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Effects of polyamine and ethylene biosynthesis inhibitors on ethylene production and polyamine levels in detached rice leaves

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Abstract. Effects of biosynthesis inhibitors of polyamines and ethylene on ethylene production and polyamine levels in detached rice leaves were investigated. Neither α-difluormethylarginine nor α-difluoromethylornithine had any effect on ethylene production. However, dicyclohexylamine (DCH) and methylglyoxal bis (guanylhydrazone) (MGBG) significantly increased ethylene production in darkness. The stimulatory effect of DCH and MGBG on ethylene production was also observed under light conditions. DCH and MGBG did not change the levels of 1-aminocyclopropane-1-carboxylic acid (ACC), but promoted the conversion of ACC to ethylene. Aminooxyacetic acid (AOA), which inhibited ethylene production, was found to decrease putrescine levels in detached rice leaves. However, AOA did not influence spermidine and spermine levels. Co^{2+}, which also inhibited ethylene production, increased putrescine level and decreased spermidine level. No effect on spermine level was found in detached rice leaves treated with Co^{2+}.

Key words: Aminooxyacetic acid; Cobalt ion; Dicyclohexylamine; Ethylene; Mthylglyoxal bis (guanylhydrazone); Polyamines.

Introduction

Polyamines have been reported to inhibit ethylene biosynthesis in plant tissues (Evans and Malmberg, 1989). However, Pennazio and Roggero (1989, 1990) found that application of polyamines to detached soybean and tobacco leaves markedly stimulated ethylene production. In a recent work, we also demonstrated that polyamines stimulated the production of ethylene in detached rice leaves via enhancement of the synthesis of ACC and/or the conversion of ACC to ethylene (Chen *et al.*, 1991). Polyamine by biosynthesis inhibitors (e. g., DFMA, DFMO, MGBG and DCH) have been widely used to change levels of polyamines in plant tissues. Thus, it would be of great interest to study the effects of polyamine biosynthesis inhibitors on ethylene production in detached rice leaves. Ethylene and polyamines are related biosynthetically. Both ethylene and polyamines share SAM as a common precursor (Miyazaki and Yang, 1987). Thus, the effects of ethylene biosynthesis inhibitors on polyamines levels in detached rice leaves were also investigated.

Materials and Methods

Plant Materials

Seedlings of rice (*Oryza sativa* cv. Taichung Native 1) were grown in hydroponic culture as described previously (Chen *et al.*, 1990). The apical 3 cm of the third leaves of 12-day-old seedlings were used for the experiments. Ten segments of such leaves were floated on 10

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Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; ADC, arginine decarboxylase; AOA, aminooxyacetic acid; DCH dicyclohexylamine; DFMA, α-difluoromethylarginine; DFMO, α-difluoromethylornithine; MGBG, methylglyoxal bis (guanylhydrazone); ODC, ornithine decarboxylase; SAM, S-adenosylmethionine.

ml of test solution in a Petri dish. All samples were kept at 27°C in the dark. Each treatment had three replicates and 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.

Determinations of Ethylene and ACC

After various periods of incubation, the leaf samples were transferred to a 14-ml test tube. The test tubes were sealed with serum caps. After 1 h of incubation at 27°C in darkness, a 1-ml gas sample was withdrawn from the head space of each test tube. Ethylene was assayed with a gas chromatography equipped with an alumina column and a flame ionization detector. Absolute levels of ethylene varied among experiments because of seasonal effects. However, the patterns of responses to test solutions were reproducible. ACC was extracted and quantified as described previously (Kao and Yang, 1982).

Determination of Polyamines

Leaf segments were homogenized in perchloric acid (5%, v/v). Polyamine levels were determined using high performance liquid chromatography after benzoylation as described previously (Chen and Kao, 1991).

Results

Effects of Polyamine Biosynthesis Inhibitors on Ethylene Production

The effects on polyamine biosynthesis inhibitors (DFMA, DFMO, DCH and MGBG) on ethylene production in detached rice leaves in darkness are presented in Table 1. Neither DFMA nor DFMO had any effect on ethylene production. However, DCH and MGBG caused significant increase in ethylene production. The ability of DCH and MGBG to stimulate ethylene production was also observed under light conditions (data not shown).

Figure 1 shows the changes with time in rates of ethylene production in detached rice leaves treated with 2.5 mM DCH and 5 mM MGBG. Promotion of ethylene production by DCH and MGBG was detected as 12 and 6 h, respectively, after the start of incubation in the dark.

The rate of ethylene production is generally controlled by the level of ACC, the immediate precur-

Table 1. *Effect of inhibitors of polyamine biosynthesis on ethylene production in detached rice leaves*

Segments of rice leaves were treated with tested inhibitors in the dark. Ethylene production was assayed 24 h after treatment. Values are average with standard errors.

Treatment	Ethylene (nl g^{-1} h^{-1})
Control	6.4±0.2
DFMA, 0.5 mM	6.2±0.4
DFMO, 0.5 mM	6.0±0.3
DCH, 2.5 mM	8.8±0.3
MGBG, 5 mM	8.0±0.2

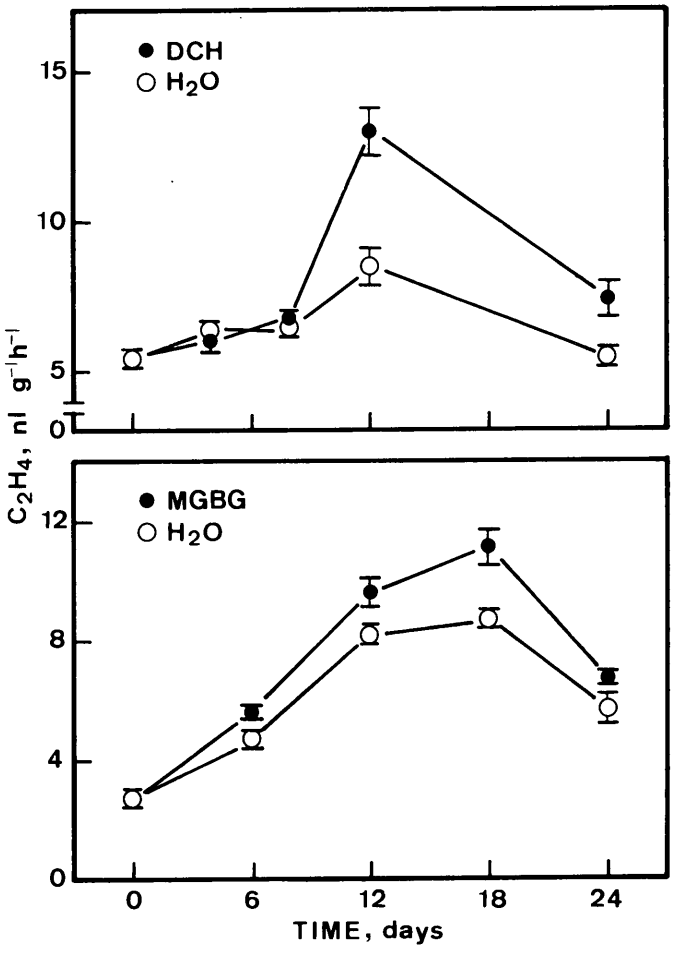

Fig. 1. Changes with time in rates of ethylene production in detached rice leaves treated with DCH and MGBG. Detached leaves were treated with either water, 2.5 mM DCH or 5 mM MGBG in the dark. Ethylene production was assayed at the times indicated. Bars indicate standard errors.

sor of ethylene (Yang and Hoffman, 1984). The effect of DCH and MGBG on the level of ACC was examined and the results are shown in Figure 2. Treatment with DCH and MGBG had no effect on the levels of ACC. Since no increase in the levels of ACC but increase of ethylene production was observed in DCH- and MGBG -treated leaf segments, the possibility that the conversion of ACC to ethylene is promoted by DCH and MGBG cannot be excluded. This possibility was tested by measuring the production of ethylene in the presence of a saturating concentration of ACC (10 mM). As indicated in Figure 2, the conversion of ACC to ethylene was promoted by DCH and MGBG.

Effects of Ethylene Biosynthesis Inhibitors on Polyamine Levels

Inhibitors of ethylene biosynthesis, AOA and Co^{2+}, as expected markedly decreased ethylene production in detached rice leaves (Table 2). AOA decreased putrescine level but did not cause a significant change in spermidine and spermine levels (Table 2). In contrast, Co^{2+} induced an increase in putrescine level and a decrease in spermidine level but did not affect spermine level (Table 2).

Discussion

It has been shown that MGBG and DFMA increase rates of ethylene production in cut carnations (Roberts *et al.*, 1984). In contrast, DFMO was reported to inhibit ethylene production in carrot cell culture (Robie and Micocha, 1989). The present investigation demostrated that DCH and MGBG stimulated ethylene production. Neither DFMA nor DFMO had influence on ethylene production. In addition to DFMA and DFMO, D-arginine and α-methyl ornithine (inhibitors of ADC and ODC, respectively) also exert no effect on ethylene production (data not shown). In rice leaves it has been shown that DFMA and DFMO reduced levels of putres-

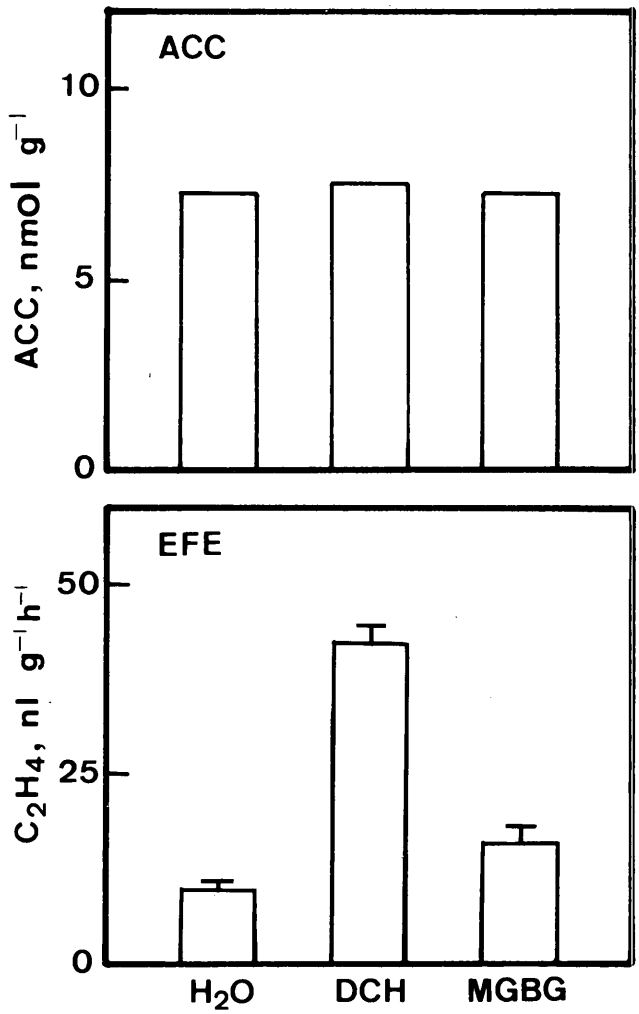

Fig. 2. Effects of DCH of MGBG on levels of ACC and the activity of ethylene-forming enzyme (EFE) in detached rice leaves. The levels of ACC were assayed after 12 h of incubation in water, 2.5 mM DCH or 5 mM MGBG in the dark. For the activity of EFE, detached leaves were pretreated with a saturating concentration of ACC (10 mM) for 2 h and then treated with water, 2.5 mM DCH, or 5 mM MGBG in the dark. Ethylene production was assayed after 12 h of treatment. Bars indicates standard errors.

Table 2. *Effect of ethylene biosynthesis inhibitors on polyamine levels in detached rice leaves*
Polyamine levels were determined 24 h after the start of incubation. Values are average with standard errors.

Treatment	Ethylene	Putrescine	Spermidine	Spermine
	(nl g^{-1} 24 h^{-1})		(nmol g^{-1})	
Control	71.0±2.4	650±34	340±48	20± 1
AOA, 5 mM	30.4±1.5	324±18	298±24	36±15
Co^{2+}, 1 mM	49.1±0.8	778±28	229± 9	15± 4

cine but caused no change in levels of spermidine and spermine, whereas DCH and MGBG increased levels of putrescine and decreased those of spermine (Chen and Kao, 1991). Thus, the stimulatory effect of DCH and MGBG on ethylene production is most likely mediated by the redirection of a portion of the pool of SAM into the ethylene pathway.

DCH and MGBG were reported to promote senescence of detached rice leaves (Chen and Kao, 1991). The question whether DCH and MGBG regulate ethylene biosynthesis directly was examined in the present study by following the time course of the DCH and MGBG effects. It is clearly evident that DCH and MGBG induced significant increase in ethylene production shortly after application. This observation suggests that DCH and MGBG induce ethylene biosynthesis directly and that the action of DCH and MGBG is not via the promotion of senescence which occurs much later.

The present investigation demonstrated that DCH and MGBG promoted the conversion of ACC to ethylene. If the conversion of ACC to ethylene is the only step promoted by DCH and MGBG, then the decrease of ACC level in DCH- and MGBG-treated rice leaves is to be expected. However, no such decrease was observed. On the basis of these results, the absence of decreased level of ACC in the presence of DCH or MGBG can be explained in terms of simultaneous promotion of both the synthesis and the conversion of ACC to ethylene 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 show that DCH- and MGBG-promoted ACC synthase activity. Minocha *et al.* (1990) have demonstrated that ACC level was higher in MGBG-treated carrot cells than the controls.

AOA, which is known to prevent ethylene biosynthesis by blocking the conversion of SAM to ACC, is reported to be inhibitor of pyridoxal phosphate mediated enzyme reaction (John *et al.*, 1978). The significant decrease in putrescine levels in AOA-treated rice leaves sugests that in addition to inhibiting ACC synthase, AOA also reduces the level of putrescine. Roberts *et al.* (1984) also observed that AOA resulted in large reduction in putrescine level in carnation flowers. Since polyamines have been shown to promote ethylene production in rice leaves (Chen *et al.*, 1991), the effect of AOA on ethylene production is most likely mediated via reduction of putrescine level and inhibition of ACC formation.

It has been shown that treatments inhibited ethylene biosynthesis resulted in increase in spermidine or spermine level (Even-Chen *et al.*, 1982; Roberts *et al.*, 1984). Biondi *et al.* (1990) also demonstrated that aminoethoxyvinylglycine, another inhibitor of ACC synthase, promoted the conversion of putrescine to spermidine in *Prunus avium* shoot cultures. However, no such increase was observed in our AOA- and Co^{2+} -treated rice leaved. Cobalt strongly inhibits ethylene -forming enzyme function, possibly by complexing with protein sulfhydryl groups (Yu and Yang, 1979). The increase in putrescine level and decrease in spermidine level in Co^{2+}-treated rice leaves suggest that the conversion of putrescine to spermidine is blocked by Co^{2+}.

Apparently compounds that inhibit ethylene biosynthesis in rice leaves (e. g., AOA and Co^{2+}) also have effect on polyamine biosynthesis.

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Literature Cited

Biondi, S., T. Diaz, I. Igleias, G. Gamberini, and N. Bagni. 1990. Polyamines and ethylene in relation to adventitious root formation in *Prunus avium* shoot cultures. Physiol, Plant. **78:** 474-483.

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.

Evans, P. T. and R. L. Malmberg. 1989. Do polyamines have roles in plant development? Annu. Rev. Plant Physiol. Plant Mol. Biol. **40:** 235-269.

Even-Chen, Z., A. K. Mattoo, and R. Goren. 1982. Inhibition of ethylene biosynthesis by aminoethoxyvinylglycine and polyamines shunts label from 3, 4-[^{14}C] methionine into spermidine in aged oranged peel disks. Plant Physiol. **69:** 385 -388.

John, R. A., A. Charteris, and L. J. Fowler. 1978. The reaction of

aminooxyacetate with pyridoxal phosphate-dependent enzymes. Biochem. J. **171:** 771-779.

Kao, C. H. and S. F. Yang. 1982. Light inhibition of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in leaves is mediated through carbon dioxide. Planta **155:** 261-266.

Minocha, S. C., C. A. Robie, A. J. Khan, N. Papa, A. I. Samuelsen, and R. Minocha. 1990. Polyamine and ethylene biosynthesis in relation to somatic embryogenesis in carrot (*Dancus carota* L.) cell cultures. *In* H. E. Flores, R. N. Arteca and J. C. Shannon (eds.), Polyamines and Ethylene: Biochemistry, Physiology, and Interactions. American Society Plant. Physiologists, Rockville, pp. 339-342.

Miyzazki, J. H. and S. F. Yang. 1987. The methionine salvage pathway in relation to polyamine biosynthesis. Physiol. Plant. **69:** 366-370.

Pennazio, S. and P. Roggero. 1989. Stimulation of ethylene production by exogenous spermidine in detached tobacco leaves in the light. Biol. Plant. **31:** 58-66.

Pennazio, S. and P. Roggero. 1990. Exogenous polyamines stimulate ethylene synthesis by soybean leaf tissues. Ann. Bot. **65:** 45-50.

Roberts, D. R., M. A. Walker, J. E. Thompson, and R. B. Dumbroff. 1984. The effects of inhbitors of polyamine and ethylene biosynthesis on senescence, ethylene production and polyamin levels in cut carnation flowers. Plant Cell Physiol. **25:** 315-322.

Robie, C. A. and S. C. Minocha. 1989. Polyamine and somatic embryogenesis in carrot. I. The effects of difluoromethylornithine and difuoromethylarginine. Plant Sci. **65:** 45-54.

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

Yu, Y. B. and S. F. Yang. 1979. Auxin-induced ethylene production and its inhition by aminoethoxyvinylglycine and cobalt ion. Plant Physiol. **64:** 1074-1077.

多元胺與乙烯合成抑制劑對水稻切離葉片乙烯形成與多元胺含量的影響

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本研究主要是探討多元胺與乙烯合成抑制劑對水稻切離葉片乙烯形成與多元胺含量的影響。α-difluoromethylarginine 與 α-difluoromethylornithine 不影響乙烯之形成。然而，dicyclohexylamine (DCH) 與 methylglyoxal bis (guanylhydrazone) (MGBG) 則顯著的增加乙烯之形成，黑暗與光線下均表現此種效應。DCH 與 MGBG 不會改變 1-aminocyclopropane-1-carboxylic acid (ACC) 含量，但促進 ACC 轉變爲乙烯。Aminooxyacetic acid (AOA) 抑制乙烯形成，亦可降低 putrescine 含量，但不影響 spermidine 與 spermine 含量。Co^{2+} 可抑制乙烯形成與降低 spermine 含量，同時增加 putrescine 含量。