Bot. Bull. Acad. Sin. (1993) **34:** 163-168

Characteristics of the induction of the ethylene by cadmium in detached rice leaves

Shu Man Hou and Ching Huei Kao[1]

Department of Agronomy, National Taiwan University, Taipei, Taiwan, Republic of China

(*Received November 30, 1992; Accepted January 19, 1993*)

Abstract. The effect of cadmium on the production of ethylene in detached rice leaves was investigated. Cadmium promoted the production of ethylene in detached rice leaves under both light and dark conditions. Cadmium stimulated the production of ethylene shortly after its application. The stimulatory effect of cadmium resulted from the enhancement of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene. Cadmium also induced the accumulation of putrescine and spermine in detached rice leaves. Both D-arginine and α-methylornithine, inhibitors of the biosynthesis of putrescine, reduced the level of putrescine and the production of ethylene induced by cadmium. Since [illegible] production of ethylene in detached rice leaves (Chen et al. Plant

ml of test solution in a Petri dish. All samples were kept at 27°C in the dark. Cadmium chloride was used throughout the experiment.

After various periods of incubation, the leaf samples were used to determine the production of ethylene and the levels of polyamines. For the production of ethylene, the leaf samples were transferred to 14-ml test tubes. The tubes were sealed with serum caps. At the indicated times, a 1-ml gas sample were withdrawn from the test tube and injected into the gas chromatograph equipped with an alumina column and a flame ionization detector. For polyamine determination, leaf samples were homogenized in 5 ml of 5% perchloric acid. Polyamine levels were determined using high performance liquid chromatography after benzoylation as described previously (Chen and Kao, 1991). Absolute levels of ethylene and polyamines varied among experiments because of seasonal effects. However, the patterns of responses to cadmium were reproducible. All data were expressed on fresh weight basis.

All experiments were repeated at least three times. Similar results and identical trends were obtained on each occasion. The data reported here are all from a single experiment.

Results and Discussion

The effect of the concentration of cadmium on the production of ethylene in detached rice leaves in darkness is presented in Fig. 1. Cadmium slightly but significantly promoted the production of ethylene in detached rice leaves. Increasing concentrations of cadmium from 0.01 to 0.5 mM progressively enhanced the production of ethylene by detached rice leaves. The ability of cadmium to stimulate the production of ethylene was also observed under light condition (data not shown).

Figure 2 shows the changes with time in the rates of production of ethylene in detached rice leaves treated with 0.1 mM cadmium. Following incubation, the rates of ethylene production in control leaf segments increased and reached a maximum in 9 h. Promotion of the production of ethylene by cadmium was detected shortly (within 4 h) after application.

In order to determine if cadmium-induced ethylene is formed via the methionine→S-adenosylmethionine→ACC→ethylene pathway, the effects of 1 mM AOA and 1 mM $CoCl_2$, which inhibit the formation of ACC and the conversion of ACC to ethylene, respectively, on cadmium-induced ethylene production were studied. After preincubation on solutions containing AOA or $CoCl_2$, ethylene production during the following cadmium treatment was inhibited (Table 1). This result indicates that cadmium-induced ethylene production is via the methionine → S-adenosylmethione → ACC → ethylene

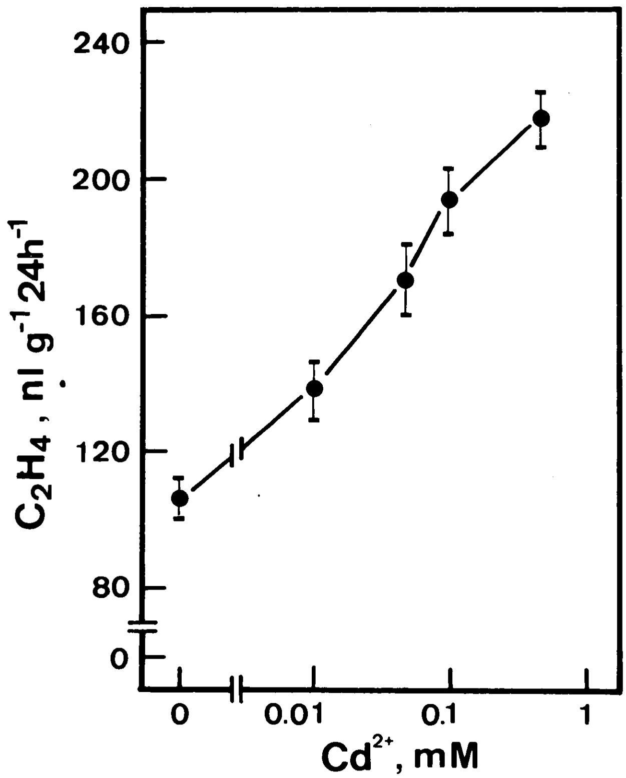

Fig. 1. Relationship between concentration of cadmium (Cd^{2+}) and the production of ethylene in segments of rice leaves. Accumulation of ethylene was determined after a 24 h incubation period in darkness. Bars indicate standard errors.

Table 1. *Effects of aminooxyacetic acid (AOA) and $CoCl_2$ on ethylene production induced by Cd^{2+} in detached rice leaves*

Measurements were carried out after a 24 h incubation on 0.1 mM Cd^{2+}. AOA (1 mM) or $CoCl_2$ (1 mM) was present during a 2h preincubation and the incubation on Cd^{2+}. Values are average with standard errors.

Treatment	Ethylene (nl g^{-1} 24 h^{-1})
No additions	109 ± 2
Cd^{2+}	269 ± 2
Cd^{2+}+AOA	35 ± 5
$Cd^{2+}+CoCl_2$	29 ± 3

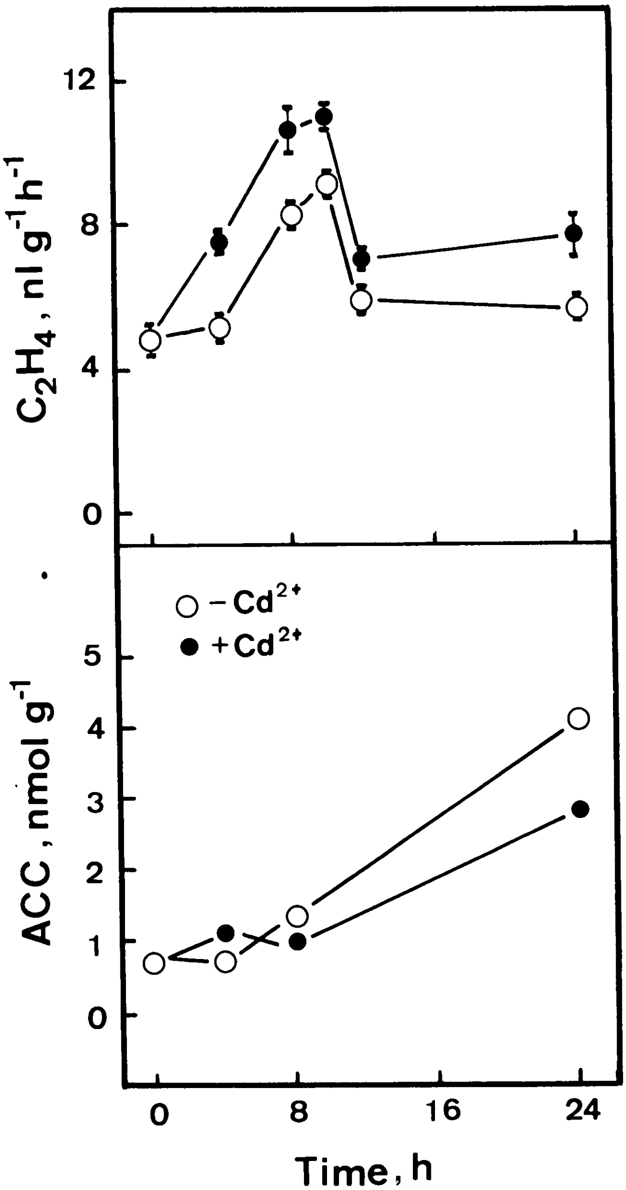

Fig. 2. Change with time in rates of ethylene production (upper) and ACC level (lower) in segments of rice leaves treated with cadmium. Segments were treated with either water (-Cd^{2+}) or 0.1 mM cadmium (+Cd^{2+}) in darkness. Production of ethylene and levels of ACC were quantitated at the times indicated.

patway.

The rate of ethylene production is generally controlled by the level of ACC, the immediate precursor of ethylene biosynthesis (Yang and Hoffman, 1984). In fact, Fuhrer (1982) reported that cadmium-enhanced production of ethylene in bean leaf discs was regulated at the step of ACC synthesis. The effects of cadmium on the levels of ACC in detached rice leaves were examined and the results are shown in Fig. 2. Cadmium had no effect on the level of ACC in the first 8 h of incubation in the dark. However, treatment with cadmium resulted in a lower levels of ACC than those in the controls at 24 h after incubtion. It should be noted that the level of conjugated ACC was not affected by cadmium during a 24 h incubation (data not shown).

Ethylene production can also be regulated at the step of the conversion of ACC to ethylene, which is catalyzed by the ethylene-forming enzyme (Yang and Hoffman, 1984). It has become possible to measure the activity of ethylene-forming enzyme *in vitro* (Ververidis and John, 1991; Ferandez-Moculet, 1992). Since cadmium reacts preferentially with SH groups of enzyme proteins (Vallee and Ulmer, 1972), it would be more meaningful to measure the activity of ethylene-forming enzyme *in vivo.* To assay the activity of ethylene-forming enzyme *in vivo,* leaf segments were pretreated with a saturating concentration of ACC (10 mM). As indicated in Fig. 3, the activity of ethylene -forming enzyme *in vivo* was promoted by cadmium

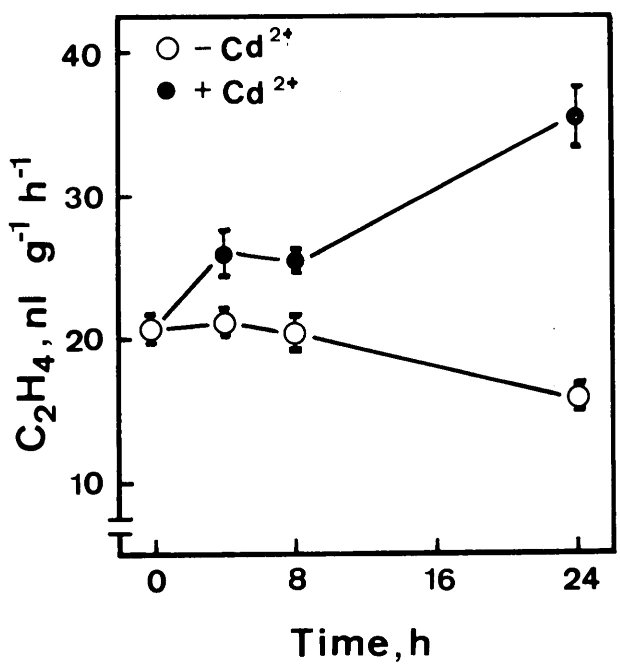

Fig. 3. Effect of cadmium on the conversion of ACC to ethylene. Segments of rice leaves were pretreated with a saturating concentration of ACC (10 mM) for 2 h and then treated either with water (-Cd^{2+}) or with 0.1 mM cadmium (+Cd^{2+}). Rates of production of ethylene were determined at the times indicated. Bars indicate standard errors.

during a 24 h incubation. Thus, cadmium promoted the conversion of ACC to ethylene in rice leaves. If the conversion of ACC to ethylene is the only step promoted by cadmium, then a decrease of ACC level in cadmium-treated leaves is to be expected. On the basis of these results, the absence of a decrease in ACC level in the presence of cadmium in the first 8 h of incubation can be explained in terms of simultaneous promotion of both the synthesis and conversion of ACC in rice leaves. For reasons not yet understood, the activity of ACC synthase in homogenates of detached rice leaves is undetectable. Thus, we were unable to present direct evidence to show the effects of cadmium on ACC synthase activity.

The effects of the concentration of cadmium of the levels of polyamines in detached rice leaves in darkness are presented in Fig. 4. Increasing concentrations of cadmium from 0.01 to 0.5 mM increased the level of putrescine. The level of spermine was increased in the range of cadmium from 0.05 to 0.5 mM. However, no significant increase in the level of spermidine was observed in cadmium-treated detached rice leaves. Weinstein *et al.* (1986) also reported that cadmium treatment resulted in a large increase in putrescine in intact and detached oat leaves and detached bean leaves. However, they observed no effect of cadmium on the level of spermidine or spermine.

In a recent report, we demonstrated that polyamines promoted the production of ethylene in

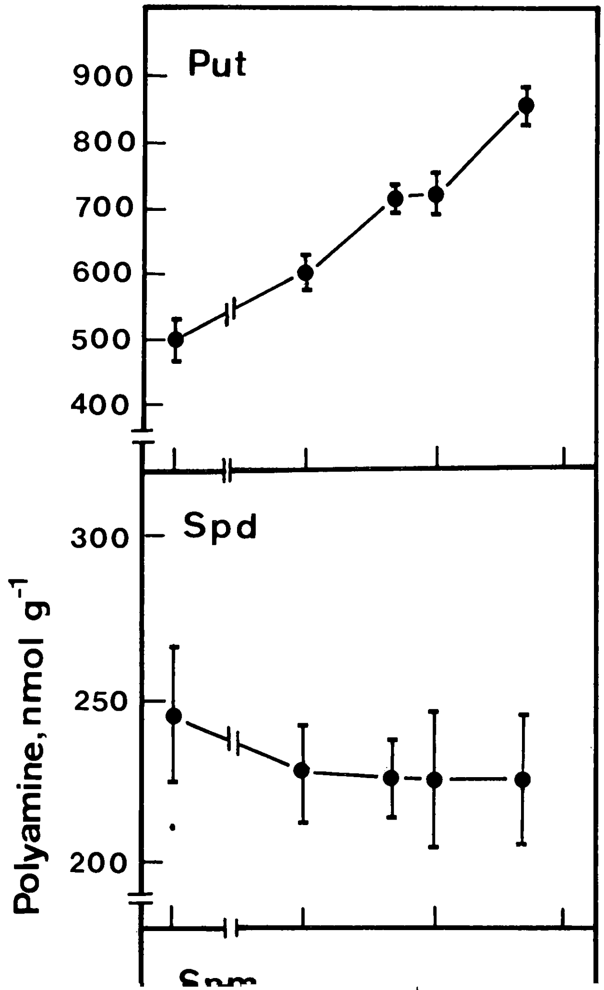

Table 2. *Effects of D-arginine or α-methylornithine with Cd on the levels of putrescine and spermine and the production of ethylene in segments of rice leaves*

Segments of rice leaves were incubated in D-arginine (5 mM) or α-methylornithin (5 mM) in the absence or presence of Cd^{2+} (0.1 mM). Putrescine and spermine levels were determined after 24 h in the dark. Accumulation of ethylene was determined after a 24 h incubation period in the dark. Values are average with standard errors.

Treatment	Putrescine (nmol g^{-1})	Spermine (nmol g^{-1})	Ethylene (nl g^{-1} 24 h^{-1})
No additions	630±17	41±2	122±7
D-arginine	362±25	44±6	120±3
α-methylornithine	485±34	39±2	118±4
Cd^{2+}	1009±74	76±6	299±13
Cd^{2+}+D-arginine	485±34	76±4	209±11
Cd^{2+}+α-methylornithine	741±7	87±7	247±7

leaves is via promotion of the conversion of ACC to ethylene. Evidence is also provided to show that the accumulation of polyamines may also regulate, at least in part, the observed increase in production of ethylene in cadmium-treated rice leaves.

Acknowledgements. This work was supported financially by the National Science Council of the Republic of China (NSC 81-0115-C002-01-161B).

Literature Cited

Chen, C. T. and C. H. Kao. 1991. Senescence of rice leaves XXX. Levels of endogenous polyamines and dark-induced senescence of rice leaves. Plant Cell Physiol. **32:** 935-941.

Chen, C. T., I. T. Chou, and C. H. Kao. 1990. Senescence of rice leaves XX. Changes of proton secretion during senescence. Plant Sci. **66:** 29-34.

Chen, S. L., C. T. Chen, and C. H. Kao. 1991. Polyamines promote the biosynthesis of ethylene in detached rice leaves. Plant Cell Physiol. **32:** 813-817.

Ferandez-Moculet, J. C. and S. F. Yang. 1992. Extraction and partial characteriztion of the ethylene-forming enzyme from apple fruit. Plant Physiol. **99:** 751-754.

Fuhrer, J. 1982. Ethylene biosynthesis and cadmium toxicity in leaf tissue of beans (*Phaseolus vulgaris* L.). Plant Physiol. **70:** 162-167.

Hogsett, W. E., R. M. Raba, and D. T. Tinger. 1981. Biosynthesis of stress ethylene in soybean seedlings: Similarities to endogenous ethylene biosynthesis. Physiol. Plant. **53:** 307-314.

Mayer, A., P. Muller, and G. Sembdner. 1987. Air Pollution and plant hormones. Biochem. Physiol. Pflanzen. **182:** 1-21.

Rodecap, K. D., D. T. Tingey, and J. H. Tibbs. 1981. Cadmium-induced ethylene production in bean plants. Z. Pflanzenphysiol. **105:** 65-74.

Tingey, D. T. 1980. Stress ethylene production- A measure of plant response to stress. Hort. Sci. **15:** 630-633.

Vallee, B. L. and D. D. Ulmer. 1972. Biochemical effects of mercury, cadmium, and lead. Annu. Rev. Biochem. **41:** 91-128.

Ververidis, P. and P. John. 1991. Complete recovery *in vitro* of ethylene-forming enzyme activity. Phytochemistry **30:** 725-727.

Weinstein, L. H., R. Kaur-Sawhney, M. V. Rajam, S. H. Wettlaufer, and A, W. Galston. 1986. Cadmium-induced accumulation of putrescine in oat and bean leaves. Plant Physiol. **82:** 641-645.

Yang, S. F. and N. E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol. **35:** 155-189.

鎘對水稻切離葉片乙烯形成的影響

侯淑滿 高景輝

國立台灣大學農藝學系

本研究主要是探討鎘對水稻切離葉片乙烯形成的影響。在黑暗與光線下，鎘顯著的促進乙烯的形成。鎘處理後在短時間(4小時)內即可促進乙烯的形成，顯示鎘可能直接影響乙烯的生合成。鎘所促進的乙烯形成效應，係由於其促進 1-aminocyclopropane-1-carboxylic acid 轉變爲乙稀。鎘亦可促進 putrescine 與 spermine 含量的增加。putrescine 合成抑制劑(D-arginine 與 α-methylornithine)可降低鎘所促進的 putrescine 含量與乙烯的形成。由於多元胺(putrescine, spermidine 與 spermine)可促進乙烯的形成(Chen *et al.* Plant Cell Physiol. 32: 813-817, 1991)，鎘所促進的 putrescine 含量增加亦可能間接影響乙烯的形成。