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Rhythmicity in Ethylene Production in Cotton Seedlings[1]

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ABSTRACT

Cotyledons of cotton (*Gossypium hirsutum* L.) seedlings grown under a photoperiod of 12 hour darkness and 12 hour light showed daily oscillations in ethylene evolution. The rate of ethylene evolution began to increase toward the end of the dark period and reached a maximum rate during the first third of the light period, then it declined and remained low until shortly before the end of the dark period. The oscillations in ethylene evolution occurred in young, mature, and old cotyledons (7 to 21 day old). These oscillations in ethylene evolution seemed to be endogenously controlled since they continued even when the photoperiod was inverted. Moreover, in continuous light the oscillations in ethylene evolution persisted, but with shorter intervals between the maximal points of ethylene evolution. In continuous darkness the oscillations in ethylene evolution disappeared. The conversion of [3,4-^{14}C]methionine into [^{14}C] ethylene followed the oscillations in ethylene evolution in the regular as well as the inverted photoperiod. On the other hand, the conversion of applied 1-aminocyclopropane-1-carboxylic acid into ethylene did not follow the oscillations in ethylene evolution, but was affected directly by the light conditions. Always, light decreased and darkness increased the conversion of applied 1-aminocyclopropane-1-carboxylic acid into ethylene. It is concluded that in the biosynthetic pathway of ethylene the conversion of 1-aminocyclopropane-1-carboxylic acid into ethylene is directly affected by light while an earlier step is controlled by an endogenous rhythm.

Many plant processes are controlled by endogenous rhythms (12). There are several reports of daily changes in the level of plant hormones, *e.g.* cytokinin levels in poplar leaves (11), ABA levels in sorghum and pearl millet leaves (10, 13), and phaseic acid in sorghum leaves (13). The daily changes in ABA levels were only partially related to the leaf water potential. Recently, Lecoq *et al.* (16) showed that in soybean leaves the oscillations in ABA level persisted in continuous light, indicating that such ABA levels were endogenously controlled. Daily changes in ethylene evolution were found in leaves of several species (7, 15) and in cotton fruits (17). El-Beltagy *et al.* (7) showed that these oscillations were not related to changes in the leaf water saturation deficit or stomatal aperture but were endogenously controlled, since they occurred also in continuous light or darkness.

In the present work we characterized the rhythmical changes in ethylene evolution in cotton seedlings mainly in relation to its biosynthesis.

MATERIALS AND METHODS

Cotton (*Gossypium hirsutum* L.) seeds were germinated and grown for 7 d in plastic pots (12.5 cm in diameter, 12 cm in height) filled with peat, and irrigated with water. The seedlings were grown in growth chambers at 29°C under photoperiods which were specified for each experiment. The light (200 μE m^{-2} s^{-1}) source was a combination of regular incandescent light and fluorescent light (F48T18-CW-VHO, Sylvania).

The production of ethylene was measured in detached cotyledons (4 g) that were enclosed immediately in flasks (63 cm^3) for 2 h. The flasks were kept in light conditions similar to those the seedlings would be in if they were not harvested. In order to prevent dehydration of the shoots, a filter paper moistened with 0.6 ml of distilled H_2O was placed on the bottom of each flask. Ethylene was determined by GC as described by Aharoni *et al.* (3).

AVG[3] (0.1 mM) was applied by spraying the seedlings until runoff. ACC (1 mM) was applied by floating 6 discs (7 mm in diameter) on 1 ml of ACC solution in 25 ml Erlenmeyer flasks. The flasks were sealed with rubber serum stoppers before ethylene production was determined.

For tracer studies, 6 discs (7 mm in diameter) were floated on 1 ml of 0.95 μCi L-[3,4-^{14}C]methionine (53 mCi/mmol) in 25-ml Erlenmeyer flasks. The flasks were sealed for 1 h before labeled ethylene from methionine was collected and determined as described by Aharoni *et al.* (2).

Each experiment was repeated at least 3 times. The data presented are of a typical experiment with at least 3 replicates in each treatment.

RESULTS

Cotyledons of cotton seedlings grown under a photoperiod of 12 h darkness and 12 h light showed daily oscillations in ethylene evolution. The rate of ethylene evolution began to increase toward the end of the dark period, it reached its maximum in the first third of the light period, and then it declined and remained low until shortly before the end of the dark period (Fig. 1). Treatment with AVG decreased ethylene evolution from the cotyledons (Fig. 1). The minimal and maximal stages of ethylene evolution, essentially, persisted in young (7-d-old) cotyledons which were not yet fully expanded as well as in fully expanded cotyledons (14-d-old) and mature cotyledons (21-d-old) (Table I).

Seedlings grown under photoperiod of 12 h darkness and 12 h light for 4 d from germination were transferred to inverted

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[3] Abbreviations: AVG, aminoethoxyvinylglycine; ACC, 1-aminocyclopropane-1-carboxylic acid; SAM, *S*-adenosylmethionine.

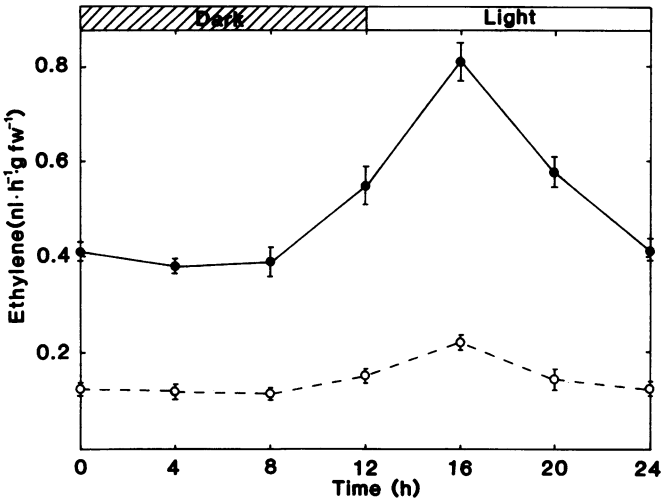

FIG. 1. Oscillations in ethylene evolution in cotton cotyledons. Seedlings were grown under photoperiod of 12 h darkness and 12 h light for 4 d from germination, then ethylene evolution was measured at 4-h intervals. AVG was applied 16 h before the ethylene measurement. (■), dark period; (▩), light period; (●), −AVG; (○), +AVG.

Table I. *Effect of the Cotyledon Age on the Minimal and Maximal Ethylene Evolution*

Seedlings were grown under photoperiod of 12 h darkness and 12 h light from germination. Ethylene was measured every 7 d at 4 and 16 h after the beginning of the dark/light cycle (minimal and maximal stages of ethylene evolution, respectively).

Time	Seedlings Age (d)		
	7	14	21
h	*nl h⁻¹ g⁻¹ fresh wt*		
4	0.34 ± 0.02	0.47 ± 0.06	0.57 ± 0.10
16	0.75 ± 0.02	1.26 ± 0.10	0.85 ± 0.04

photoperiod, *i.e.* the dark period was changed to light period and the light period was changed to dark period. Under such conditions the rate of ethylene evolution continued to oscillate according to the chronological time, irrespective of the immediate light conditions. Under the inverted photoperiod, as in the regular photoperiod, the maximal rate of ethylene evolution was about 16 to 20 h after the beginning of the cycle, and then it declined toward the end of the cycle and remained low at its beginning (Fig. 2). Under continuous light the changes in ethylene evolution continued. However, the maximal ethylene evolution occurred about each 12 h instead of each 24 h under the regular photoperiod (Fig. 3). Under continuous darkness the changes in ethylene evolution damped (Fig. 3).

The conversion of [3,4-^{14}C]methionine into ethylene followed the rhythmical changes in ethylene evolution. It was low when the rate of ethylene evolution was low, and high when the rate of ethylene evolution was high (Table II). The same pattern of conversion of [3,4-^{14}C]methionine into ethylene was observed in the regular dark/light cycle and in inverted photoperiod (Table II).

The conversion of ACC into ethylene was not affected by the time in the dark/light cycle. It was affected only by the light conditions during the conversion of ACC into ethylene. Darkness stimulated the conversion of ACC into ethylene while light inhibited it at all times during the dark/light cycle (Table III).

DISCUSSION

Cotyledons of cotton seedlings grown under a photoperiod of 12 h darkness and 12 h light showed daily oscillations in ethylene

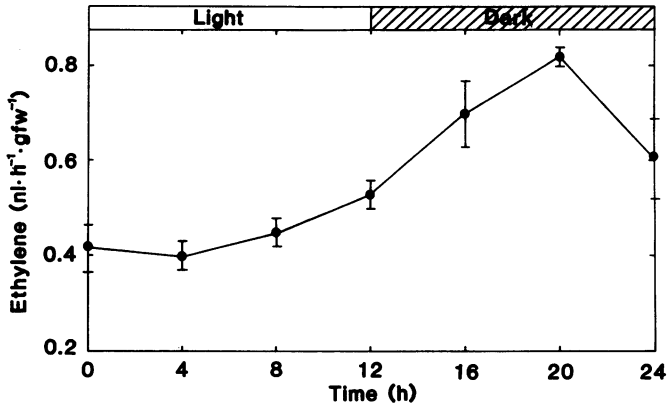

FIG. 2. Oscillations in ethylene evolution in cotton cotyledons under inverted photoperiod. Seedlings were grown under photoperiod of 12 h darkness and 12 h light for 4 d from germination, then the dark period was changed to light period and the light period was changed to dark period. Ethylene evolution was measured at 4-h intervals during the last light and dark periods. (■), dark period; (▩), light period.

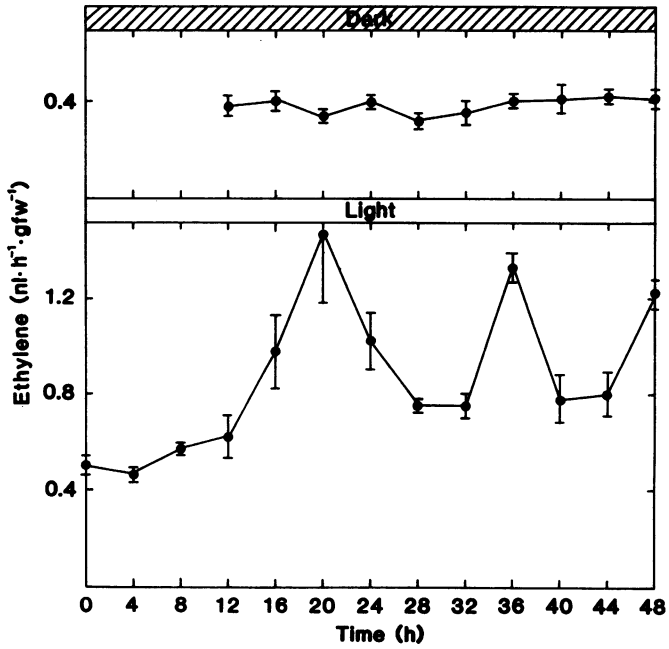

FIG. 3. Oscillations in ethylene evolution in cotton cotyledons under continuous light or darkness. Seedlings were grown under photoperiod of 12 h darkness and 12 h light for 4 d from germination, then after the regular dark or light periods the seedlings were kept on continuous darkness or light, respectively. Ethylene was measured at 4-h intervals during the continuous dark and light periods. (■), dark period; (▩), light period.

evolution. Similar phenomena were found in leaves of several other species (7, 15) and cotton fruits (17). The source of the ethylene in the cotton cotyledon was from its regular biosynthetic pathway from methionine to SAM to ACC and to ethylene as suggested by Adams and Yang (1) since [3,4-^{14}C]methionine was converted into [^{14}C]ethylene, and it was inhibited by AVG, an inhibitor of the enzyme ACC synthase which catalyzes the conversion of SAM to ACC. The high and low levels of ethylene evolution were correlated with high and low rates of [3,4-^{14}C] methionine conversion into [^{14}C]ethylene, indicating that the oscillations in ethylene evolution reflected oscillations in its biosynthesis.

The oscillations in the biosynthesis of ethylene (as indicated

Table II. *Conversion of [3,4-^{14}C]Methionine into Ethylene by Cotton Cotyledons at the Minimal and Maximal Stages of Ethylene Evolution*

Seedlings were grown under photoperiod of 12 h darkness and 12 h light for 4 d from germination. Conversion of [3,4-^{14}C]methionine into ethylene was measured in cotyledon discs at 4 h and 16 h after the begininng of the dark/light cycle (minimal and maximal stages of ethylene evolution, respectively).

Time	Light	Conversion of [3,4-^{14}C]Methionine into Ethylene
h		*dpm h^{-1} g^{-1} fresh wt*
4	−	1190 ± 60
16	+	1690 ± 40
4[a]	+	890 ± 100
16[a]	−	1450 ± 180

[a] Time in inverted photoperiod, *i.e.* the dark period was changed to light period and the light period was changed to dark period.

Table III. *Conversion of ACC into Ethylene by Cotton Cotyledons at the Minimal and Maximal Stages of Ethylene Evolution*

Seedlings were grown under photoperiod of 12 h darkness and 12 h light for 4 d from germination. Conversion of ACC into ethylene by cotyledon discs was measured at 4 h and 16 h after the beginning of the dark/light cycle (minimal and maximal stages of ethylene evolution, respectively).

Time	Light	Conversion of ACC into Ethylene
h		*nl h^{-1} g^{-1} fresh wt*
4	−	55.1 ± 3.6
16	+	12.1 ± 0.1
4[a]	+	14.5 ± 0.5
16[a]	−	85.1 ± 8.2

[a] Time in inverted photoperiod, *i.e.* the dark period was changed to light period and the light period was changed to dark period.

by conversion of [3,4-^{14}C]methionine into [^{14}C]ethylene) and in the evolution of ethylene seemed to be endogenously controlled since they occurred irrespective of the immediate light conditions. However, the damping of the oscillations in continuous darkness implies that their control mechanism must be reset by light. In continuous light the period of the oscillations was changed from 24 h to 12 to 16 h. Similar change in the period of the oscillations upon transfer to continuous light was found also in the activity of (NADP) glyceraldehyde-3-P dehydrogenase during chloroplast maturation (5).

The rates of conversion of [3,4-^{14}C]methionine into [^{14}C] ethylene always correlated with the oscillations in ethylene evolution during the regular and inverted photoperiods. On the other hand, the conversion of applied ACC (the immediate precursor of ethylene in plant tissue [1]) into ethylene, did not correlate with the oscillations in ethylene evolution. The conversion of ACC into ethylene was always affected by the immediate light conditions, light decreased the conversion of ACC into ethylene, while darkness increased it. A similar effect of light on the conversion of ACC into ethylene was found in several other plants systems (6, 8, 14). These results suggested that, in the biosynthetic pathway of ethylene in cotton cotyledons, the steps from methionine to ACC are affected by an endogenous rhythm while the step from ACC to ethylene is affected primarily by the immediate light conditions.

There are contradictory reports about the effect of light on ethylene evolution. In cucumber seedlings light stimulated ethylene evolution (18), while in wheat leaves light inhibited ethylene evolution (19). It seems that the conversion of ACC into ethylene is inhibited by light (6, 8). Also, it was suggested that light exerted its effect by changing the internal level of CO_2 which directly modulated the conversion of ACC into ethylene (4, 9, 14). In cotton cotyledons the maximal evolution of ethylene occurred during the light period although the last stage in the biosynthetic pathway of ethylene, the conversion of ACC into ethylene, was probably inhibited by the light. It seemed that the observed level of ethylene evolution at any time is determined by a combination of rhythmical changes that occurred in the biosynthetic pathway between methionine and ACC and the immediate effect of light on the conversion of ACC into ethylene.

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