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Full Length Research Paper

Assessment of Benzoic and Cinnamic Acids' Effects on Root Oxidative Injury in Tomato Seedlings

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A systematic experiment was conducted to examine the effects of main autotoxic substances (benzoic acid and cinnamic acid), which was separated in our previous study, on roots oxidative damage of tomato seedlings. Potted tomato seedlings were cultured in perlite and treated with benzoic acid (BA) and cinnamic acid (CA) as exogenous autotoxins. Changes of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and malonaldehyde (MDA) in roots were measured. The results showed that MDA contents were enhanced when treated with both BA and CA, especially in BA with a concentration of 10 mM on the 20th day. Activities of SOD, CAT and POD varied depending on autotoxins (BA or CA), their time of action and concentration. The SOD activity was increased by BA on the 5th day but decreases on the 10th and 20th day, which was increased by CA on the 5th and 10th day but decreases on the 20th day. The POD activity was enhanced on the 5th and 10th day (except the 10 mM CA on the 10th day), but reduced on the 10th day. With the application of both BA and CA, the CAT activity presented a peal on the 10th day and was inhibited on the 20th day and even under the level of the control. Results indicated the adverse effects of exogenous BA and CA on enzymes of antioxidant defence system resulting in lipid peroxidation in roots of tomato seedlings.

Key words: Exogenous autotoxins, enzymes of antioxidant defence system, lipid peroxidation, tomato seedling.

INTRODUCTION

Autotoxicity exists both in natural and agricultural ecosystems which have attracted increasingly scientist's attention (Liu et al., 2007). It is defined as a process in which a species or its decomposing residues release phytotoxins into the environment to inhibit germination and growth on the same species (Miller, 1996; Cruz-Ortega, 2008; Sannigrahi and Chakrabortty, 2005; Singh et al., 1999). Autotoxicity is commonly observed in continuous cropping systems and pure stands of perennial crops and it has reductive effects on production both in agriculture and forestry (Batish et al., 2001; Baziramakenga et al., 1995; Bonanomi et al., 2007; Cao and Luo, 1996; Chou and Lin, 1976). In monocropping system, Autotoxicity is considered for sure to be responsible for the growth and yield loss to some extent. The detrimental effects of autotoxicity including deterioration, regeneration failure and subsequent yield declines, are commonly observed in rice (Fageria and Baligar, 2003), tomato (Bonanomi et al., 2007), cucumber (Yuan et al., 2003), tea tree Cao and Luo (1996) and Chinese fir (Huang et al., 2002).

It was reported decades ago that soil problems caused by autotoxicity related closely to tomato productivity. With the intensive growing and supply of off-season green house tomato in North China, continuous cropping barrier has become or is becoming the bottleneck for tomato production. Previous study shows that aqueous extracts of tomato leaves had inhibitory effects on the seedlings growth and biomass accumulating of tomato (5). Root exposed to soil directly have close contacts with autotoxic substances released by plants during their growth, therefore, it is the key organ for exploring autotoxicity. Benzoic acid and cinnamic acid (0.02 g/L) that inhibited seed germination and seedling growth of tomato are the main allelopathic substances (Yao, 2007). Plants release allelochemicals as mixture, rather than a single compound. The effects of individual constituents are often different from that of mixtures in which the synergic

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and antagonistic effects involved. However, the mechanism of action of individual constituents in tomato seedlings is not known. Exogenic BA and CA were applied to investigate the oxidative damage on the base of previous separation of them from the root exudates. Root damage indexes (including the activity of SOD, POD, CAT and the MDA content) were measured to discuss the mechanism between exogenic autotoxins and membrane lipid peroxidation.

MATERIALS AND METHODS

Preparation of exogenic BA and CA

Chemical BA and CA (purchased from Shenyang Shendong Chemical Factory) were applied as the major autotoxic substances on the base of previous study. The chemicals were firstly dissolved in methanol and then diluted with distilled water when they dissolved completely. Series concentration of 1, 5 and 10 mM of each chemical solution was made and ready for next use.

Potted plant trial

Hybrid tomato of Liaoyuanduoli (the hybrid name) was used for oxidative damage testing. At 4-leaf stage, tomato seedlings were transplanted in 13 cm × 13 cm in pots, in a plastic greenhouse. The hydroponic system was filled with perlite as substrate and irrigated with 50% Hoagland nutrient solution with or without the chemicals. Control seedlings were irrigated with 80 ml nutrients together with 80 ml water every two days. For the treatments, BA or CA at three concentrations was used instead of water. The treatments were arranged in a randomized complete block design with 3 replications and 20 plants in each replication.

Preparation of root samples

Tomato roots were sampled 2^{td}, 5^{th}, 10^{th} and 20^{th} days after transplanting. Roots were gathered, immediately frozen with liquid nitrogen and stored in a fridge for further enzyme analysis. Crude antioxidant enzyme solutions were extracted as described in previous study (Fernandez-Garcia et al., 2004). 0.5 g root was taken and ground in 1 ml, 50 mM phosphate buffer (pH 7.8) with liquid nitrogen. After addition of 3 ml phosphate buffer, the ground roots were centrifuged (4000 rpm) at 4°C for 20 min and the supernatant was used for the determination of enzyme activities with Beckman UV/Visible light spectrophotometer.

Enzyme analyses

SOD solution was prepared using modified Marklund method (Yuan et al., 2003). Crude extract was added to 4.5 ml reaction solution containing 100 mM Tris-HCl buffer (pH 8.2), 1 mM EDTA·2Na and 4.5 mM pyrogallol-HCl solution. Then absorbance was measured at 325 nm at the beginning and 1 min later.

For POD activity measurement, 1 ml crude extract was added in 4 ml reaction medium containing 200 mM phosphate buffer (pH 6.0), 19 ul guaiacol (100) and 28ulc H_2O_2 (30%). The absorbance at 420 nm was measured within 5 min for POD activity (Fernandez-Garcia et al., 2004).

CAT solution was made by adding 0.2 ml crude extract in 3 ml reaction solution including 200 mM phosphate buffer, 100 mM H_2O_2. CAT activity was measured after 4 min H_2O_2 consumption at 240 nm (Fernandez-Garcia et al., 2004).

Total 4 ml solution was prepared by adding 1.5 ml crude extract into a mixture containing 20% trichloroacetic solution and 0.5% thiobarbitric acid for MDA testing. The above-mentioned solution was boiled for 30 min and immediately cooled down and then centrifuged at 1800 g for 10 min. The supernatant were used for MDA measurement at 532 and 600 nm, respectively (Hodges et al., 1999).

Data analysis

Experiments were repeated thrice and results are the means of three independent replicates and for comparison among treatments, Duncan's multiple range test was used, followed by Data Processing System (DPS) software (Refine Information Tech. Co., Ltd, China). Means followed by different letters are significantly different between treatments at P 0.05 level.

RESULTS

Effects of BA and CA on SOD activity

The activity of SOD was increased by the application of both BA and CA on the 5^{th} day, but was decreased on the 20^{th} day (Figures 1 and 2). For instance, treated with 5 mM BA and CA, the SOD activities can be increased 1.4 and 1.7 times when compared to the control 5 days after the treatment, respectively. On the 10^{th} day, the activity was highly reduced by BA under the concentration of 5 and 10 mM, but increased by the application of CA compared with control. The stimulatory effects (BA at 1 mM and CA at all concentrations on 10^{th} day) then decreased and finally reversed to inhibitory effect on the 20^{th} day when the SOD activity was entirely depressed and under the value of the control.

Effects of BA and CA on POD activity

BA and CA showed positive effects on POD activity over the control on the 2^{nd}, 5^{th} and 10^{th} day. The POD activity peak appeared on the 10^{th} day for both BA and CA except that it appeared on the 5^{th} for CA at 10 mM. For both substances sharp decrease of POD activity comes out 20 days after treatments for all the concentrations. Stronger inhibitory effects of CA were found than that of BA due to more resistance of POD to BA (Figures 3 and 4). Nearly, the uniform fluctuation of the three concentrations of BA and CA led to the conclusion that the POD activity was more dependent on time of their action than concentration.

Effects of BA and CA on CAT activity

The activity of CAT was finally under the level of the control on the 20^{th} day after the treatment (Figures 5 and 6). In the control, the CAT activity in roots of tomato

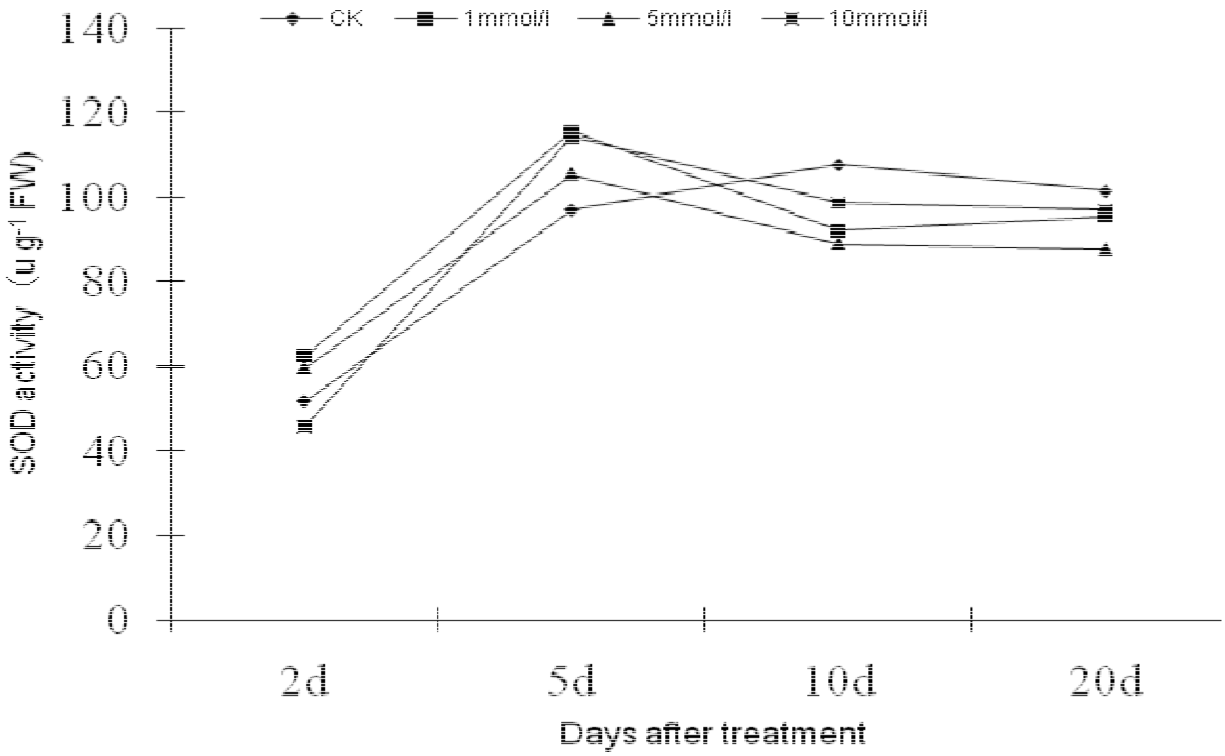

Figure 1. Changes of superoxide dismutase activity in tomato seedling roots under benzoic acid at different concentrations.

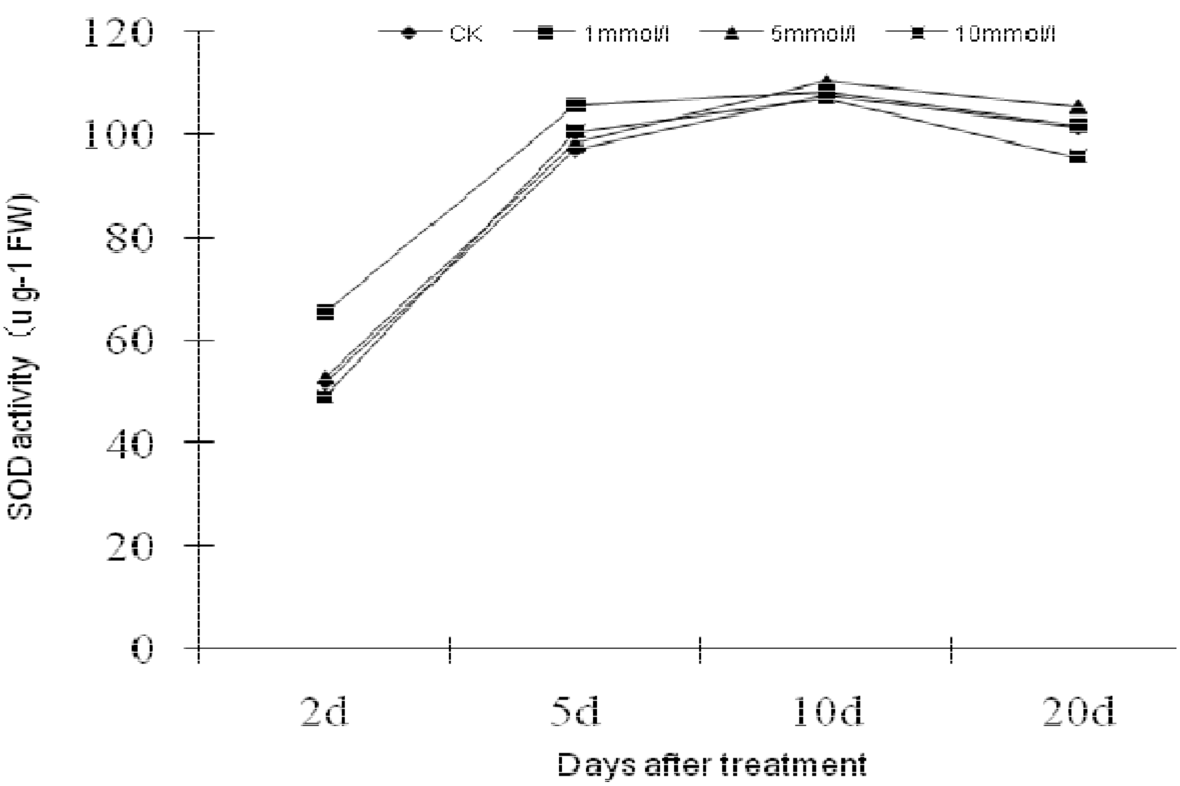

Figure 2. Changes of superoxide dismutase activity in tomato seedling roots under cinnamic acid (CA) at different concentrations.

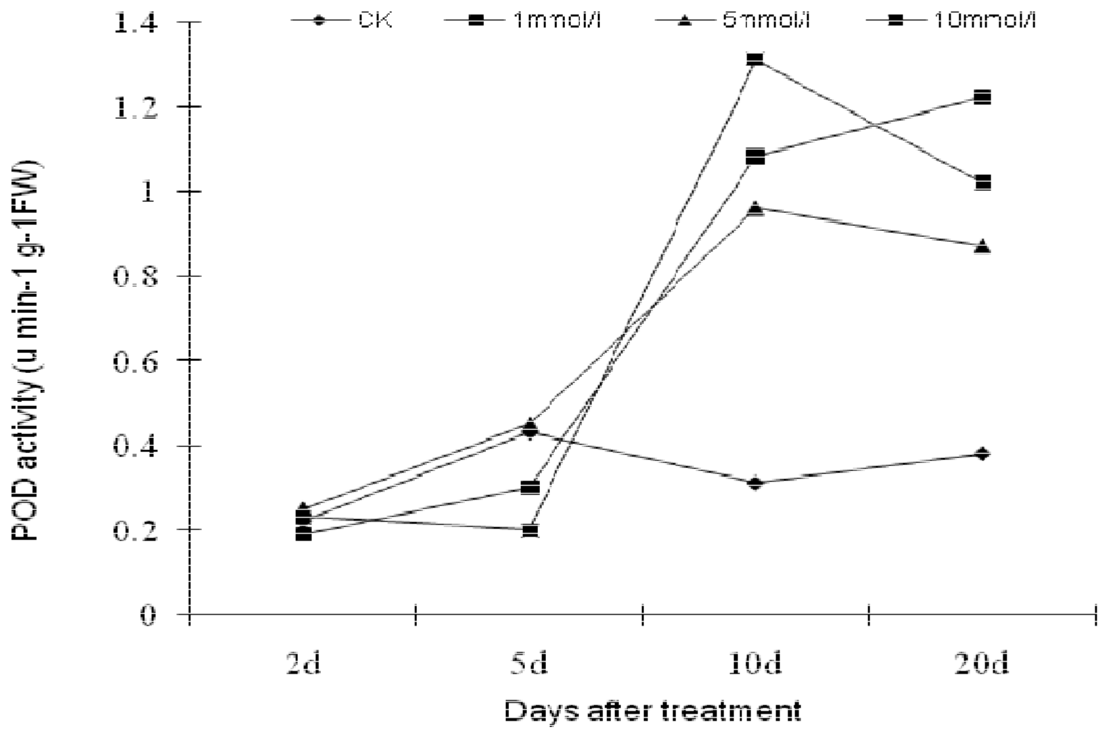

Figure 3. Changes of peroxidase activity in tomato seedling roots under benzoic acid (BA) at different concentrations.

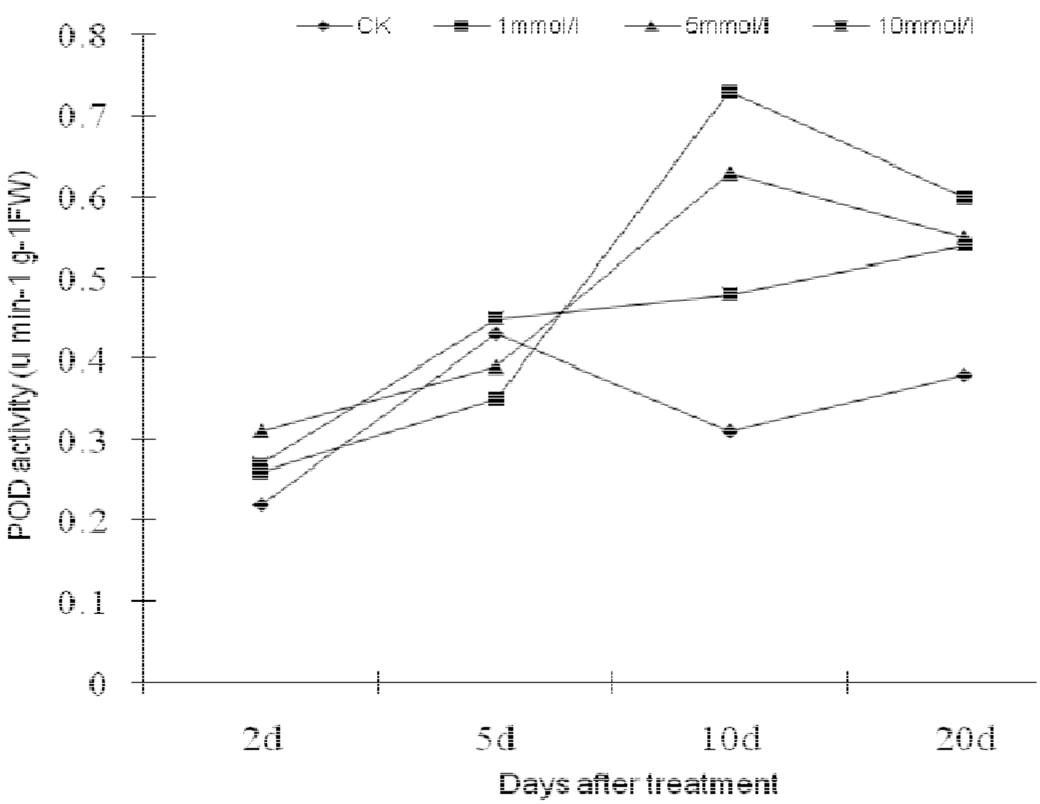

Figure 4. Changes of peroxide activity in tomato seedling roots under cinnamic acid (BA) at different concentrations.

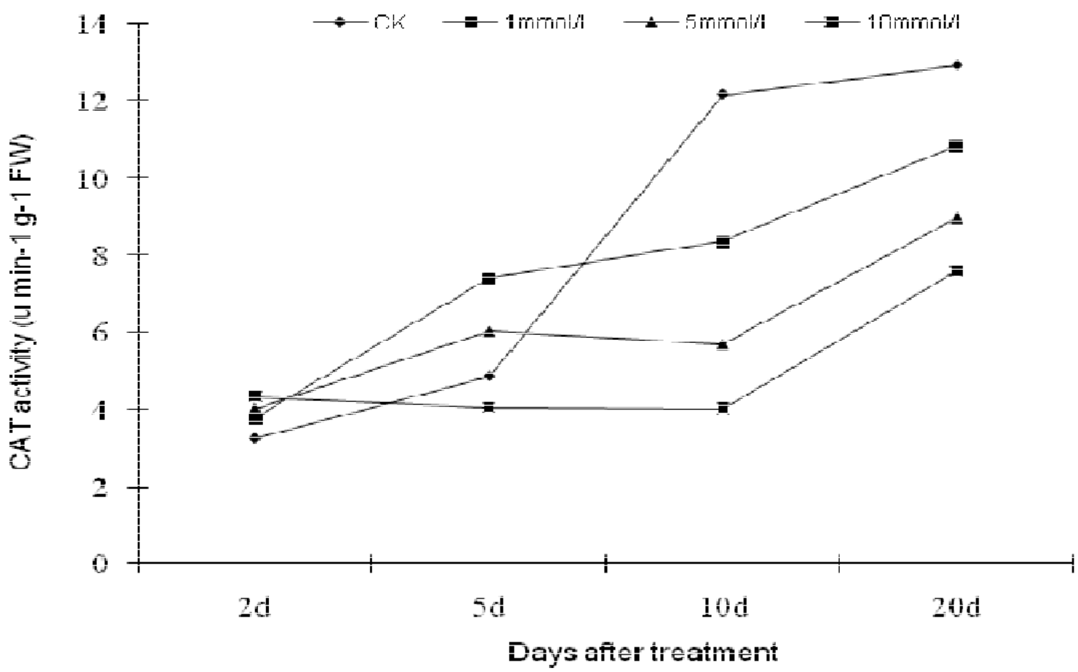

Figure 5. Changes of catalase activity in tomato seedling roots under benzoic acid (BA) at different concentrations.

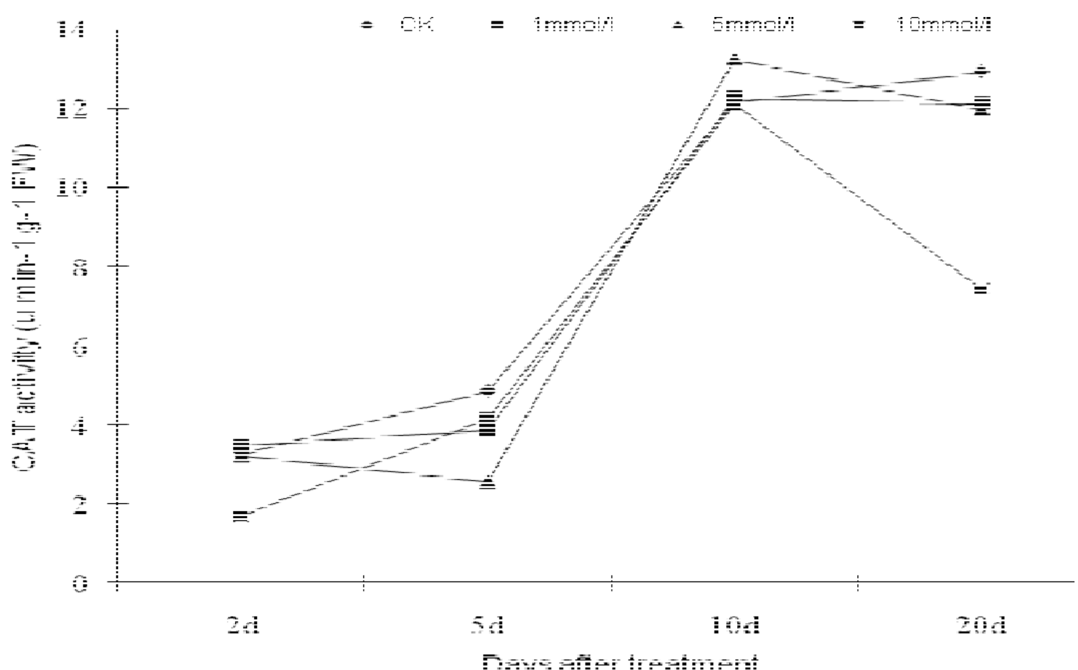

Figure 6. Changes of catalase activity in tomato seedling roots under cinnamic acid (CA) at different concentrations.

seedlings maintained increasing trend with time under natural conditions, without any environmental stress. The CAT activity was inhibited by BA till 5 days after the treatment, but was stimulated by CA during the same time. It was notable that it was enhanced on the 10th day for BA and CA (except for 10 mM). On the 20th day, it seemed that there was no significant difference in BA among all concentrations, but that of CA decreased with

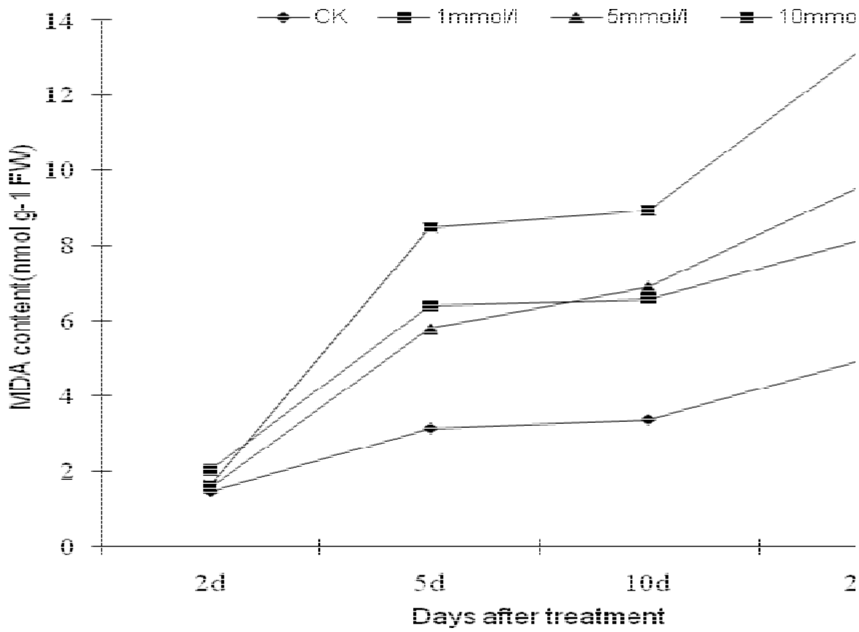

Figure 7. Changes of MDA content in tomato seedling roots under benzoic acid (BA) at different concentration.

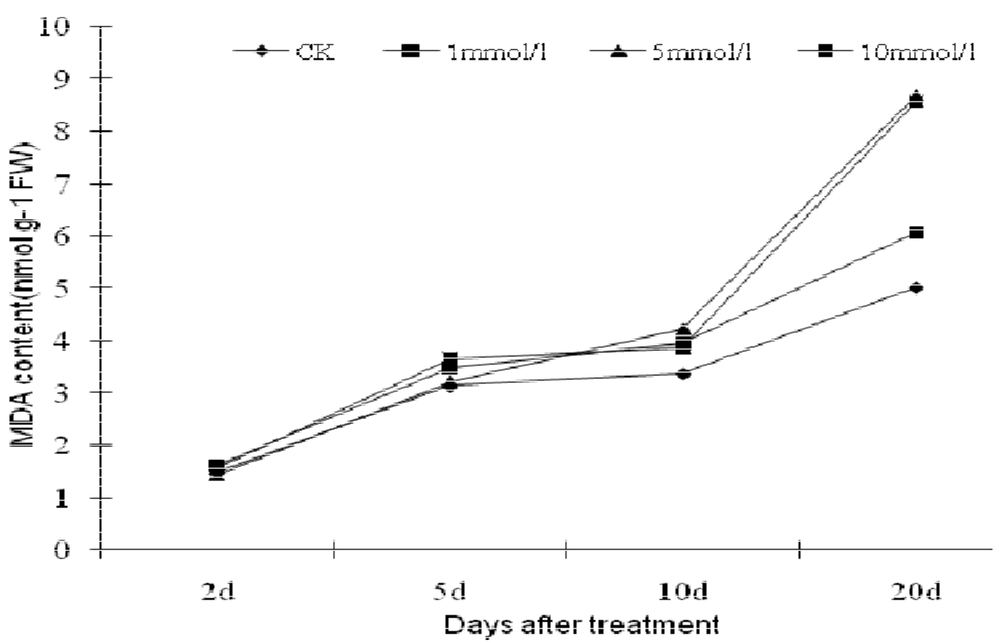

Figure 8. Changes of MDA content in tomato seedling roots under cinnamic acid (CA) at different concentration.

increase in concentration.

Effects of BA and CA on MDA content

The oxidative effects of BA and CA on the lipid degradation were indicated by MDA contents (Figures 7 and 8). The MDA contents can be increased by the application of BA and CA. The longer the roots treated, the higher the concentration, the more the MDA content found. Significant effects of BA and CA on MDA contents were observed compared to the control. On the 5th day, the MDA contents in BA treatments were 128% (1 mM), 149% (5 mM), 189%(10 mM) higher than control. As for CA treatments, the MDA contents reached 381% (1 mM), 262% (5 mM), 456% (10 mM), respectively. MDA accumulated with duration extension and with concentration increase.

DISCUSSION

Poor germination rate and plant growth under allelochemical stresses were observed in tomato seedlings in previous studies (Sannigrahi and Chakrabortty, 2005). Application of BA and CA considerably increased the production of peroxides and active oxygen species (AOS) in allelochemical- treated root tips (Yang et al., 2006) and this forced the plant to over synthesize O_2^- and H_2O_2 followed by perturbations of antioxidant enzymes system.

$$\pi\ 2O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \quad \text{(Equation 1)}$$

$$\theta\ 2\,H_2O_2 \xrightarrow{CAT} 2\,H_2O + O_2 \quad \text{(Equation 2)}$$

$$\rho\ AH_2 + H_2O_2 \xrightarrow{POD} A + H_2O \quad \text{(Equation 3)}$$

The antioxidant enzymes and MDA content did not change much over the control on the 2nd day. It indicated that defensive system of the plant prevailed due to slight overloading and the free radicals were alleviated by scavengers due to the relative short endurance.

Furthermore (on the 5th day), the synthesis of free radicals predominated over the defensive quality of the system, the antioxidant enzyme system was activated and the activity increased. Similar findings were reported by other scientists (Bais et al., 2003; Xiao et al., 2006) . It was shown that the activity of SOD was stimulated to decrease the content of O_2^- when the plant was exposed to BA and CA and the harmful damage was softened. The same results were also obtained with other allelochemicals (Cruz-Ortega, 2008). Equation 1 showed the process of O_2^- removal and H_2O_2 generation by SOD catalysis. Then H_2O_2 was transformed into H_2O and O_2 (Equations 2 and 3). POD activity greatly increased reacting to H_2O_2 accumulation. The CAT activity in BA was depressed when compared to the control. Although that of CA was improved, the harmful effect accumulated before that was not compensated. As a result of mem-brane lipid peroxidation, MDA contents increased in all treatments. Same findings were observed in soybean roots when it was subjected to BA and CA (Baziramakenga et al., 1995).

On the 10th day, under the stress of BA and CA, the plant over synthesize O_2^- , but without the ability to scavenge them due to the damaged system of enzymes. Increased MDA contents were observed in the treat-ments. It was notable that it was higher in CA at 10 mM than others which was associated with the CAT activity in that was under the level of control, and this probably because the plant was deficient in CAT to scavenge the excess H_2O_2. In addition, the MDA content was lower in BA at 5 mM than others and this may have resulted from the higher activity of SOD whose responsibility was to decrease the content of O_2^-.

At the end of the treatment (the 20th day), the allelochemicals caused deep oxidative stresses in target tissues and degraded the antioxidant mechanisms (Aenavoli et al., 2006). In addition, the activities of SOD, POD, CAT in the control was increased with the time prolonged, it probably because of the environmental stress such as climate, nutrient and so on, which was also observed in other findings (Jin et al., 2008). The plant produced lots of O_2^- under the stress of BA and CA during this period and the activities of SOD, POD and CAT were inhibited and damage of O_2^- was not counteracted, leading to the more MDA contents which trigger the peroxidation in membrane lipid of tomato roots.

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