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Role of Ethylene in the Senescence of Detached Rice Leaves[1]

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CHING HUEI KAO AND SHANG FA YANG
Department of Agronomy, National Taiwan University, Taipei, Taiwan, Republic of China (C. H. K.); and Department of Vegetable Crops, University of California, Davis, California 95616 (S. F. Y.)

ABSTRACT

The role of ethylene in the senescence of detached rice leaves in relation to their changes in 1-aminocyclopropane-1-carboxylic acid (ACC) content and ethylene production was studied. In freshly excised rice leaf segments, ACC level and ethylene production rates were very low. Following incubation, the rates of ethylene production increased and reached a maximum in 12 h, and subsequently declined. The rise of ethylene production was associated with a 20- to 30-fold increase in ACC level.

Ethylene seems to be involved in the regulation of the senescence of detached rice leaves. This conclusion was based on the observations that (a) maximum ethylene production preceded chlorophyll degradation, (b) ACC application promoted chlorophyll degradation, (c) inhibitors of ethylene production and ethylene action retarded chlorophyll degradation, and (d) various treatments such as light, cycloheximide, α,α-dipyridyl, Ni^{2+}, and cold temperature, which retarded chlorophyll degradation, also inhibited ethylene production.

Abscisic acid promoted senescence but significantly decreased ethylene production, whereas benzyladenine retarded senescence but promoted ethylene production. This is interpreted to indicate that abscisic acid treatment increased the tissue sensitivity to ethylene, whereas benzyladenine treatment decreased it.

The senescence of detached rice leaves is characterized by a decrease in Chl and protein contents and an increase in α-amino nitrogen content (12). Senescence of detached rice leaves has been reported to be retarded by Ag^+, Co^{2+}, Ni^{2+}, or by anaerobic conditions (11, 15, 24). Since the discovery of ACC[2] as the key intermediate in the pathway of ethylene biosynthesis, our understanding of the regulation of ethylene production has been greatly clarified (1, 23). Cobalt ion and anaerobiosis are known to interfere with the conversion of ACC to ethylene (23). Like Co^{2+}, Ni^{2+} inhibits ethylene production in many plant tissues (16). In contrast, Ag^+ is a potent inhibitor of ethylene action in plants (4, 5). Recent work of Aharoni and Lieberman (3) and Gepstein and Thimann (9) suggested that endogenous ethylene played a significant role in the senescence process of detached tobacco and oat leaves.

In the present investigation, we examined the role of ethylene in inducing senescence of detached rice leaves and the changes of ethylene production in relation to their senescence as influenced by various environmental and external factors, such as light, CO_2, cytokinin, and ABA treatments, which may either accelerate or retard senescence. Since ACC is the immediate precursor of ethylene and its level regulates ethylene production rate (1), the changes of endogenous ACC level in relation to ethylene production rates during senescence were also studied.

MATERIALS AND METHODS

Plant Materials and Incubation Conditions. Rice (*Oryza sativa* L. 'Taichung' Native 1) seedlings were cultured as previously described (14). The apical 3-cm segments excised from the third leaves of 8-d-old seedlings were used. A group of 10 segments was floated in a 50-ml flask containing 10 ml test solution. Incubation was carried out at 30°C either under light (80 μmol quanta m^{-2} s^{-1}) provided by a mixture of cool-white and Grolux lamps or in darkness. In experiments with Ag^+ treatment, leaf segments were pretreated with 10 mg/l silver nitrate for 30 min, and then transferred to 50-ml flasks containing test solution without Ag^+. For the experiments in which various concentrations of CO_2 were used, all flasks were flushed with CO_2-free air and sealed with rubber serum caps. To obtain desired concentrations of CO_2, known amount of CO_2 was injected into each sealed flask; a CO_2-free atmosphere was achieved by hanging in the flask a center well containing a filter paper wick wetted with 0.2 ml of 20% KOH. All experiments were repeated at least three times. Similar results and identical trends were obtained each time. The data reported here were from a single experiment.

Chl Determination. Chl was extracted and determined as described before (12). Chl was expressed as A_{665} per 10 segments in 10 ml 80% ethanol.

Determination of Ethylene. The flasks were flushed with fresh air, sealed with rubber serum caps and incubated at 30°C. For the time-course experiment (Fig. 1), 1-ml gas sample was withdrawn from the headspace of the flask at specified times. The flasks were then flushed with fresh air and resealed until the next ethylene determination. For those experiments with treatments that included ABA, ACC, BA, CHI, DP, low temperature, inhibitors of ethylene production or inhibitors of ethylene action, ethylene that accumulated in the first 6 h of incubation under light or dark condition was determined. Ethylene was assayed using a gas chromatograph equipped with an alumina column and a flame ionization detector.

Determination of ACC. ACC was extracted and determined as described previously (14).

Determination of Respiration Rates. Leaf segments were placed into 14-ml test tubes containing 0.5 ml of deionized H_2O, and sealed with rubber serum caps after flushing with fresh air. Respiration rates were determined by measuring the CO_2 accumulated in darkness during a 2-h period; respiration rates were linear for 4 h. Gas samples were taken as described for ethylene and determined with a gas chromatograph (model 800, Carle

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[2] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; AOA, aminooxyacetic acid; AVG, aminoethoxyvinylglycine; CHI, cycloheximide; DP, $\alpha\alpha$-dipyridyl.

Instruments, Inc.) equipped with a silica-gel column and a thermoconductivity detector.

RESULTS

Changes in Ethylene Production, Respiration Rate, and Chl and ACC Contents of Detached Leaves during Senescence in Light or Darkness. The senescence of rice leaves was followed by measuring the decrease of Chl. Figure 1 shows the time courses of Chl and ACC contents and ethylene and CO_2 production rates of leaf segments floating on water in the light or dark. The decrease of Chl content was evident at 24 h after leaf detachment under both light and dark conditions. It is also clear from Figure 1 that light was effective in retarding senescence of rice leaf segments. Under both light and dark conditions, the rates of ethylene production increased immediately after excision and reached a maximum in 12 h, and subsequently declined. Light substantially inhibited the endogenous ethylene production. The increase of ACC level in the leaf segments coincided closely with the increase of ethylene production under both light and dark conditions. The endogenous level of ACC rapidly increased and peaked at 9 and 12 h after excision in the light and dark, respectively, and then declined. The rise of ethylene production was associated with a 20- and 30-fold increase in ACC content in light and darkness, respectively. During the first 9 h incubation, no significant difference could be found in ACC level between light and dark treatments, but subsequently ACC contents in the light were lower.

The relationship between leaf senescence and respiration rate is not a simple one. If senescence is viewed as a general decline in function (20), a steady decline of respiration rate would be expected. On the other hand, some senescence-related changes, such as the synthesis of degradative enzymes (20), seem to require higher respiratory activity. In detached leaves, the rise of respiration rate is usually preceded by a steep fall or a constant rate (2, 17, 18). It is also true in rice leaf segments as shown in Figure 1. Respiration rate decreases during early stage of incubation, but it started to increase after about 12 and 48 h incubation in the light and dark, respectively. Under both light and dark conditions, respiration peaked at a later stage (about 72 h after incubation), during which time leaf segments had low Chl level. It seems that the rise of respiration in the present system is the result rather than the cause of leaf senescence. In climacteric fruits, the onset of respiratory increase is accompanied by the onset of ethylene production, whereas the ethylene production in rice leaves occurs considerably earlier than maximal respiration rate. Thus, it is unlikely that ethylene plays any causative role in inducing the rise of respiration rate during the senescence of rice leaf segments. Figure 1 also shows that light substantially promoted respiration rate throughout the course of senescence. Since respiration was determined under dark condition, the higher respiration rate is unlikely due to photorespiration.

Effects of Inhibitors of Ethylene Production and ACC on the Senescence of Detached Leaves. If ethylene plays a regulatory role in senescence, it is expected that inhibitors of ethylene

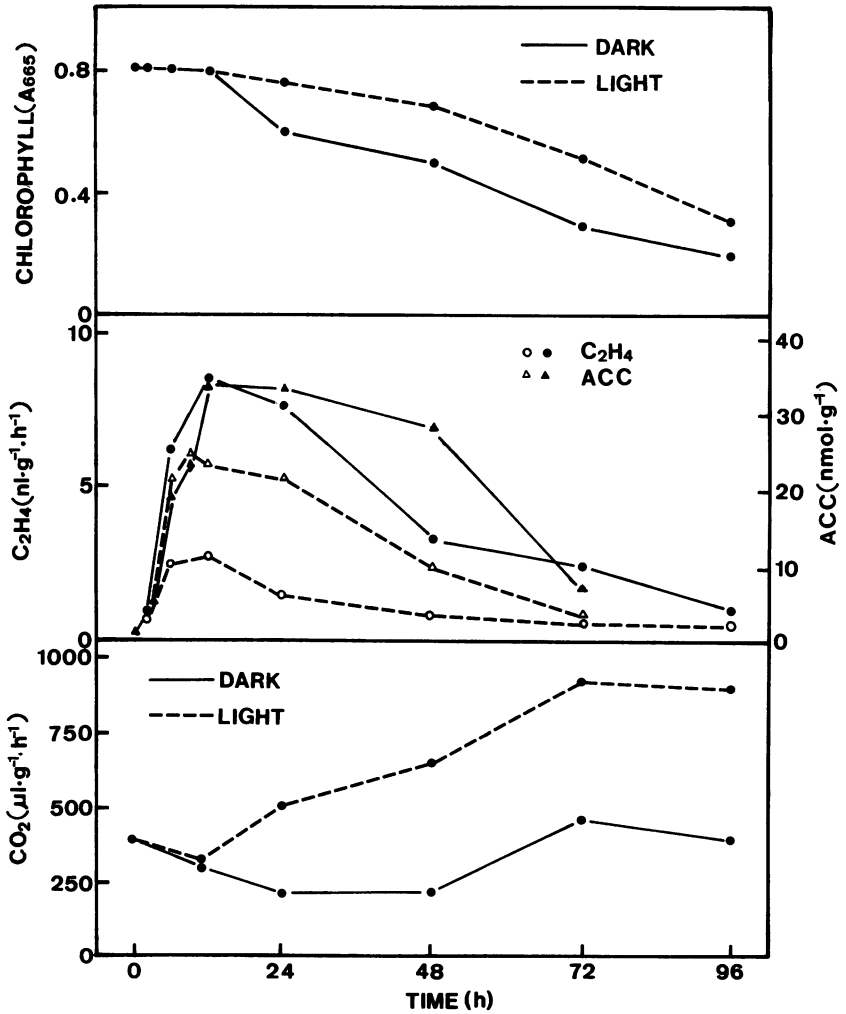

FIG. 1. Changes in ethylene production, respiration rate, ACC, and Chl contents of detached rice leaves during senescence in light and darkness.

Table I. *Effects of ACC, AVG, and AOA on Ethylene Production and Chl Content of Detached Rice Leaves in Light and Darkness*

Chl was determined after 3 and 4 d in darkness and light, respectively.

Treatment	Darkness		Light	
	C_2H_4	Chl	C_2H_4	Chl
	$nl\ g^{-1}\ h^{-1}$	A_{665}	$nl\ g^{-1}\ h^{-1}$	A_{665}
Control	7.88	0.383 ± 0.021	3.62	0.403 ± 0.034
ACC, 1 mM	15.95	0.334 ± 0.002	9.37	0.292 ± 0.008
AVG, 0.2 mM	1.22	0.452 ± 0.031	1.23	0.521 ± 0.006
AOA, 1 mM	2.22	0.458 ± 0.019		

biosynthesis would retard senescence. AVG and AOA, which are known to inhibit ethylene production by blocking ACC formation (23), significantly retarded the senescence of detached leaves incubated in the light or dark (Table I). Table I also shows the effect of ACC, the immediate precursor of ethylene (1), which significantly promoted ethylene production, and accelerated senescence of detached leaves under both dark and light conditions. Co^{2+}, an inhibitor of ethylene production (5), which blocks the conversion of ACC to ethylene (25), also retarded senescence and ethylene production in rice leaves (Fig. 2).

The effects of Co^{2+} are more pronounced in darkness than in the light. It should be noted that the effective concentration of Co^{2+} to retard senescence and to inhibit ethylene production in the light is much lower than that in darkness.

Effects of Inhibitors of Ethylene Action on the Senescence of Detached Leaves. Ag^+ and CO_2 are known to inhibit ethylene action in many physiological responses (4, 5, 7, 24). Both Ag^+ and CO_2 effectively retarded senescence of detached rice leaves, although they promoted ethylene production (Table II). The promotive effect of Ag^+ and CO_2 on ethylene production in other plant systems has been well documented (3, 14). Ag^+ was, however, less effective than CO_2 on retardation of Chl degradation. These observations are in contrast to those of Aharoni and Lieberman (3), who reported that Ag^+ was more effective than CO_2 in retarding senescence in tobacco leaves.

Effects of Senescence Retardants on Ethylene Production and Senescence of Detached Leaves. CHI and Ni^{2+} have been reported to retard senescence in detached rice leaves (11, 15). The chelator, DP, and low temperature (5°C) were also known to delay senescence in oat leaf tissues in the dark (19, 20). All these treatments are expected to inhibit ethylene production, if ethylene plays a role in regulating senescence. Indeed, it is the case. Table III shows the effects of CHI, DP, Ni^{2+}, and low temperature (5°C) on leaf senescence and ethylene production. All these treatments not only effectively retarded leaf senescence, but also, inhibited ethylene production.

Effects of BA and ABA on Ethylene Production and Senescence of Detached Leaves. It has long been recognized that cytokinins are effective in retarding the senescence of most, if not all, leaves. The effect of cytokinins in retarding senescence is species- or variety-specific; for the rice variety used in this investigation, BA has been found to be the most active cytokinin in retarding senescence in the dark (11). The effect of BA on leaf senescence in relation to ethylene production is presented in Figure 3. Although BA effectively retarded senescence in the light, as well as in the dark, BA significantly promoted ethylene production. Among the known promoters of senescence, ABA has been studied most widely. Recently, Gepstein and Thimann (8) claimed that ABA is an endogenous factor in leaf senescence. Figure 4 shows the effect of ABA on ethylene production and leaf senescence in the light. Ethylene production was greatly decreased by ABA. In contrast, leaf senescence increased as the concentration of ABA were increased. In dark, ABA also promoted leaf senescence and decreased ethylene production (data not shown).

DISCUSSION

The changes of ACC content and ethylene production have been studied in ripening fruit and senescing flowers (6, 10). In senescing rice leaves, the relationship between ACC and ethylene production is generally similar to that found in ripening fruits and senescing flowers. In freshly excised rice leaf segments, both ACC content and ethylene production rate were very low. Subsequently, ethylene production rate increased which was accompanied by an increase in ACC content, suggesting that ACC is an intermediate in the biosynthesis of ethylene throughout the senescence process. Thus, the biosynthetic pathway in senescing rice leaves appears to be the same as that established in pple tissue (1).

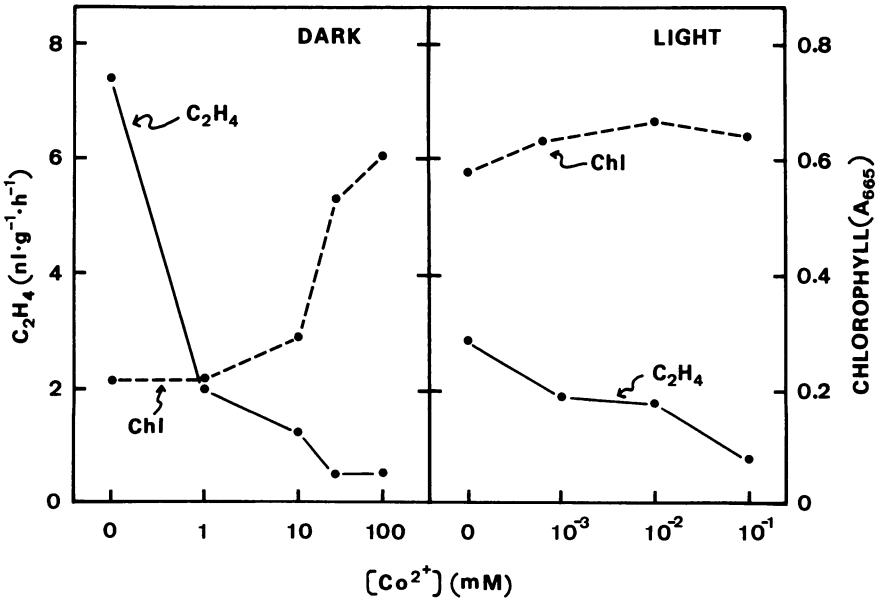

FIG. 2. Effects of the concentrations of Co^{2+}, applied as $CoCl_2$, on Chl content and ethylene production of detached rice leaves. Chl was determined after 3 d.

Table II. *Effects of $AgNO_3$ or CO_2 Treatment on Ethylene Production and Chl Content of Detached Rice Leaves in Light and Darkness*

Chl was determined after 3 d in darkness or 4 d in the light in experiment 1 and after 3 d in experiment 2. For Ag^+ treatment, the detached leaves were pretreated for 30 min in 10 mg/l $AgNO_3$ solution; for CO_2 treatment, the leaves were incubated in 5% CO_2. Ethylene accumulated in the first 6 h of incubation was determined.

Treatment	Darkness		Light	
	C_2H_4	Chl	C_2H_4	Chl
	$nl\ g^{-1}\ h^{-1}$	A_{665}	$nl\ g^{-1}\ h^{-1}$	A_{665}
Exp. 1				
Control	7.04	0.348 ± 0.004	2.40	0.385 ± 0.005
Ag^+	15.58	0.491 ± 0.031	9.75	0.544 ± 0.055
Exp. 2				
Control	5.81	0.216 ± 0.021	4.64	0.494 ± 0.034
CO_2	8.03	0.570 ± 0.022	16.76	0.650 ± 0.009

Table III. *Effects of CHI, DP, Ni^{2+}, and Low Temperature on Ethylene Production and Chl Content of Detached Rice Leaves in Darkness*

Chl was determined after 3 d in darkness. The concentrations of CHI, DP, and Ni^{2+} employed were 0.5, 0.1, and 10 mM, respectively. Low temperature treatment was at 5°C.

Treatment	C_2H_4	Chl
	$nl\ g^{-1}\ h^{-1}$	A_{665}
Control	6.42	0.374 ± 0.007
CHI	3.40	0.752 ± 0.002
DP	5.44	0.810 ± 0.004
Ni^{2+}	1.80	0.739 ± 0.014
Low temperature	3.87	0.842 ± 0.024

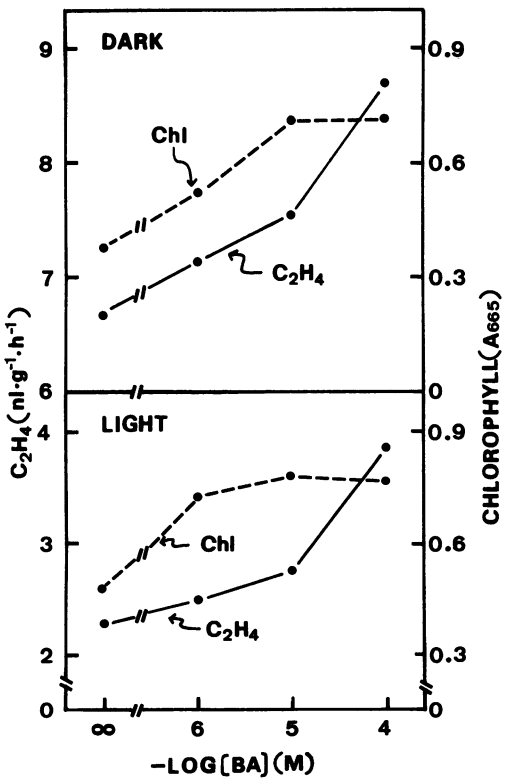

FIG. 3. Effects of BA concentrations on ethylene production and Chl content of detached rice leaves. Chl was determined after 3 d.

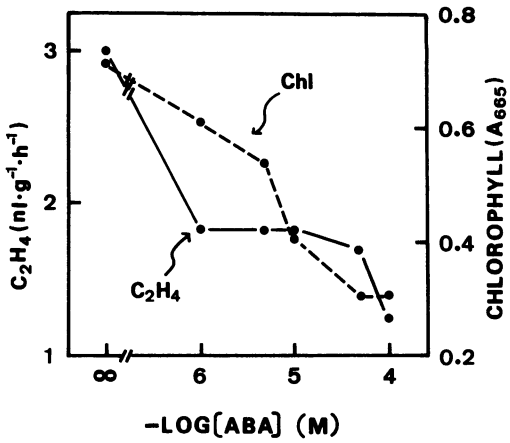

FIG. 4. Effects of ABA concentrations on ethylene production and Chl content of detached rice leaves in light. Chl was determined after 2 d in light.

Light substantially inhibited ethylene production throughout the senescence process. During the first 9-h incubation, there was no significant difference in ACC content between light and dark conditions (Fig. 1), indicating that the inhibition of ethylene production by light is exerted not at the level of ACC but at the conversion of ACC to ethylene. Using detached rice and tobacco leaves, Kao and Yang (14) found that inhibition of ethylene production by light was mediated through the internal level of CO_2, which directly modulated the activity of the enzyme converting ACC to ethylene.

Since the increase of ethylene production in rice leaves is rapid following excision, one may argue that the ethylene production is probably a wound or other stress response. When detached leaves were used to study senescence and determine ethylene production, wounding is always a problem. However, in the present study, each long and narrow rice leaf was cut once transversely, the area of wounding was very small. It has been reported that there was a slight but significant increase in the fresh weight of rice leaf segments floated on water (13). Therefore, the rapid increase of ethylene production could not result from water stress. The onset of ethylene production is therefore thought to be an excision-related response.

In the study of hormonal control of leaf senescence, two approaches are commonly employed. The first and most widely used is external application of plant hormones or chemicals which are known to affect the synthesis or to exert an effect on the action of the hormone. The second is to correlate changes in endogenous hormone content with senescence. The present study indicated that ethylene participates in the regulation of rice leaf senescence. This conclusion was based on observations (under both light and dark conditions) that (a) endogenous ethylene production preceded Chl degradation (Fig. 1); (b) inhibitors of ethylene production such as AVG and CO^{2+} retarded senescence (Table I; Fig. 2); (c) ACC, the immediate precursor of ethylene, promoted senescence (Table I); (d) inhibitors of ethylene action as Ag^+ and CO_2 retarded senescence (Table II); and (e) other treatments such as light, CHI, DP, Ni^{2+}, and low temperature, which retarded senescence, also inhibited ethylene production (Fig. 1; Table III). Recently, the importance of ethylene in regulating senescence of detached leaves has also been reported by Aharoni and Lieberman (3) and Gepstein and Thimann (9).

Using oat leaves, Gepstein and Thimann (9) found kinetin did not affect ethylene production under dark conditions, but promoted ethylene production under light conditions. They also found that ABA promoted ethylene production, which is in contrast to our results (Fig. 4). The effect of ABA on ethylene production found by them was probably a wound response, since

they used fine abrasive to scrub leaf tissues in order to enhance the entry of ABA. Wright (22), however, reported that ABA significantly inhibited ethylene production from both excised wilted and nonstressed wheat leaves.

When ABA and cytokinins are applied exogenously, they are effective in promoting and retarding, respectively, leaf senescence. Furthermore, the endogenous level of ABA and cytokinins increases and decreases, respectively, before the onset of senescence symptoms (20). Thus, it is probable that leaf senescence is regulated by many factors including interactions among cytokinins, ABA, and ethylene. Recently, Trewavas (21) developed a concept of using variations of quantitative tissue sensitivity to explain the developmental phenomena. It seems that ethylene effect is governed not only by the ethylene production rate of the tissue, but also by the tissue sensitivity to ethylene. In the present system, ABA promoted senescence but inhibited ethylene production, whereas BA retarded senescence but promoted ethylene production. A simple explanation which accounts for these seemingly contradictory observations is that ABA increases tissue sensitivity to ethylene, whereas BA decreases it. Consequently, even though ABA inhibits and BA promotes ethylene production, the overall ethylene effects may not parallel with their ethylene production rates.

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