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Effects of 6-methoxy-2-benzoxazolinone on the germination and α-amylase activity in lettuce seeds

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Summary

Germination of lettuce seeds was inhibited by 6-methoxy-2-benzoxazolinone (MBOA) at concentrations greater than 0.03 mmol/L. MBOA also inhibited the induction of α-amylase activity in the lettuce seeds at concentrations greater than 0.03 mmol/L. These two concentration–response curves for the germination and α-amylase indicate that the percentage of the germination was positively correlated with the activity of α-amylase in the seeds. Lettuce seeds germinated around 18 h after incubation and inhibition of α-amylase by MBOA occurred within 6 h after seed incubation. These results show that MBOA may inhibit the germination of lettuce seeds by inhibiting the induction of α-amylase activity.

Introduction

A number of plants have been reported to possess inhibitory effects on the growth and population of neighboring or successional plants by releasing allelopathic substances into the soil, either as exudates from living plant tissues or by decomposition of plant residues (Rice, 1984; Putnam and Tang, 1986; Inderjit, 1996; Narwal, 1999). A variety of secondary plant metabolites are associated with allelopathic effects of plants, and some of them play an important role in chemical interactions in natural plant communities (Einhellig, 1996; Seigler, 1996; Dayan et al., 2000).

6-Methoxy-2-benzoxazolinone (MBOA) has been isolated from several Gramineaus plant species such as wheat, maize and rye (Niemeyer, 1988), and is involved in plant resistance to insects, fungi and bacteria because of its phytotoxic activity (Frey et al., 1997; Yue et al., 1998; Bravo and Copaja, 2002; Glenn et al., 2002). MBOA has also attracted attention because of involvement in

Abbreviations: MBOA 6-methoxy-2-benzoxazolinone

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allelopathic effects (Barnes and Putnam, 1987; Inderjit and Duke, 2003; Belz and Hurle, 2004). In several studies, MBOA inhibited the germination and growth of several plant species (Pérez, 1990; Hayashi et al., 1994; Kato-Noguchi et al., 1998; Kato-Noguchi, 2000). However, the mode of action of MBOA on the inhibition is not fully understood. In this study, a possible mechanical action of MBOA on the inhibition of plant germination was found.

Materials and methods

Plant material

Seeds of lettuce (*Lactuca sativa* L. cv. Grand Rapids) were sterilized in a 2% (w/v) solution of sodium hypochlorite for 15 min and rinsed four times in sterile distilled water. All further manipulations were carried out under sterile conditions. MBOA (0, 0.001, 0.03, 0.1, 0.3, 1 and 3 mmol/L MBOA) was dissolved in a small volume of methanol and added to two sheets of filter paper (No 1; Merck) in a 9-cm Petri dish and dried. The filter paper in the Petri dish was moistened with 4 mL 0.05% (v/v) aqueous Tween 20. Fifty seeds of lettuce were arranged on the filter paper in the Petri dish and germinated in the dark at 25 °C for 36 h. Following this step, the germinated seeds were counted. Control seeds were treated with the plain solution (0 mmol/L MBOA). For determination of α-amylase activity, lettuce seeds were harvested at 0, 6, 12, 18, 24 and 36 h after sowing, frozen immediately with liquid N_2 and freeze-dried.

Extraction and assay of α-amylase

Freeze-dried lettuce seeds (10 seeds for one determination) were ground to a fine powder in a mortar using a pestle. The powder was then homogenized with 1.5 mL of ice-cold solution of 100 mmol/L HEPES-KOH (pH 7.5) containing 1 mmol/L EDTA, 5 mmol/L $MgCl_2$, 5 mmol/L DTT, 10 mmol/L $NaHSO_3$ and 50 mmol/L bovine serum albumin. The homogenate was centrifuged at 30,000*g* for 30 min, and the supernatant was heated with 3 mmol/L $CaCl_2$ at 75 °C for 15 min to inactivate β-amylase and α-glucosidase (Sun and Henson, 1991; Guglielminetti et al., 1995), and used for α-amylase assay.

α-Amylase was assayed by measuring the rate of generation of reducing sugars from soluble starch. The heat-treated supernatant (0.2 mL) was added to 0.5 mL of 100 mmol/L Na-acetate (pH 6.0) containing 10 mmol/L $CaCl_2$. Reaction was initiated with 0.5 mL 2% (w/v) soluble starch. After incubation at 37 °C for 15 min, the reaction was terminated by adding 0.5 mL of 40 mmol/L dinitrosalicylic acid solution containing 400 mmol/L NaOH and 1 M K–Na tartrate, and then placing immediately into a boiling water bath for 5 min. After dilution with distilled water, the A_{530} of the reaction mixture was measured, and reducing power evaluated using a standard curve obtained with glucose (Guglielminetti et al., 1995).

Results and discussion

MBOA inhibited lettuce germination at concentrations greater than 0.03 mmol/L (Fig. 1). When the percentage of germination was plotted against logarithm of MBOA concentrations, concentration–response curves were linear between 20% and 80% inhibition. The concentration required for 50% inhibition was 0.15 mmol/L as interpolated from the concentration-response curve. These results suggest that MBOA inhibited the lettuce germination and the inhibition was increased with increasing MBOA concentrations. The germination inhibition by MBOA has also been found previously in several other plant species (Pérez, 1990; Kato-Noguchi, 2000).

MBOA inhibited the activity of α-amylase in lettuce seeds at concentrations greater than 0.03 mmol/L (Fig. 2). When α-amylase activities were plotted against logarithm of the concentrations, concentration–response curves were linear. The concentration required for 50% inhibition of

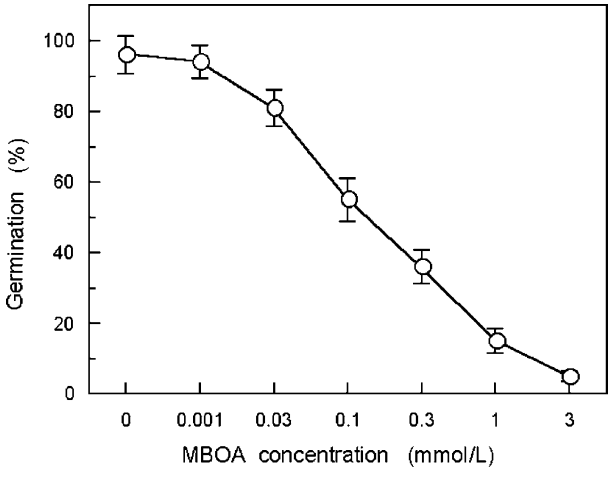

Figure 1. Effects of MBOA on germination of lettuce seeds. Lettuce seeds were incubated with MBOA in the dark at 25 °C for 36 h. Means ± SE from 4 independent experiments with 50 plants for each determination are shown.

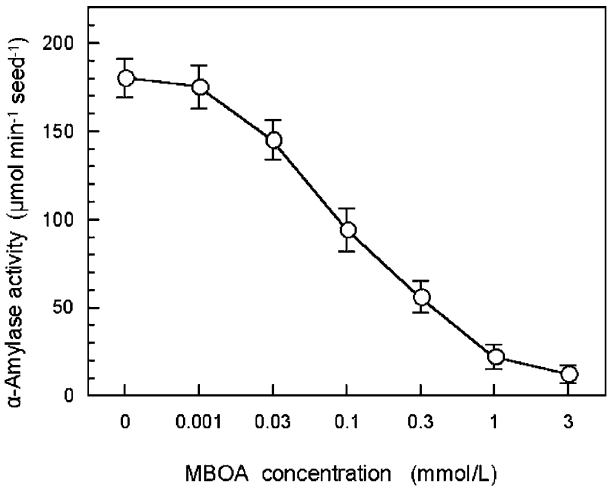

Figure 2. Effects of MBOA on α-amylase activity of lettuce seeds. Lettuce seeds were incubated with MBOA in the dark at 25 °C for 36 h. Means ± SE from 4 independent experiments with 3 assays for each determination are shown.

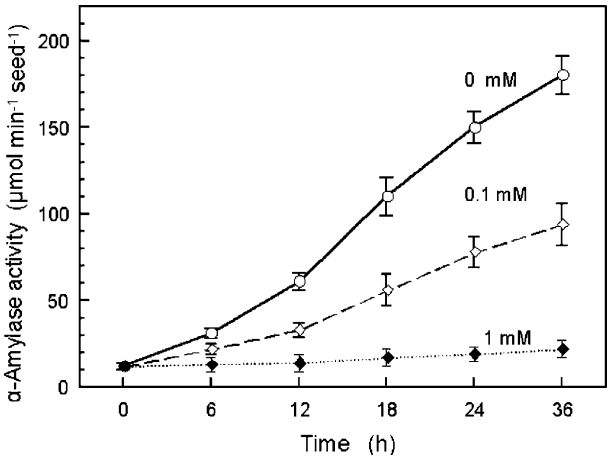

Figure 3. Changes in α-amylase activity of cress seeds. Lettuce seeds were incubated with MBOA in the dark at 25 °C. Means ± SE from 4 independent experiments with 3 assays for each determination are shown.

the activity was 0.12 mmol/L, as interpolated from the concentration–response curve, and this value was almost the same as the concentration required for 50% inhibition of seed germination (Fig. 1). These results suggest that MBOA inhibited α-amylase activity, and that the inhibition was increased with increasing MBOA concentrations.

Fig. 3 shows changes in α-amylase activity in lettuce seeds after sowing. The activity in control seeds (0 mmol/L MBOA) was low at time 0, and increased as the process of germination occurred, where radicles of lettuce seeds emerged around 18 h after sowing. MBOA inhibited the induction of

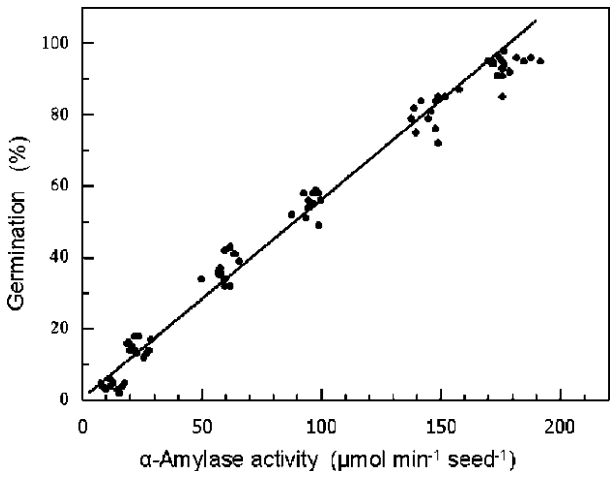

Figure 4. Relationship between the α-amylase activity and the percentage germination percentage of lettuce seeds. The data points are derived from the samples in Figs. 1 and 2.

α-amylase activity within 6 h after sowing, and the inhibition was greater with increasing MBOA concentrations. At 36 h, the activities in seeds treated with 0.1 mmol/L MBOA was 52% of that in control seeds, and the activity in seeds treated with 1 mmol/L MBOA remained almost unchanged.

The induction of α-amylase in many plant seeds is regulated by gibberellin at the transcriptional level (Ritchie and Gilroy, 1998). The inhibition of the α-amylase activity by MBOA occurred within 6 h after seeds sowing. Considering germination process where radicles of the seeds emerged around 18 h after sowing, this inhibition may be not too late to inhibit any translation process for α-amylase protein. Thus, MBOA may inhibit α-amylase induction in antagonism with gibberellin-induced events in α-amylase translation process.

Two concentration–response curves (Figs. 1 and 2) indicate that the percentage of the germination is positively correlated with the activity of α-amylase in lettuce seeds. The relationship between the germination and α-amylase activity in lettuce seeds is linear (Fig. 4), and its regression coefficient is 0.961 (significant at 0.01 level).

For germination, plant seeds accelerate respiratory metabolism to produce metabolic energy and biosynthetic precursors (Perata et al., 1997). To maintain respiratory metabolism and germination, readily respiratory carbohydrates, soluble sugars, must be supplied constantly. However, the amount of readily utilizable soluble sugars in plant seeds is usually very limited, with starch being the main reserve carbohydrate (Ricard et al., 1998; Saglio et al., 1999; Guglielminetti et al., 2000). α-Amylase is considered to play a major role in degradation of

reserve carbohydrate to soluble sugars during germination (Perata et al., 1997; Vartapetian and Jackson, 1997). Therefore, induction of α-amylase is essential to maintain active respiratory metabolism, which allows germination of plant seeds.

MBOA inhibited the germination (Fig. 1) and the induction of α-amylase activity in lettuce seeds (Figs. 2 and 3). The extent of the germination was positively correlated with the activity of α-amylase in the seeds (Fig. 4). These results suggest that MBOA may inhibit the germination of lettuce seeds by inhibiting the induction of α-amylase activity. This may be the possible mechanical action of MBOA on the inhibition of plant germination.

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