Excess Boron Reduces Polyphenol Oxidase Activities in Embryo and Endosperm of Maize Seed during Germination

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The effects of increasing concentrations of boron (0, 0.1, 1, 10 and 20 mM) as boric acid on the rate of germination and polyphenol oxidase activities in embryo and endosperm tissues of maize seeds (*Zea mays* L. cv. Arifiye) were studied. The germination percentage of maize seeds was not affected by boron concentrations up to 10 mM, and decreased by 20 mM. Distilled water and lower boron concentrations (0.1 and 1 mM) increased polyphenol oxidase activities at the beginning of germination up to 12 h whereas its excess levels (10 and 20 mM) decreased polyphenol oxidase activities in embryos and endosperm during germination. Polyphenol oxidase activities with *o*-diphenolic substrates (caffeic acid, catechol and dopa) were found to be higher than with a monophenolic substrat (tyrosine) in both embryos and endosperms. Further, caffeic acid oxidizing polyphenol oxidase was found to show more activity in embryos of the seeds germinating in distilled water when compared to other substrates.

Key words: Boron, Maize Seed Germination, Polyphenol Oxidase Activities

Introduction

Boron is an essential micronutrient required for growth and development of plants (Marschner, 1995). Boron deficiency or toxicity is a widespread and agriculturally important micronutrient disorder affecting the productivity of cultivated crops in many parts of the world (Shorrocks, 1997; Nable *et al.*, 1997). It is thought that effects of B on plant growth and development processes depend on specific complexing between boron and a variety of substrate or reactant compounds. As boric acid has an outstanding capacity to form stable complexes with compounds having diols and polyols, particularly with *cis*-diols, the compounds include a number of sugars and their derivates (*e.g.* sugar alcohols and uranic acid) and some diphenols (*e.g.* caffeic acid and hydroxyferulic acid). In addition stable complexes are formed with *cis*-diols on apiose which is an important component of the cell wall, with ribose, the principle sugar component of RNA, and also with NAD^+ (Çakmak and Römheld, 1997). Boron is one of the nutrients responsible for the changes in concentration and metabolism of phenolic compounds in vascular plants (Marschner, 1995). It is well known that B deficiency causes an accumulation of phenolics (Camacho-Cristóbal *et al.*, 2004; Chatterjee *et al.*, 2005) and an increase in polyphenol oxidase

(PPO) activity (Çakmak and Römheld, 1997; Pfeffer *et al.*, 1998). Polyphenol oxidases catalyze the hydroxylation of monophenols to *o*-dihydroxyphenols (E.C. 1.14.18.1), and the oxidation of *o*-dihydroxyphenols to *o*-quinones (E.C. 1.10.3.2) which play an important role in the respiratory chain is one of a number of oxidation systems reported in seeds and seedlings (Bewley and Black, 1983). Although there are many studies showing the effect of B on PPO activities in root and leaf tissues, there is no data avaible in the literature showing the effect of boron deficiency or toxicity on PPO activities during germination in different seed fractions. Therefore the aim of this study was to establish changes in PPO activities that might occur upon increasing concentrations of B during maize seed germination.

Materials and Methods

Seed germination

Maize seeds (*Zea mays* L. cv. Arifiye) were surface-sterilized with 1% of sodium hypochloride for 10 min and washed three times with distilled water. Then the seeds were imbibed in distilled water for 6 h. Seeds were sown in 9 cm Petri dishes lined by two layers of Whatman No 1 filter paper containing 12 mL of distilled water (accepted as 0 mM

B) or 0.1, 1, 10 and 20 mM H_3BO_3 solutions prepared in distilled water. Petri dishes were left in an incubator at 25 °C in continuous dark. Germination percentages were determined at intervals of 12 h up to 60 h. Seeds were separated into embryo and endosperm fractions after 6 h of imbibition in distilled water (0 h) and at 12 h intervals up to 60 h for all B treatments, and all the samples were stored at −85 °C until further analyses.

Enzyme extraction and determination of PPO activities

Seed fractions – embryo and endosperm – tissues were ground in ten volumes of chilled 0.1 mM phosphate buffer (pH 6.5). The homogenate was centrifuged at 18,000 rpm at 4 °C for 15 min. The supernatant was used for determination of the enzyme activities.

Polyphenol oxidase activities were determined by using four substrates, namely catechol (pyrocatechol), caffeic acid (3,4-dihydoxycinnamic acid), L-dopa (3,4-dihydroxyphenylalanine) and tyrosine (3,4-hydroxyphenylalanine). Tyrosine (2.5 mM) and each of the other substrates (10 mM) were preapared in 0.1 mM (pH 6.5) phosphate buffer solution. The reaction mixture containing 1 mL of substrate and 50 μL of crude extract was incubated at 30 °C for 3 min or 3 h in the case of tyrosine. The absorbance was measured by a spectrophotometer at 490 nm for o-diphenolase activity using catechol, caffeic acid or dopa and at 430 nm for monophenolase activity using tyrosine. The mixture without crude extract served as a blank. The absorbance values at the mentioned wavelength were expressed as PPO activity units per g seed fraction per min (A_{490} or A_{430} g tissue $^{-1}$ min^{-1}) (Jennings and Duffus, 1977).

Results and Discussion

Germination of maize seeds was not affected by B concentrations up to 10 mM but decreased by 20 mM B (Fig. 1). Other authors also reported a negative effect of excess B on seed germination. For example, the germination percentage of maize seeds was shown to decrease by 15 and 20 mM B treatments (İsmail, 2003). Besides, 93 μM B inhibited the germination of pea seeds compared to 9.3 and 55.8 μM B (Bonilla et al., 2004).

The patterns of change in PPO activity measured using catechol were almost similar in embryos of maize seeds treated with 0 and 0.1 mM B
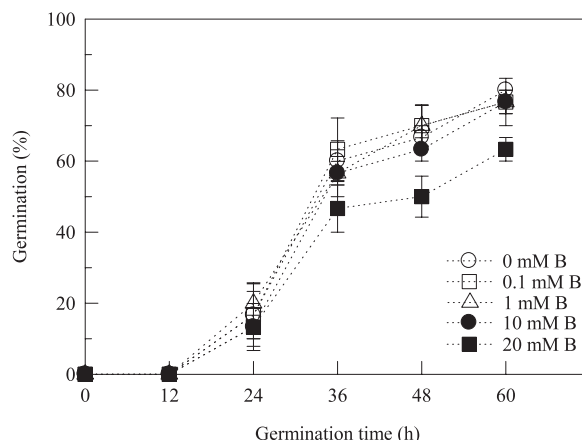

Fig. 1. Time course of maize seed germination. Vertical bars represent standard error of means of three independent experiments.

during a 60 h period. In addition, between 0 h and 12 h, PPO activity was found to be increased and after a sharp decline at 24 h it was not shown an important change up to 60 h (Fig. 2A). The changes in PPO activity measured using dopa were similar to catechol for the all treatments (Fig. 2B). The activity of PPO oxidizing caffeic acid was higher in embryos of 0 mM B treated seeds than in all other treatments during 24 h after imbibition and it decreased to the level of the other treatments at 60 h (Fig. 2C). On the other hand, there were no significant changes in PPO activity of embryos both during the experimental period and in all treatments when tyrosine was used as substrate. However, in this experiment there was a slight increase in 0.1 mM B treatment between 0–12 h (Fig. 2D).

The activities of PPO in the endosperm tissue showed a slightly different pattern than in the embryonic tissue. The activities in the endosperm of 0, 0.1 and 1 mM B treated seeds began to increase markedly after 0 h, and reached a maxium at 12 h, then started to decrease gradually with time below the 0 h level. In contrast, PPO activities of endosperm were lowered markedly by 10 and 20 mM B treatments from 0 h to 12 h (Figs. 2E–H).

The present results showed that the activities with diphenolic substrates were higher than with monophenolic subtrate (tyrosine) in both embryo and endosperm tissues. Furthermore, caffeic acid oxidizing PPO had more activity in embryos of the seeds germinating in distilled water compared to other subtrates in this study (Fig. 2C). It has also
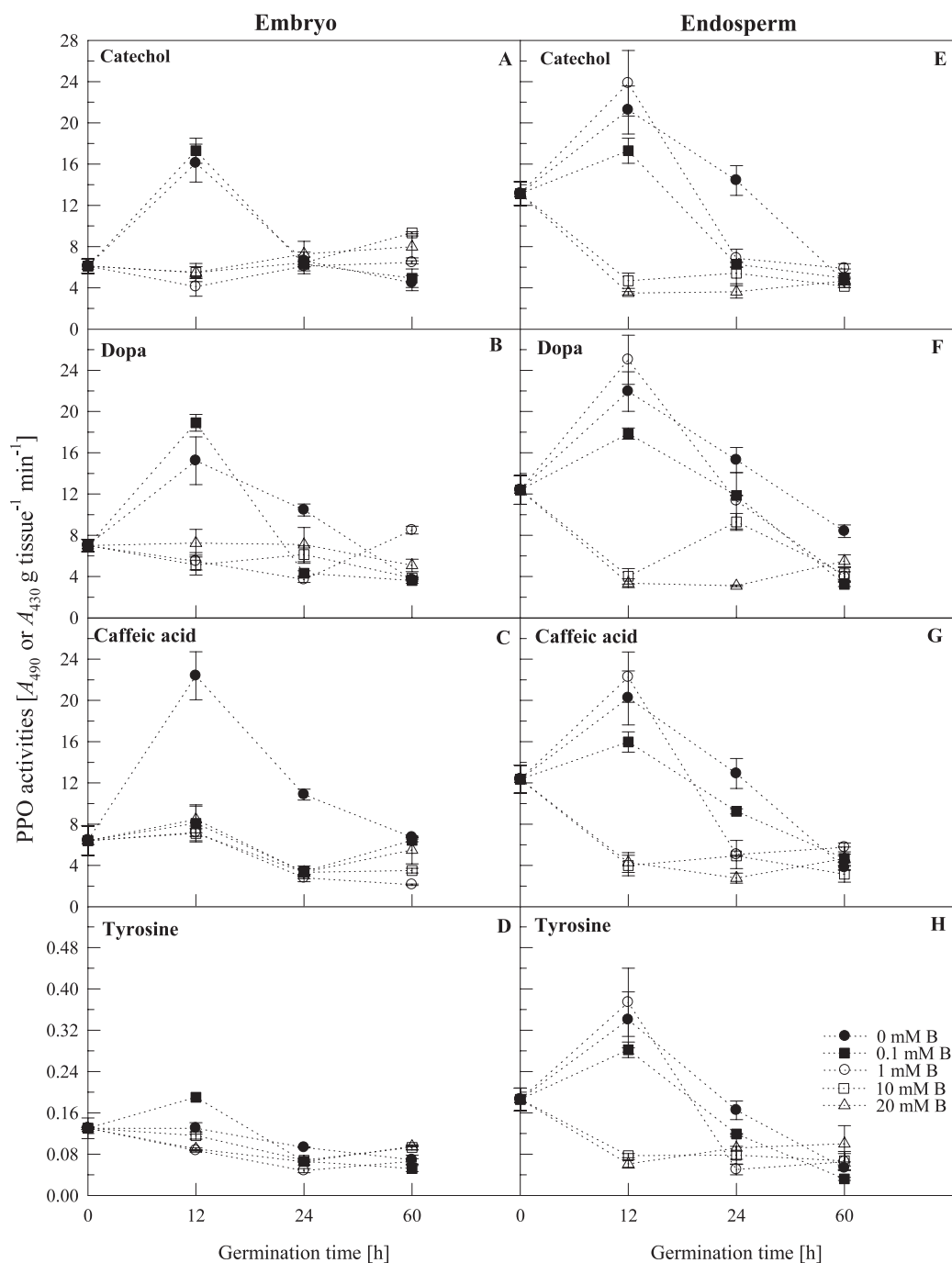
Fig. 2. Time course of PPO activities in embryo and endosperm of maize seeds treated with boron during and following germination. The activities were determined using catechol (A, E), dopa (B, F), caffeic acid (C, G) and tyrosine (D, H). Vertical bars represent standard error of means of three independent experiments.

been reported in a previous paper that caffeic acid oxidizing PPO had more activity in embryos of germinating maize seeds than with the substrates catechol, dopa and tyrosine (Kocaçalışkan *et al*., 1995). This case indicates that caffeic acid oxidizing PPO probably plays a more effective role in embryos of the seeds during germination. On the other hand, since the activity had the highest level at 12 h PPO might play an important role during germination, especially before radicle emergence namely in the pre-germinative stage. There might exist a relationship between PPO activity and respiration in this stage and ATP synthesis in the respiratory chain is probably stimulated by supplying quinone produced as a result of PPO activity in embryos. Thus, in embryos of the seeds, respiration is higher during initial germination because of pre-existing substrates found in embryos are used by respiration to produce ATP required for radicle growth (Stiles, 1960).

Notably, PPO activity increased in our study suggesting its defensive response against wounding of the seed tissues by radicle penetration in the 0, 0.1 and 10 mM B treated seeds. Support for this suggestion comes from the fact that a general increase in PPO activity before and after radicle emergence occurs as shown in wheat (Demeke *et al*., 2001) and tomato (Maki and Morohashi, 2006) seeds, where PPO activity was localized mainly in the micropylar endosperm of tomato seeds. On the other hand, although the *o*-diphenolase activity was prevalent in embryo and endosperm parts of germinating seeds, the monophenolase activity was markedly higher in the endosperm than in embryos of 0, 0.1 and 1 mM B treated seeds. These data are also consistent with other studies (Taneja and Sachar, 1974a, b; Jennings and Duffus, 1977).

However, excess levels of B (10 and 20 mM) either decreased or unchanged PPO activities in germinating and germinated maize seeds (Figs. 2A–H). In some studies, PPO activity increases by B deficiency in leaves of sunflower (Pfeffer *et al*., 1998) and tobacco (Camocho-Cristóbal *et al*., 2002), whereas in roots of squash it was contrary (Cara *et al*., 2002). It has also been shown that both low and excess B decreases the PPO activity in leaves of gram (Chatterjee *et al*., 2005). Similarly, studies with the mineral nutrient copper showed that both low and excess Cu decreases the PPO activity in leaves of sugarbeet (Agarwala *et al*., 1985) whereas the activity increases by excess Cu in *Panax ginseng* roots (Ali *et al*., 2006).

There is substantial variation in the PPO activity among plant species, cultivars and tissues even with seed size, and PPO studies concerned in germination are not enough. However, this study reveals that excess B lowers the PPO activity in seed tissues during germination of maize. Therefore, application of B at high concentrations (10 and 20 mM) to maize seeds or even application very near to the seeds is not advisable.

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