Prior temperature exposure affects subsequent Cd-induced ethylene production in rice leaves

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Abstract

We investigated the effects of prior temperature exposure of rice seedlings on the subsequent Cd-induced ethylene production in detached leaves. A reduction of Cd-induced ethylene production in detached leaves from heat (45°C) shocked rice seedlings was observed. Cd-induced ethylene production in detached leaves from seedlings grown at 35°C was lower than that from seedlings grown at 25°C. In contrast to heat pretreatment, chilling (4°C) pretreatment of seedlings sensitized detached leaves to subsequent ethylene production by Cd.

Keywords: Cadmium; Ethylene; *Oryza sativa*; Temperature

1. Introduction

Cd is one of the most toxic of the heavy metals. There are reports that Cd induces the production of ethylene in several plant species [1–6]. The most important step in Cd-induced production of ethylene was considered to be the stimulation of ACC (1-aminocyclopropane-1-carboxylic acid) formation in bean leaf discs [1], the conversion of ACC to ethylene in detached rice leaves [3] or both in soybean cuttings [6].

It has been shown that exposure of wheat seedlings to heat shock temperatures resulted in the acquisition of protection against Cd toxicity [7].

Abbreviation: ACC, 1-aminocyclopropane-1-carboxylic acid.

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Since Cd-induced ethylene production is one of the early effects of Cd toxicity [3,8], heat shock pretreatments are expected to decrease Cd-induced ethylene production. It is not known whether chilling pretreatment also affect subsequent Cd-induced ethylene production. The present investigation was thus conducted to test if prior temperature exposure of rice seedlings affects subsequent Cd-induced ethylene production in detached leaves.

2. Materials and methods

Rice (*Oryza sativa* L. cv. Taichung Native 1) seedlings were grown in hydroponic culture for 10 days as described previously [9]. Briefly, seedlings were planted on a stainless net floating on half-strength Johnson's modified nutrient solution [10]

in a 500-ml beaker. The nutrient solutions (pH 4.5) were replaced every 3 days. Rice seedlings were grown for 10 days in a greenhouse with natural day light at 30°C day/25°C night and humidity 95%.

To examine the effect of the concentration of Cd on ethylene production, 10 leaf segments (apical 3 cm) excised from the third leaves were placed vertically in test-tubes which contained 1 ml of distilled water or CdCl₂. Test-tubes were closed with rubber stoppers and incubated for 24 h at 27°C in the light (31.5 W/m²). The ethylene in the gas phase of the closed tubes was determined by analysis of 1 ml of sample withdrawn with a hypodermic syringe as described elsewhere [11].

For temperature pretreatments, seedlings were exposed to 4, 27 and 45°C in the dark and sampled at intervals over a 24-h period. Apical 3-cm segments excised from the third leaves were then used to determine the effect of Cd on ethylene production as described above.

3. Results and discussion

The effect of the concentration of Cd on the production of ethylene in detached rice leaves in the light is shown in Fig. 1. Cd significantly promoted ethylene production in detached rice leaves.

Fig. 1. Relationship between concentration of CdCl₂ and the production of ethylene in detached rice leaves. Accumulation of ethylene was determined after a 24-h incubation period in light. Bars indicate standard errors ($n = 4$).

Fig. 2. Effects of CdCl₂ on ethylene production of detached leaves from rice seedlings pretreated with 27 and 45°C for 6, 12 and 24 h. The concentration of CdCl₂ was 0.5 mM. Accumulation of ethylene was determined after a 24-h incubation period in light. Bars indicate standard errors ($n = 4$).

Increasing concentrations of Cd from 0.01 to 0.5 mM progressively enhanced ethylene production by detached rice leaves. The ability of Cd to stimulate ethylene production has also been observed under dark conditions [3].

To test if prior temperature exposure of rice seedlings would affect subsequent Cd-induced ethylene production in detached leaves, seedlings were pretreated at 27 and 45°C for 6, 12 and 24 h, respectively, under dark conditions. Irrespective of pretreatment time, the induction of ethylene production by Cd became less evident in detached leaves from seedlings pretreated at 45°C compared with those pretreated at 27°C (Fig. 2). For example, detached leaves from seedlings pretreated with

Fig. 3. Effect of CdCl₂ on ethylene production of detached leaves from rice seedlings grown at 25 and 35°C. Detached leaves from 13- and 9-day-old seedlings grown at 25 and 35°C, respectively, were used to determine Cd-induced ethylene production. The concentration of CdCl₂ was 0.5 mM. Accumulation of ethylene was determined after a 24-h incubation period in light. Bars indicate standard errors.

45°C for 6 h resulted in no induction of ethylene production by Cd. Our results indicate that the heat shocked leaf had a lower ability to show induction of ethylene production by Cd.

Additional experiments were conducted to examine the effects of Cd on ethylene production in detached leaves from seedlings grown at 25 and 35°C, respectively. To do this, seedlings were grown at 25 and 35°C for 13 and 9 days, respectively, when the third leaves were fully expanded. As indicated in Fig. 3, Cd-induced ethylene production in detached leaves from seedlings grown at 35°C was lower than that from seedlings grown at 25°C.

As a cereal crop of tropical origin, rice is susceptible to chilling injury [12]. No data are available to show that chilling pretreatment affects subsequent Cd-induced ethylene production in detached leaves. Thus, it is of great interest to know whether low temperature pretreatment would affect subsequent Cd-induced ethylene production in detached leaves. To test this, seedlings were exposed to 4°C for 6, 12 and 24 h, respectively, and then Cd-induced ethylene production in detached leaves was determined. As indicated in Fig. 4, irrespective of pretreatment time, Cd-induced ethylene production became more pronounced in detached

Fig. 4. Effects of CdCl₂ on ethylene production of detached leaves from rice seedlings pretreated with 27 and 4°C for 6, 12 and 24 h. The concentration of CdCl₂ was 0.5 mM. Accumulation of ethylene was determined after a 24-h incubation period in light. Bars indicate standard errors.

leaves from seedlings pretreated at 4°C compared to those pretreated at 27°C.

Ethylene production is one of early effects of Cd toxicity [3,8]. If ethylene production by Cd is taken as an indicator of Cd toxicity, our results indicate that heat shock and chilling pretreatments increase and decrease, respectively, Cd tolerance of rice leaf tissue. Our results are in general consistent with those of Orzech and Burke [7], who demonstrated that exposure of wheat seedlings to heat shock temperatures result in the acquisition of protection against metal, including Cd, toxicity. Plant cells subjected to Cd rapidly synthesize phytochelatins, i.e. Cd-binding peptides, whose function is to sequester or to detoxify excess Cd ions [13]. One mechanism of increasing and

decreasing tolerance to Cd may be the formation of phytochelatins. If phytochelatins function as a protection system, then they may be induced by heat shock and reduced by chilling. Clearly a deeper knowledge of heat shock-induced and chilling-inhibited phytochelatin accumulation in rice leaves is necessary for a better understanding of temperature-induced changes of Cd tolerance.

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