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The effects of 2,2′-bipyridine and other metal chelators on the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in rice

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Abstract

Effects of metal chelators, 2,2′-bipyridine, 8-hydroxyquinoline and 1,10-phenanthroline, on the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene in detached leaves of light-grown rice (*Oryza sativa*) seedlings and detached shoots of etiolated rice seedlings were investigated. Metal chelators strongly inhibited the *in vivo* ACC oxidase activity in detached leaves and detached etiolated shoots. This inhibition could be partially recovered by Fe^{2+}. Our results support the notion that Fe^{2+} is an essential cofactor for the conversion of ACC to ethylene *in vivo*.

Abbreviations: ACC = 1-aminocyclopropane-1-carboxylic acid; BP = 2,2′-bypyridine; HQ = 8-hydroxylquinoline; MJ = methyl jasmonate; PA = 1,10-phenanthroline; Put = putrescine.

1. Introduction

The final step in the biosynthesis of ethylene is catalyzed by ACC oxidase [12]. The strong inhibition of the conversion of ACC to ethylene by several chelators in intact tissues [1] indicates the involvement of a metal in the activity of ACC oxidase operating *in vivo*. The participation of a transition metal for the conversion of ACC to ethylene has been suggested [2, 3, 10, 12]. Recent work indicates that *in vitro* and *in vivo* ACC oxidase activity requires divalent iron as a cofactor [4, 8, 11].

Unlike many other plant systems, ethylene biosynthesis in detached rice leaves was found to be promoted by polyamines [7] and inhibited by water stress [5]. Thus, rice leaves seem to be an interesting and unusual system to study ethylene biosynthesis. In the present study detached leaves of light-grown rice seedlings and detached shoots of etiolated rice seedlings were used to examine the effects of BP and other metal chelators (HQ and PA) on the conversion of ACC to ethylene *in vivo*.

2. Materials and methods

Seedlings of rice (*Oryza sativa* cv. Taichung Native 1) were grown in hydroponic culture as described previously [6]. The apical 3 cm of the third leaves of 12-day-old light-grown seedlings were used for the experiments. For those experiments in which etiolated shoots of seedlings were used, the seeds, after sterilization, were allowed to germinate in Petri dishes containing filter papers moistened with Tris buffer (pH 7.0) at 27 °C in the dark. After 3 days of germination, seedlings were transferred to a second Petri dish containing Tris buffer or 2 m*M* metal chelators in Tris buffer and incubated under dark conditions for a further 3 days. Etiolated shoots were then excised and used for the experiments.

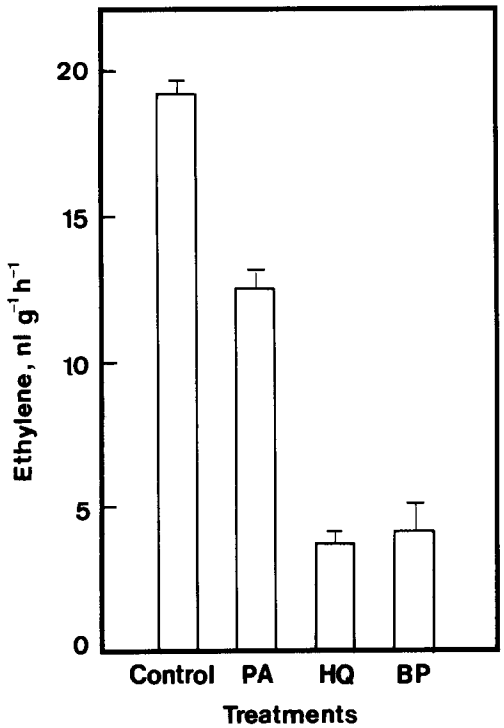

Fig. 1. Effects of BP, HQ and PA on *in vivo* ACC oxidase activity in detached rice leaves. Detached rice leaves were pretreated with ACC (10 m*M*) for 2 h and then treated wtih Tris buffer (10 m*M*, pH 7.0) or Tris buffer containing metal chelators (5 m*M*) in darkness. Ethylene production was assayed after 6 h of treatment. Bars indicate standard error ($n = 4$).

For the determination of *in vivo* ACC oxidase activity, leaf segments or excised shoots were pretreated with 10 m*M* ACC for 2 h, then washed, blotted dry and transferred to test tubes (12.4 ml) containing treatment solution (1 ml). The test tubes were capped immediately and incubated at 27 °C in the dark. Ethylene was measured by gas chromatography at the times indicated as described previously [9].

3. Results

All metal chelators tested significantly inhibited the conversion of ACC to ethylene in detached rice leaves (Figure 1). However, PA was less effective than HQ and BP in inhibiting *in vivo* ACC oxidase activity. Inhibitory effect on *in vivo* ACC oxidase activity caused by BP was detected within 2 h of application (Figure 2).

The reversal of the inhibition of the *in vivo* ACC oxidase activity in detached leaves caused by BP

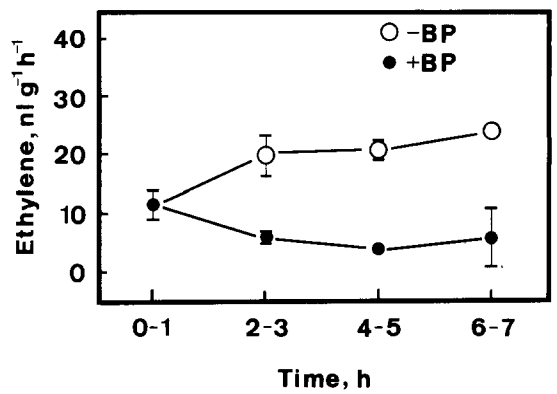

Fig. 2. Changes with times in *in vivo* ACC oxidase activity in detached rice leaves treated with BP. Detached rice leaves were pretreated with ACC (10 m*M*) for 2 h and then treated with BP (5 m*M*) in darkness. Rates of ethylene production were determined at the times indicated. Bars indicate standard errors ($n = 4$).

was studied using a range of divalent cations (Figure 3). Inhibition caused by BP could be partially reversed by Fe^{2+} and Cu^{2+}. However, Zn^{2+} and Mn^{2+} had no effect.

Our previous work showed that MJ and putescine (Put) were effective in promoting the conversion of ACC to ethylene [5, 7]. If *in vivo* ACC oxidase requires a metal as cofactor, then MJ or Put would be unable to promote *in vivo* ACC oxidase activity in BP-pretreated detached

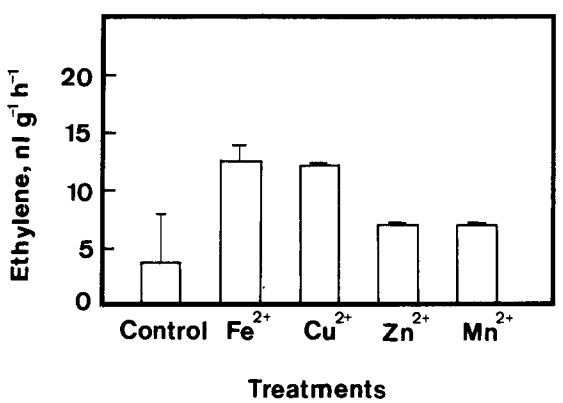

Fig. 3. Reversal of the inhibitory effect on *in vivo* ACC oxidase activity of BP by various metals in detached rice leaves. Detached rice leaves were pretreated with BP (2 m*M*) for 2 h and then treated wtih sulfate salts of various metals (1 m*M*) for 3 h in the dark. Detached rice leaves were then fed with ACC (10 m*M*) and their ethylene production was determined after 2 h in the dark. Bars indicate standard errors ($n = 4$). Ethylene production in excised leaves pretreated with water and treated in the absence of metals was 23.3 ± 1.6 nl g^{-1} h^{-1}.

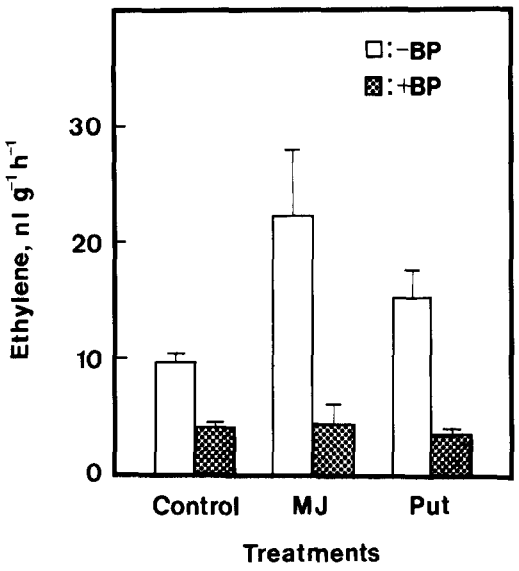

Fig. 4. Effect of BA on MJ- and Put-enhanced *in vivo* ACC oxidase activity in detached rice leaves. Detached rice leaves were pretreated with or without BP (2 m*M*) for 2 h and then treated with MJ (45 μM) or Put (5 m*M*) for 3 h in the dark. Detached rice leaves were fed with ACC (10 m*M*) and their ethylene production was determined after 2 h in the dark. Bars indicate standard errors ($n = 4$).

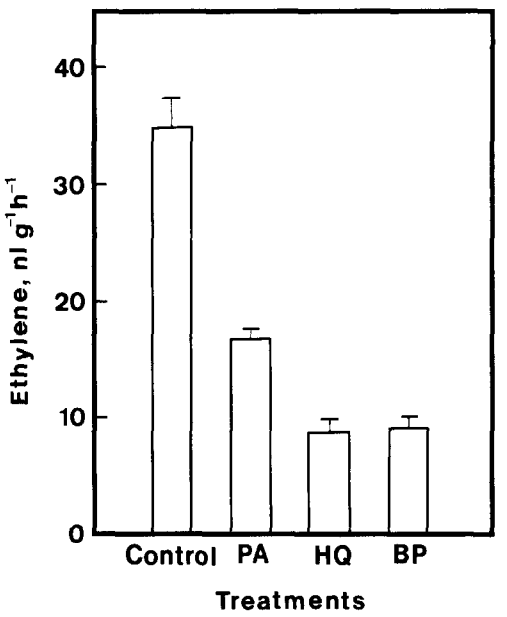

Fig. 5. Effect of BP, HQ and PA on *in vivo* ACC oxidase activity in detached shoot of rice seedlings germinated in darkness. Etiolated shoots were excised from 6-day-old rice seedlings growing in Tris buffer (10 m*M*, pH 7.0). Detached shoots were pretreated with ACC (10 m*M*) and then treated with Tris buffer (10 m*M*, pH 7.0) or Tris buffer containing metal chelators (5 m*M*) in darkness. Ethylene production was determined after 2 h of treatement. Bars indicate standard errors ($n = 4$).

leaves. As indicated in Figure 4, MJ and Put were indeed unable to promote the conversion of ACC to ethylene in BP-pretreated detached rice leaves.

To understand whether the effects of metal chelators on *in vivo* ACC oxidase activity are specific for green leaf tissue, the effects of metal chelator on *in vivo* ACC oxidase activity in etiolated shoots from 6-day-old dark-grown seedlings were also studied. As found for light-grown leaves, all metal chelators tested were effective in inhibiting the *in vivo* ACC oxidase activity in etiolated shoots (Figure 5).

Metal deficiency in shoots can be achieved by growing rice seedlings in the presence of metal chelators. With these shoots were excised and their *in vivo* ACC oxidase activity was measured, ACC oxidase activity was found to be significantly less than that of shoots excised from seedlings growing in the absence of metal chelators (Figure 6). *In vivo* ACC oxidase activity in shoots starved for metals could be partially recovered by Fe^{2+} or Cu^{2+} (Figure 7). Both Fe^{2+} and Cu^{2+} were able to recover *in vivo* ACC oxidase activity in shoots from seedlings grown in PA. However, *in vivo* ACC oxidase activity in shoots excised from seedlings grown in BP or HQ could only be partially recovered by Fe^{2+}.

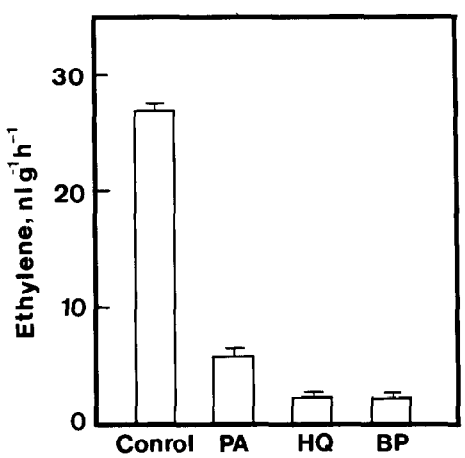

Fig. 6. In vivo ACC oxidase activity in detached shoots starved for metals. Starvation of metals in shoots was achieved by growing seedlings in metal chelators (2 m*M*) in darkness. Detached shoots were treated with ACC (10 m*M*) and their ethylene production was measured after 2 h in darkness. Bars indicate standard errors ($n = 4$).

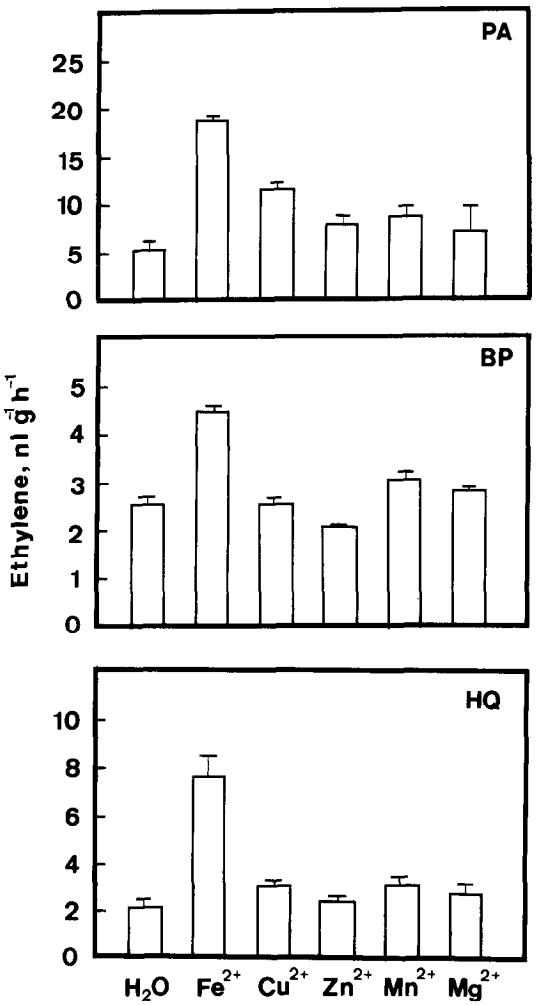

Fig. 7. Reversal of *in vivo* ACC oxidase activity by metals in etiolated shoots excised from seedlings grown in metal chelators (2 m*M*). Detached shoots were pretreated with ACC (10 m*M*) for 2 h and then treated with sulfate salts of various metals (1 m*M*) in the dark. Ethylene production was measured at 2 h after treatment. Bars indicate standard errors ($n = 4$). Ethylene production in etiolated shoots excised from seedlings grown in buffer solution and then treated in the absence of metals was $28.5 \pm 0.9\,\text{nl}\,\text{g}^{-1}\,\text{h}^{-1}$.

4. Discussion

Apelbaum *et al.* [1] reported that metal chelators strongly inhibited EFE activity. They concluded that the copper ion was involved in the conversion of ACC to ethylene. However, recent investigation by Bouzayen *et al.* [4] showed that Fe^{2+} was required for *in vivo* ACC oxidase activity. The present investigation demonstrated that metal chelators (BP, PA and HQ) tested were effective in inhibiting *in vivo* ACC oxidase activity in both detached leaves and etiolated-shoots. Our results also showed that inhibition of ACC oxidase activity caused by metal chelators was partially recovered by Fe^{2+}. The partial recovery of *in vivo* ACC oxidase activity by adding Cu^{2+} to BP-treated detached leaves and to shoots excised from seedlings grown in the presence of PA most likely resulted from the displacement of Fe^{2+} from PA or BP complex by Cu^{2+} as suggested by Bouzayen *et al.* [4]. It is concluded that Fe^{2+} is essential for the conversion of ACC to ethylene in rice.

Acknowledgements

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