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BRIEF COMMUNICATION

Inhibition of germination and α-amylase induction by 6-methoxy-2-benzoxazolinone in twelve plant species

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Abstract

6-Methoxy-2-benzoxazolinone (MBOA) inhibited germination of rice (*Oryza sativa* L.), wheat (*Triticum aethiopicum* Jakubz), rye (*Secale cereale* L.), onion (*Allium cepa* L.), wild oat (*Avena fatua* L.), barnyard grass [*Echinochloa crus-galli* (L.) Beauv.], ryegrass (*Lolium rigidum* Gaudin), cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), tomato (*Lycopersicum esculentum* Mill.), carrot (*Daucus carota* L.) and amaranth (*Amaranthus retroflexus* L) and the inhibition increased with increasing MBOA concentrations. MBOA also inhibited the induction of α-amylase in these plant seeds and the inhibition increased with increasing MBOA concentrations. There were variations in sensitivity of these plant species to MBOA, and species of family *Poaceae* (barnyard grass, wild oat, rice, rye, ryegrass, and wheat) were less sensitive to MBOA than the other plant species.

Additional key words: allelopathy, germination inhibitor, *Poaceae.*

6-Methoxy-2-benzoxazolinone (MBOA) is an important secondary metabolite of *Poaceae* plants involved in plant resistance to pests and diseases (Frey *et al.* 1997, Yue *et al*. 1998, Bravo and Copaja 2002, Glenn *et al.* 2002). This compound has also associated with allelopathy because of its inhibiting activity of growth and germination against several plant species (Pérez 1990, Inderjit and Duke 2003, Belz and Hurle 2004). However, the physiological mechanism of MBOA on the inhibition is not fully understood.

Induction of α-amylase is considered to be essential for seed germination because this enzyme triggers starch degradation in the endosperm of seeds and enables the seeds to germinate and grow (Perata *et al.* 1997, Vartapetian and Jackson 1997). MBOA has recently shown to inhibit germination of lettuce seeds and induction of α-amylase in the seeds at concentration greater than 0.03 mM, and the germination rate was positively correlated with the activity of α-amylase in the seeds (Kato-Noguchi and Macías 2005). Therefore, MBOA might inhibit the germination of lettuce seeds by inhibiting the induction of α-amylase activity. We reported here effects of MBOA on germination and α-amylase activity in several monocotyledonous and dicotyledonous plant species.

Monocotyledonous plant seeds of rice (*Oryza sativa* L.), wheat (*Triticum aethiopicum* Jakubz), rye (*Secale cereale* L.), onion (*Allium cepa* L.), wild oat (*Avena fatua* L.), barnyard grass [*Echinochloa crus-galli* (L.) Beauv.], ryegrass (*Lolium rigidum* Gaudin), and dicotyledonous plant seeds of cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), tomato (*Lycopersicum esculentum* Mill.), carrot (*Daucus carota* L.), and amaranth (*Amaranthus retroflexus* L.) were used for assay of seed germination and determination of α-amylase activity as test plants.

Seeds of test plants were sterilized in 25 mM 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 was firstly

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Abbreviations: DTT - dithiothreitol; EDTA - ethylenediaminetetraacetic acid; MBOA - 6-methoxy-2-benzoxazolinone.
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disolved in a small volume of methanol and in concentration 0, 0.1, 0.3, 1.0 or 3.0 mM and added to three sheets of filter paper (No 1, *Merck*, Darmstadt, Germany) in a 9-cm Petri dish and dried. The filter paper in the Petri dish was moistened with 6 cm^3 0.05 % (v/v) aqueous *Tween 20*. Fifty seeds of each test plant were arranged on the filter paper in the Petri dish and germinated in the dark at 25 °C for 3 - 6 d. Then, the germinated seeds were counted. The experiment was repeated five times and the means and standard errors were calculated. For determination of α-amylase activity, these seeds were harvested, frozen immediately with liquid N_2 and freeze-dried.

Freeze-dried seeds (10 seeds for one determination) were homogenized with 1.5 cm^3 of ice-cold solution of 100 mM HEPES-KOH (pH 7.5) containing 1 mM EDTA, 5 mM $MgCl_2$, 5 mM DTT, 10 mM $NaHSO_3$ and 50 mM bovine serum albumin. The homogenate was centrifuged at 30 000 *g* for 30 min, and the supernatant was heated with 3 mM $CaCl_2$ at 75 °C for 15 min to inactivate β-amylase and α-glucosidase (Sun and Henson 1991, Guglielminetti *et al.* 1995). α-Amylase was then assayed by measuring the rate of generation of reducing sugars from soluble starch as described by Kato-Noguchi and Macías (2005). The experiment was repeated five times, with three assays for each determination.

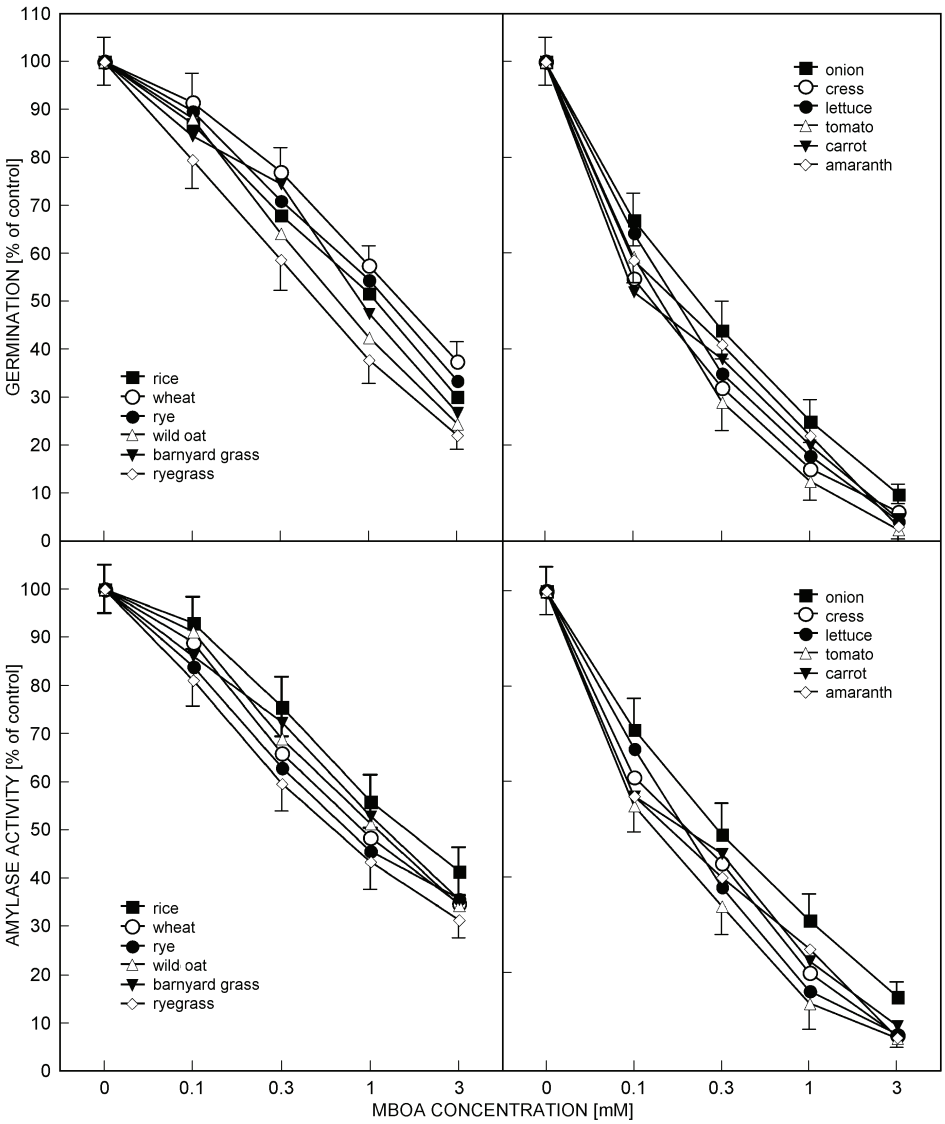

Fig. 1. Effects of MBOA on germination and α-amylase activity in twelve plant seeds. Seeds were incubated with MBOA in the dark at 25 °C for 3 - 6 days. Means ± SE from 5 independent experiments with 50 plants for each determination are shown. Germination and α-amylase activity of control seeds was 87 ± 6, 93 ± 8, 76 ± 7, 51 ± 6, 69 ± 6, 47 ± 5, 86 ± 7, 96 ± 7, 95 ± 7, 94 ± 6, 81 ± 7, 71 ± 7 % and 1650 ± 140, 2150 ± 180, 2310 ± 190, 2750 ± 220, 2340 ± 180, 2350 ± 180, 508 ± 41, 456 ± 37, 427 ± 35, 468 ± 36, 285 ± 27, 375 ± 32 nmol $seed^{-1}$ min^{-1} for rice, wheat, rye, wild oat, barnyard grass, ryegrass, onion, cress, lettuce, tomato, carrot and amaranth, respectively.

MBOA inhibited germination of all test plant seeds and the inhibition increased with increasing MBOA concentrations (Fig. 1). However, *Poaceae* species (rice, wheat, rye, wild oat, barnyardgrass and ryegrass) were less sensitive to the inhibition by MBOA than other plant species (onion, cress, lettuce, tomato, carrot and amaranth). Although onion is monocotyledonous plant species, its sensitivity was similar to other dicotyledonous plant species. When the percentage of germination was plotted against logarithm of MBOA concentrations, concentration-response curves were linear between 40 and 80 % germinations for *Poaceae* species, and between 20 and 60 % germination for the other plant species. The concentrations required for 50 % inhibition of germination, as interpolated from the concentration-response curves, were 0.51 - 1.6 mM and 0.12 - 0.24 mM for *Poaceae* and the other plant species, respectively. Comparing these values, the sensitivities of the other plants were 4.3- to 6.7-fold greater than those of *Poaceae* plants.

It was reported that the detoxification capacity of benzoxazolinone differed in plant species, and *Poaceae* species possessed great capacity of the detoxification (Sicker *et al.* 2003). Benzoxazolinones including MBOA and their precursor hydroxamic acids were found in several *Poaceae* plant species, which indicates that these plants posses metabolic pathway of these compounds including these detoxification process (Niemeyer 1988, Von Rad *et al.* 2001). Therefore, it might be possible that *Poaceae* species were less sensitive to germination inhibition by MBOA than other plant species (Fig. 1) because of greater detoxification capacity of MBOA.

MBOA inhibited the induction of α-amylase activity in all test plant seeds and the inhibition increased with increasing MBOA concentrations (Fig. 2). When α-amylase activities were plotted against logarithm of the concentrations, concentration-response curves were linear between 40 and 80 % activity of α-amylase for *Poaceae* species, and between 20 and 60 % activity of α-amylase for the other plant species. The concentrations required for 50 % inhibition on the activity were 0.63 - 1.8 mM and 0.13 - 0.31 mM for *Poaceae* and the other plants, respectively, which values were close to the concentration required 50 % inhibition on the germination of these plants (Fig. 1). Furthermore, the extents of the germination of all plant species were positively correlated with the activity of α-amylase in their seeds (Fig. 1).

Plant germination is a complex phenomenon (Bogatek *et al.* 2006), and many genes and enzymes participate in this event. Amylolytic breakdown of reserve starch in the seeds is thought to be a prerequisite for seed germination and subsequent seedling growth (Beck and Ziegler 1989, Thomas 1993, Conley *et al.* 1999). During germination, respiration accelerates to produce metabolic energy and biosynthetic precursors for constructing cell structures (Beck and Ziegler 1989, Perata *et al.* 1997). Therefore, soluble sugars that can be readily used in respiration must be supplied constantly to maintain respiratory metabolism. However, the amount of readily utilizable soluble sugars in plant seeds is usually very limited (Ricard *et al.* 1998, Saglio *et al.* 1999, Guglielminetti *et al.* 2000). α-Amylase plays a major role in degradation of reserve saccharides to soluble sugars during germination (Beck and Ziegler 1989, Vartapetian and Jackson 1997). Therefore, induction of α-amylase is a prerequisite for not only seed germination but also subsequent seedling growth, until the seedlings produce carbohydrate by photosynthesis (Beck and Ziegler 1989, Thomas 1993, Conley *et al.* 1999).

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 induction by MBOA occurred within 6 h after seeds sowing and radicles of the seeds emerged after 18 h (Kato-Noguchi and Macías 2005). Considering that radicles emerged around 18 h after sowing, this inhibition may not be too late to inhibit translation process of α-amylase. While it is possible that MBOA inhibits gibberellin biosynthesis, inhibitors of gibberellin biosynthesis do not inhibit the increase in α-amylase production during germination (Groselindemann *et al.* 1991). During germination, gibberellin precursors stored in the seeds are mobilized, and the gibberellin that is produced triggers α-amylase induction (Groselindemann *et al.* 1991, Ritchie and Gilroy 1998). Thus, MBOA may inhibit α-amylase induction in antagonism with gibberellin-induced events by affecting the α-amylase translation process rather then inhibition of gibberellin biosynthesis.

In conclusion, MBOA inhibited the germination of all plant seeds and the induction of α-amylase in these seeds in similar manner (Fig. 1). These results suggest that MBOA may inhibit the germination of these seeds by inhibiting the induction of α-amylase activity.

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