johnson's modified nutrient solution (Johnson et al. 1957), pH 4.5. The solution was replaced every 3 d. Tobacco plants (*Nicotiana tabacum* L. cv. Havana 425; seeds kindly provided by Department of Plant Pathology, University of California, Davis) were grown in a greenhouse under natural light and a temperature of no less than 20° C. Fully grown leaves of 11-week-old plants were used for the experiments.

Incubation conditions. Ten rice leaf segments (the apical 3 cm of the third leaves), weighing about 50 mg, or seven tobacco leaf discs (1 cm diameter), weighing about 150 mg, were floated on 10 and 1 ml, respectively, of test solution in a 50-ml flask. The flasks were flushed with air, sealed with rubber serum caps, and incubated at 30° C either under light (80 μmol quanta m^{-2} s^{-1}) provided by a mixture of cool-white and Grolux tubes, or in darkness. For the experiments in which various concentrations of CO_2 were used, all flasks were flushed with CO_2-free air and sealed. To obtain desired concentrations of CO_2, a known amount of CO_2 was injected into each sealed flask; a CO_2-free ("minus CO_2") atmosphere was achieved by hanging in the flask a center well containing a filter paper wick wetted with 0.2 ml of 20% KOH. The concentrations of CO_2 in the flasks were determined with a gas chromatograph (Model 800; Carle Instruments, Fullerton, Cal. USA) equipped with a Silica-gel column and a thermoconductivity detector. After each determination of ethylene, the flasks were flushed with fresh air or CO_2-free air as described and when required, CO_2 was reintroduced. The CO_2 concentration in the CO_2-free air treatment was below 0.01%.

Determination of ethylene. A 1-ml gas sample was withdrawn from the head space of the flask with a hypodermic syringe, and ethylene was assayed using a gas chromatograph equipped with an alumina column and a flame ionization detector.

Determination of ACC. Rice leaf segments were extracted twice with boiling 80% ethanol. The ethanol was evaporated under vacuum at 40° C. The residue was dissolved in 2 ml of water and the pigments were removed by adding 0.5 ml of chloroform. An aliquot of the aqueous solution was used for assay of ACC according to the method of Lizada and Yang (1979).

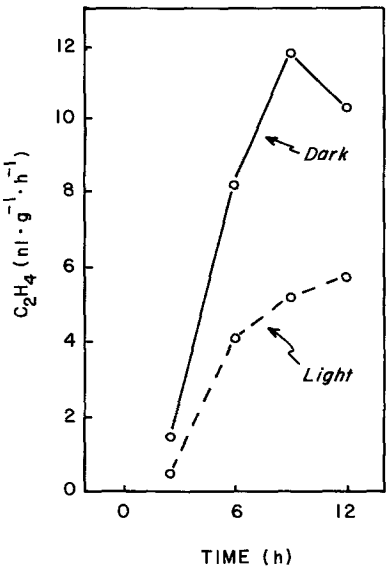

Fig. 1. Effects of light and darkness on the time course of ethylene production in rice leaf segments

The efficiency of the conversion of ACC to ethylene was between 65 and 70%. The amount of ACC was calculated from the quantity of ethylene liberated and the conversion efficiency.

Results

Effects of light and CO_2 on the basal ethylene production in rice leaves. The effects of light and darkness on the rate of endogenous ethylene production by excised rice leaf segments is shown in Fig. 1. Although the rates increased with time under light or darkness, it is clear that the ethylene production rates in the light were lower. The increase in ethylene production rates with time is probably a wound response. Since the experiment was carried out in sealed flasks, the concentration of CO_2 in flasks under light would be lower than that under darkness, because of CO_2 fixation by photosynthesis and possibly a reduction in CO_2 production because of light inhibition of dark respiration (see e.g. Graham 1980). For example, when rice leaf segments were enclosed in a flask and incubated for 6 h under light conditions, CO_2 concentration decreased from 0.08 to 0.01%, whereas under dark conditions CO_2 increased about five-fold from 0.08 to 0.39% (Table 1).

In order to examine whether ethylene production rates were sensitive to CO_2 concentrations, the effect of CO_2 concentration on the endogenous ethylene production during the first 6-h incubation period under light was studied. The ethylene production rates were markedly influenced by the CO_2 concentration. Ethylene production increased with increasing concentrations of CO_2 and reached a

Table 1. Effect of CO_2 on basal and ACC-dependent ethylene production by rice leaf segments. Leaf segments were enclosed in flasks under air, 3% CO_2, or CO_2-free air, and incubated in the presence or absence of ACC (1 mM) in the light or darkness. Ethylene production was assayed after 6 h incubation. The concentrations of CO_2 in the air flasks at the beginning and at the end of incubation were 0.08 and 0.39% (v/v), respectively, under darkness, and 0.08 and 0.01%, respectively under light

Treatment	C_2H_4 (nl g^{-1} 6 h^{-1})	
	−ACC	+ACC
Darkness		
Air	41	106
CO_2 (3%)	35	111
CO_2-free air	16	30
Light		
Air	13	62
CO_2 (3%)	71	237
CO_2-free air	13	54

Table 2. Effect of light-dark treatments or CO_2 treatment on ethylene production and the endogenous ACC level in rice leaf segments. For Exp. I the leaf segments were incubated in the light or darkness for 6 h and ethylene accumulated during this period was determined. In Exp. II, the leaf segments were first incubated for 3 h in the light and then enclosed for 1 h under light in the CO_2-free ($-CO_2$) or 3% CO_2 ($+CO_2$) atmosphere. Ethylene accumulated during this 1 h period was determined. Immediately after ethylene determinations, the same samples were assayed for ACC

Treatment	C_2H_4 (nl g^{-1} h^{-1})	ACC (nmol g^{-1})
Experiment I		
Light	2.4	20.5
Darkness	7.0	19.3
Experiment II		
$-CO_2$	5.2	14.5
$+CO_2$	20.4	17.9

plateau at about 2–3% of CO_2 concentration (data not shown).

The effect of 3% CO_2 on ethylene production by rice leaf segments under light and darkness were compared. The results shown in Table 1 clearly indicate that the reduced ethylene-production rate in the ambient air under light as compared to that under darkness was the consequence of the limited CO_2 concentration under the light treatment. This conclusion was based on observations that (a) 3% CO_2 greatly enhanced the ethylene produced under light but had little effect under darkness, and (b) when CO_2 was removed from the flasks, the ethylene produced under light was not affected, but that under dark was greatly reduced to a rate comparable to that under light. Since the air-filled flask

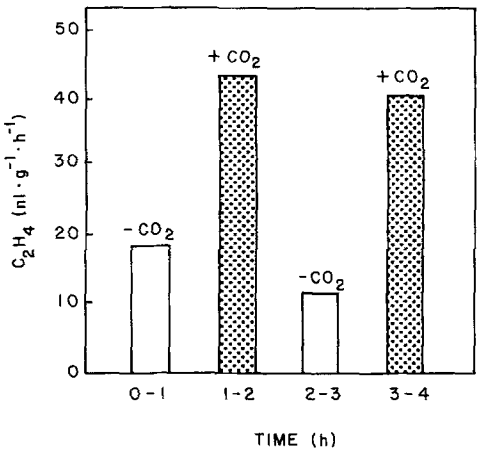

Fig. 2. Influence of plus- and minus-CO_2 alternations on ACC-dependent ethylene production in rice leaf segments. After leaf segments were preloaded with ACC (3 mM) for 3 h under light, they were enclosed in a flask and incubated alternately in CO_2-free air (open bar) and 3% CO_2 (hatched bar) for 1 h

under light contained 0.01% of CO_2 at the end of incubation and as little ethylene had been produced as in the flask filled with CO_2-free air, it may be assumed that 0.01% CO_2 was too low to stimulate appreciably ethylene production. Conversely, the air-filled flask under darkness contained 0.39% CO_2 at the end of incubation and as much ethylene had been produced as in the flask initially filled with 3% CO_2, indicating the 0.39% CO_2 exerted maximal or nearly maximal activity.

Effects of light and CO_2 on ACC-dependent ethylene production and the endogenous ACC levels in rice leaves. Adams and Yang (1979) have studied ethylene biosynthesis in apple tissue and established the following pathway: methionine→S-adenosylmethionine→ACC→ethylene. Therefore, it would be interesting to determine at which step in the ethylene biosynthetic pathway CO_2 exerts its promotive effect on ethylene production. The effects of light-dark or CO_2 treatment on ethylene production rates and the endogenous ACC level in rice leaf segments was examined. Although dark treatment or CO_2 treatment under light increased ehtylene production several-fold, these treatments did not change the internal levels of ACC appreciably (Table 2), indicating that dark condition or CO_2 exerts its promotive effect mainly through enhancing the conversion of ACC to ethylene. When ACC was applied to rice leaf segments incubated in the light, ethylene production increased markedly with the increase in external ACC concentration up to at least 3 mM (data not shown).

The conclusion that CO_2 exerts its promotive effect through enhancing the conversion of ACC

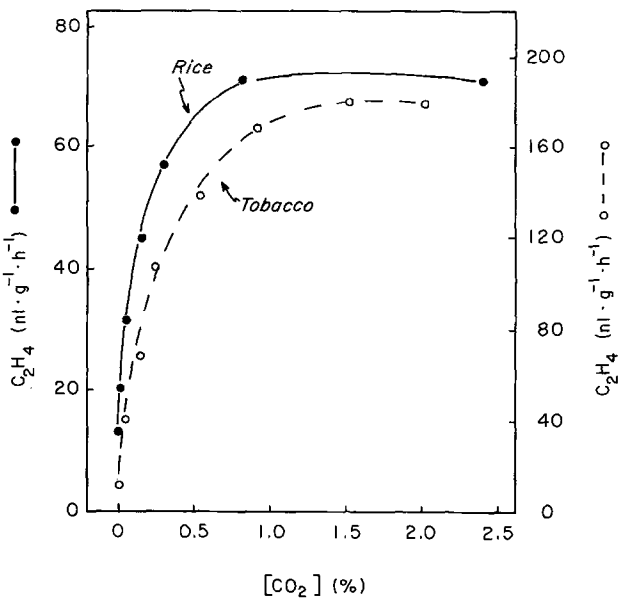

Fig. 3. Effect of CO_2 concentration on ACC-dependent ethylene production in rice leaf segments and tobacco leaf discs. After leaf segments or discs were preloaded with ACC (3 mM) for 3 h in the light, they were enclosed in flasks containing various concentrations of CO_2 and incubated for 1 h in the light. Ethylene was assayed after 1 h of CO_2 treatment. The CO_2 concentration indicated was the average of the CO_2 concentrations present in the flask immediately before and after 1-h incubation

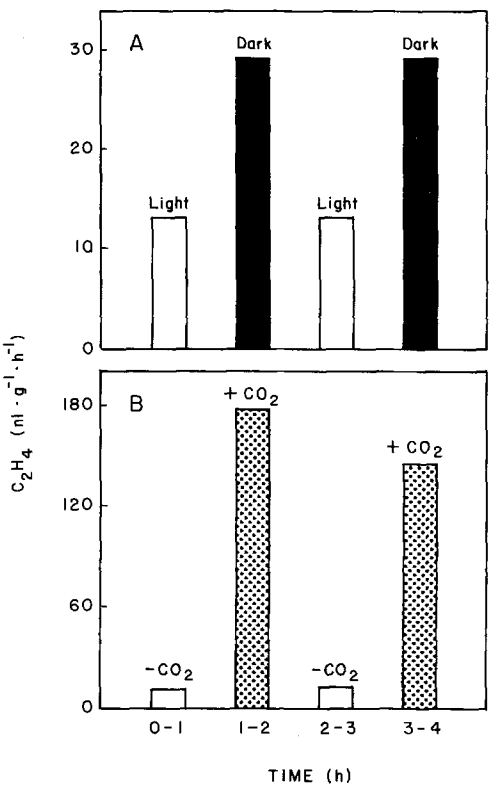

Fig. 4A, B. Effects of light-darkness alternations and minus- and plus-CO_2 alternations on ACC-dependent ethylene production in tobacco leaf discs. After leaf discs were preloaded with ACC (3 mM) for 3 h in the light, they were enclosed in a flask, **A** containing ambient air and incubated alternately under light and darkness for 1 h each, or **B** incubated alternately in the absence (with KOH solution) or presence of 2% CO_2 for 1 h each under light

to ethylene was further supported by the observation that the ACC-dependent ethylene production in the light by rice leaf segments was also greatly stimulated by CO_2 (Table 1). Similar to the basal ethylene production system (Table 1), light markedly inhibited ACC-dependent ethylene production under air, and the promotive effect of CO_2 on ACC-dependent ethylene production was more pronounced under light than under darkness. Once an effective concentration of CO_2 was provided, the rate of ACC-dependent ethylene production became greater in the light than in darkness, indicating that light per se did not inhibit but stimulates the conversion of ACC to ethylene.

The stimulation of ethylene production in the light by CO_2 appeared to be readily reversible, as shown in Fig. 2. These data indicate that CO_2 promotes the conversion of ACC to ethylene not by inducing the synthesis of the enzyme involved in the conversion but by activating the enzyme.

The dependence of ACC-dependent ethylene production on CO_2 concentration under light was examined using rice leaf segments. The data in Fig. 3 show that ACC-dependent ethylene production increased as the CO_2 concentration increased, and reached a maximal rate of CO_2 concentration of about 0.8%; the half-maximal activity of CO_2 was estimated to be about 0.06%.

Effects of light and CO_2 on ACC-dependent ethylene production in tobacco leaves. Since tobacco leaf discs were employed by both Gepstein and Thimann (1980) and de Laat et al. (1981) in their studies of the light inhibition of ACC conversion to ethylene, we have examined whether the light inhibition of ACC conversion to ethylene in tobacco leaf discs was also mediated through CO_2. As in rice leaf segments ACC-dependent ethylene production rate in tobacco leaf discs was inhibited by light (Fig. 4A), but when 2% CO_2 was provided ethylene production was stimulated more than ten times (Fig. 4B). Furthermore this effect of CO_2 on ACC-dependent ethylene production under light was fully reversible (Fig. 4B). The effect of CO_2 concentration on ACC-dependent ethylene production by tobacco leaf discs is illustrated in Fig. 3. Carbon dioxide saturated the system at

1.5%, and the half-maximal activity was obtained at 0.18% CO_2.

Discussion

Gepstein and Thimann (1980) were the first to report that light inhibited ACC-dependent ethylene production. Based on the observation in tobacco leaf discs that mercaptoethanol reversed the light inhibition of ACC conversion to ethylene, they suggested that light exerted its inhibitory effect by oxidizing the essential SH group(s) of the enzyme converting ACC to ethylene. This explanation was questioned by de Laat et al. (1981), who were not able to confirm the finding that mercaptoethanol reversed the light inhibition. Although no specific explanation was offered, de Laat et al. (1981) suggested that light inhibited ACC-converting enzymes at the level of both synthesis and activation.

Our present study clearly shows that light inhibits ethylene production by inhibiting the conversion of ACC to ethylene, and this inhibition is mediated through lowering the internal CO_2 concentration. Since our results indicate that the effects of plus-minus-CO_2 alternation and light-dark alternation are rapid and fully reversible (Figs. 2, 4), it is most unlikely that CO_2 or light regulates ACC conversion to ethylene at the level of synthesis of the enzyme. It is therefore suggested that the light effect is mediated through CO_2 which in turn regulates ethylene production by activating the enzyme involved in the ACC conversion to ethylene. The inhibition of ACC conversion to ethylene by light appears to be a general phenomenon since this effect was observed in leaf tissue of both a monocotyledon (rice) and a dicotyledon (tobacco). It has been reported that dichlorophenyldimethylurea, an inhibitor of photosynthetic electron transport, countereacted the inhibitory action of light on ethylene production (de Laat et al. 1981; Grodzinski et al. 1981). Such results are in accord with our conclusion since this inhibitor would result in the accumulation of CO_2 within the cell, and thereby relieve the inhibitory effect of light. Moreover, our conclusion explains well the previous observation that ACC-induced ethylene production was inhibited by light in green *Pharbitis* cotyledons but not in cotyledons that lacked chlorophyll (de Laat et al. 1981).

In the "CO_2-free atmosphere" there always exists a very low but detectable concentration (0.005–0.01%) of CO_2 in the incubation tubes. It is therefore unlikely that the basal ethylene production in the "CO_2-free" atmosphere (Fig. 3) represents an ethylene-production system which does not require the presence of CO_2, but rather that the low concentration of CO_2 existing in the tissues activates the ethylene-production system. The mechanism by which CO_2 modulates the conversion of ACC to ethylene is not understoood. Elucidation of such a regulatory mechanism awaits the successful isolation of this enzyme in cell-free form. Another type of modulation of ethylene production by light is mediated through phytochrome during phototropic curvature and elongation growth; in this case, ethylene production was inhibited by a short exposure to red light, and the inhibition was reversed by far-red irradiation (Goeschl et al. 1967; Kang and Burg 1972; Samimy 1978). In contrast, the light effect described in this paper and others resulted from long exposure to white light and was related to the photosynthetic system. It should be noted that ethylene production rates under optimal CO_2 are greater in the light than in darkness (Table 1). Thus light per se is a promoting rather than an inhibiting factor with respect to ethylene production from ACC.

This work was supported by a research grant from the National Science Foundation (PCM-8114933).

References

Adams, D.O., Yang, S.F. (1979) Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad. Sci. USA **76**, 170–174

Aharoni, N., Lieberman, M. (1979) Ethylene as a regulator of senescence in tobacco leaf discs. Plant Physiol. **64**, 801–804

Craker, L.E., Abeles, F.B., Shropshire, W. (1973) Light-induced ethylene production in sorghum. Plant Physiol. **51**, 1082–1083

de Laat, A.M.M., Brandenburg, D.C.C., van Loon, L.C. (1981) The modulation of conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene by light. Planta **153**, 193–200

Dhawn, K.R., Bassi, P.K., Spencer, M.S. (1981) Effects of carbon dioxide on ethylene production and action in intact sunflower plants. Plant Physiol. **68**, 831–834

Gepstein, S., Thimann, K.V. (1980) The effect of light on the production of ethylene from 1-aminocyclopropane-1-carboxylic acid by leaves. Planta **149**, 196–199

Gepstein, S., Thimann, K.V. (1981) The role of ethylene in the senescence of oat leaves. Plant Physiol. **68**, 349–354

Goeschl, J.D., Pratt, H.K., Bonner, B.A. (1967) An effect of light on the production of ethylene and the growth of the plumular portion of etiolated pea seedlings. Plant Physiol. **43**, 1077–1080

Graham, D. (1980) Effects of light on "dark" respiration. In: The biochemistry of plants, vol. 2: Metabolism and respiration, pp. 525–579, Stumpf, P.K., Conn, E.E., eds. Academic Press, New York London

Grodzinski, B., Boesel, I., Horton, K. (1981) Effect of light and CO_2 on the release of ethylene from leaves of Xanthium *strumarium*. (Abstr.) Plant Physiol. [Suppl.] **67**, 272

Johnson, C.M., Stout, P.R., Broyer, T.C., Carlton, A.B. (1957) Comparative chlorine requirements of different plant species. Plant Soil **8**, 337–353

Kang, B.G., Burg, S.P. (1972) Relation of phytochrome-enhanced sensitivity to ethylene production. Plant Physiol. **50**, 132–135

Lizada, M.C.C., Yang, S.F. (1979) A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal. Biochem. **100**, 140–145

Saltveit, M.E., Pharr, D.M. (1980) Light stimulated ethylene production by germinating cucumber seeds. J. Am. Soc. Hort. Sci. **105**, 364–367

Samimy, C. (1978) Effect of light on ethylene production and hypocotyl growth of soybean seedlings. Plant Physiol. **61**, 772–774

Wright, S.T.C. (1981) The effect of light and dark periods on the production of ethylene from water-stressed wheat leaves. Planta **153**, 172–180

Young, R.E., Romani, R.J., Biale, J.B. (1961) Carbon dioxide effects on fruit respiration. II. Response of avocados, bananas and lemons. Plant Physiol. **37**, 416–422

Received 23 March; accepted 20 April 1982