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Toxic Reactivity of Wheat (*Triticum aestivum*) Plants to Herbicide Isoproturon

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The herbicide isoproturon is widely used for controlling weed/grass in agricultural practice. However, the side effect of isoproturon as contaminants on crops is unknown. In this study, we investigated isoproturon-induced oxidative stress in wheat (*Triticum aestivum*). The plants were grown in soils with isoproturon at 0−20 mg/kg and showed negative biological responses. The growth of wheat seedlings with isoproturon was inhibited. Chlorophyll content significantly decreased at the low concentration of isoproturon (2 mg/kg), suggesting that chlorophyll was rather sensitive to isoproturon exposure. The level of thiobarbituric acid reactive substances (TBARS), an indicator of cellular peroxidation, showed an increase, indicating oxidative damage to plants. The isoproturon-induced oxidative stress resulted in a substantial change in activities of the majority of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). Activities of the antioxidant enzymes showed a general increase at low isoproturon concentrations and a decrease at high isoproturon concentrations. Activities of CAT in leaves showed progressive suppression under the isoproturon exposure. Analysis of nondenaturing polyacrylamide gel electrophoresis (PAGE) confirmed these results. We also tested the activity of glutathione *S*-transferase (GST) and observed the activity stimulated by isoproturon at 2−10 mg/kg.

KEYWORDS: Antioxidant enzymes; herbicide stress; isoproturon; wheat (*Triticum aestivum*)

1. INTRODUCTION

Isoproturon [3-(4-isopropylphenyl)-1,1-dimethylurea, IPU] is a phenylurea-derived systemic herbicide for pre- and postemergence control of annual grasses and broad-leaved weeds for wheat, barley, and rye (*1, 2*). It is known that isoproturon inhibits the electron transport in photosystem II (PS II) by binding to the D1 protein in the thylakoid membrane (*3*). Isoproturon is one of the herbicides used worldwide. In China alone, more than thousands of tons of isoproturon are annually applied to the winter and spring cereal crops. However, because of its relatively high solubility in water and low chemical and biological degradation rates (*4*), it has been over-accumulated in soils as residues and consequently become one of the biological and environmental concerns (*5*). Previous studies have demonstrated that the herbicide entered aquatic ecosystems and imposed undesirable side effects on biological properties (*6*). Also, the toxic effect of isoproturon has been shown in phytoplankton algae (*7–10*) and rooted macrophytes (*11*). Thus far, little is known about its adverse chemical behaviors in agricultural systems, particularly in the aspect associated with biological responses.

Higher plants function as one of the essential producers in ecosystems, with important roles in sustaining the integrity of ecosystems. However, the import of various herbicides to the system results in the abnormal biochemical/physiological metabolisms in plants (*12, 13*). Toxic tests for synthetic chemicals have demonstrated that higher plants are rather sensitive to herbicides as compared to phytoplankton (*14*). It is suggested that root elongation of the land vegetable lettuce was more sensitive to the toxicity of wastewater than alga *Selenastrum capricornutum*, the standard specie of alga for toxic tests (*15*). Thereby, toxic stress of agrochemicals on higher plants is a key challenge in ecosystem health.

Recent evidence indicates that pollutants, such as herbicides, are able to induce the intracellular production of reactive oxygen species (ROS), thus damaging plant cells (*16–18*). All living organisms have endogenous defense systems against the oxidative damage (*19, 20*). ROS, such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$), are inevitable byproducts of all aerobic lives under normal conditions, because of the leakage of the electron-transport chain and subsequent cascades of reaction, but they are tightly controlled in cells at an acceptable level (*21*). Environmental stresses, such as heavy metals and herbicide exposure, are commonly stimuli

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that promote the generation of ROS and cause imbalance between ROS generation and removal in plants (*13, 16, 21–23*). To prevent the ROS-induced oxidative damage, plant cells have developed multiple defense systems, including antioxidant enzymes, such as superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6), and ascorbate peroxidase (APX, EC 1.11.1.1), as well as nonenzymatic antioxidants, such as polyphenols, ascorbic acid, and carotenoids (*24*). Because of the difficulty of directly detecting short-lived ROS in plants (*25, 26*), ROS generation induced by stresses is usually deduced from changes in the antioxidant system (*21*). These biomarkers for soil−plant systems include the changes in antioxidant enzyme activities, along with an increase of thiobarbituric acid reactive substances (TBARS) (*18, 27*). Additionally, the intracellular level of glutathione *S*-transferase (GST, EC 2.5.1.18) can be induced by a variety of contaminants (*28*). This enzyme catalyzes the conjugation of glutathione (GSH) to several electrophilic substrates and is believed to be a part of the detoxification system of xenobiotics and oxygen radicals in organisms; therefore, it is considered as a good biomarker of xenobiotic-induced stress (*28*).

In this study, wheat (*Triticum aestivum*) was selected as a mode plant to investigate the isoproturon-induced oxidative stress. This crop has been one of the most important economic crops worldwide. Contamination with herbicides is most likely to be linked to the crop production, quality, and human health. Also, as an important crop, wheat is frequently used as an ecotoxicological indicator. Thus, the objective of the study is (1) to elucidate the effect of excess isoproturon on the growth, physiological, and biochemical responses of wheat plants and (2) to seek sensitive biomarkers for diagnosing potential adverse effects on ecosystems at biochemical levels under the stressful condition. The outcome of the work may improve our understanding of toxic process and developing strategies for reducing the risks of the herbicide to the crop production.

2. MATERIALS AND METHODS

2.1. Materials. Isoproturon was obtained from the Institute of Pesticide Science, Academy of Agricultural Sciences in Jiang Su, Nanjing, China. Wheat seeds (*T. aestivum*, cv. Ning 13) were provided by the Institute of Crop Science, Academy of Agricultural Sciences in Jiang Su, Nanjing, China. The tested soil was collected from the surface layer (0−20 cm) of an uncontaminated field in the Experimental Station of Nanjing Agricultural University. The collected soils were then air-dried at room temperature, ground, and sieved through a 3 mm mesh before use. The soil was mixed with isoproturon, and the final concentrations of the herbicide were set at 0, 2, 3.5, 5, 10, and 20 mg/kg soil. Each treatment was set in triplicate.

2.2. Plant Culture and Treatment. Wheat seeds were surface-sterilized with 5% sodium hypochlorite solution for 10 min, then thoroughly washed with distilled water, and germinated at 25 °C. After 8 h, the seeds were sown in each plastic pot (1 L). The pots were watered daily to maintain 60−70% relative water content in the soil. Seedlings were grown in a growth chamber under the conditions of a 12 h photoperiod, 300 μmol of photons m^{-2} s^{-1}, and 25/20 °C day/light temperature. After growth for 10 days (the third leaf emergence), different tissues were separately harvested and immediately frozen in liquid nitrogen or stored in a −80 °C freezer for analysis. For the determination of dry mass, tissues were oven-dried at 70 °C for 72 h and then weighted.

2.3. Analyses of Soil Properties. Soil samples were air-dried, ground, and passed through a 2 mm mesh sieve for analysis of properties. The soil pH was measured with a glass electrode (1:1 soil/water) (*29*). Organic matter was determined by the method of Mebius (*30*). Total nitrogen was determined by the Kjeldahl digestion procedure (salicylic acid modification) (*31*). Available phosphorus was measured by the method described by Olsen et al. (*32*), and available potassium was measured by the method by Knudsen et al. (*33*). Some chemical properties of the soil were listed as followings: pH 7.65; organic carbon, 0.81%; total N, 1.26 g/kg; available P, 34.3 mg/kg; and available K, 91.5 mg/kg (*13*).

2.4. Determination of Chlorophyll and TBARS. The chlorophyll concentration in tissues was measured according to the method of Porra et al. (*34*). A total of 0.1 g of flesh leaf samples was homogenized in 8 mL of 80% acetone (pH 7.8 adjusted with sodium phosphate buffer), followed by centrifugation at 500*g* for 10 min. The supernatant was used for a spectrophotometric assay, and total chlorophyll was calculated.

Accumulation of lipid peroxides in tissues was determined in terms of TBARS based on the method of Wang et al. (*20*). Fresh tissues (0.5 g) frozen in liquid nitrogen were ground and dissolved in 3 mL of 0.1% (w/v) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 10000*g* for 30 min, and 2 mL of the supernatant was mixed with 2 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated in boiling water for 30 min, cooled to room temperature, and centrifuged at 15000 *g* for 5 min. The absorbance of the supernatant was measured at 532 nm. The value for nonspecific absorbance at 600 nm was subtracted. The amount of TBARS was calculated using the extinction coefficient of 155 mM^{-1} cm^{-1}.

2.5. Enzyme Activity Assay. Wheat leaves and roots (0.3 g) were separately homogenized in 1.5 mL of ice-cold extraction buffer containing 50 mM Tris-HCl (pH 7.8), 1 mM ethylenediaminetetraacetic acid (EDTA), and 1.5% (w/w) polyvinylpyrrolidone. The homogenate was centrifuged at 15000*g* for 20 min. The supernatant was used as the crude extract for the assay of enzyme activities.

CAT (EC 1.11.1.6) activity was determined by consumption of H_2O_2 (extinction coefficient of 39.4 mM/cm) at 240 nm for 30 s (*35*). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2, and 50 μL of leaf or root extract in a 3 mL volume.

POD (EC 1.11.1.7) activity was measured following the change of absorbance at 470 nm because of guaiacol oxidation. The activity was assayed for 1 min in a reaction solution (3 mL final volume) composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H_2O_2, and 50 μL of crude extract (*22*).

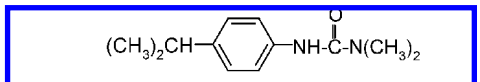
Activities of SOD (EC 1.15.1.1) were assayed by measuring its capacity of inhibiting the photochemical reduction of nitroblue tetrazolium (NBT) (*20*). A total of 3 mL of reaction mixture contained 50 mM phosphate buffer (pH 7.8), 10 mM methionine, 1.17 mM riboflavin, 56 mM NBT, and 30 μL of enzyme extract. The absorbance of solution was measured at 560 nm. One unit of SOD was defined as the enzyme activity that inhibited the photoreduction of NBT to blue formazan by 50%.

Ascorbate peroxidase activity was determined according to the previous method (*36, 37*), with the following modification: 0.2 g of fresh root was homogenized in 1.5 mL of 50 mM ice-cold phosphate buffer (pH 7.8) containing 2 mM ascorbate and 5 mM EDTA. The homogenate was centrifuged at 10000*g* at 4 °C for 30 min. The reactive solution (3 mL) contained 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, and 0.1 mM H_2O_2, and finally, 50 μL of enzyme extract was added. The decrease in absorbance at 290 nm was read.

GST activity was assayed in 3 mL of reaction mixture containing 100 mM potassium phosphate buffer (pH 7), 1 mM GSH, 1 mM 1-chloro-2,4-dinitro-benzene (CDNB) (10 mM CDNB dissolved in 50% acetone stock solution), and proteins from root extract (*38*). The reaction was initiated by adding CDNB. The increase in absorbance at 340 nm was measured within 15 min.

2.6. PAGE. A total of 3 g of tissue were homogenized with 50 mM potassium phosphate buffer (pH 7.0), including 1 mM 2-mercaptoethanl, 0.5 mM phenylmethyl, and 1 mM EDTA. The homogenate was centrifuged at 10000*g* at 4 °C for 20 min. The supernatant was used for the detection of isoenzymes (*22*). The isoenzymes of SOD, CAT, and POD were separated on the discontinuous polyacrylamide gels (stacking gel of 5% and separating gel of 10%) under the nondenaturing conditions. Proteins were electrophoresed at 4 °C and 10 mA in the stacking gel, followed by 15 mA in the separating gel.

SOD activity was determined on the gel as described by Wang and Yang (*22*). The gels were rinsed in water and incubated in the dark for



properties	
Molecular formula	$C_{12}H_{18}N_2O$
Molecular weight	206.29 g/mol
Melting point	155-156 °C
Water solubility (20 °C)	55 mg/L
Vapor pressure (20 °C)	0.0033 mPa
Standard specific gravity	1.16 g/cm^3
Partition coefficient (22°C)	2.5

Figure 1. Structure and some properties of isoproturon.

30 min at room temperature in the assay mixture containing 50 mM potassium phosphate buffer (pH 7.8), 1 mM EDTA, 0.05 mM riboflavin, 0.1 mM NBT and 0.3% *N,N,N′,N′*-tetramethylethylenediamine (TEMED). After that, the gels were rinsed with water and exposed on a light box at room temperature for 10 min until the development of colorless bands of SOD activity in a purple-stained gel was visible. For peroxidase isoforms, the gels were stained for 20 min in 0.2 M acetate buffer (pH 5.5) with 5 mM benzidine and 5 mM H_2O_2 (*23*).

For the detection of CAT isoenzyme activity, the gel was soaked in deionized water for 15 min. Subsequently, the gel was incubated in 0.03% H_2O_2 for 25 min and then carefully washed with deionized water to remove the residual H_2O_2. After that, the gel was stained in the solution of 1% (w/v) potassium ferricyanide and 1% (w/v) ferric chloride (*39*). This caused the gel to turn blue, except at positions exhibiting CAT activity. When maximum contrast was achieved, the reaction was stopped by rinsing the gel with deionized water.

3. RESULTS

3.1. Effect of Isoproturon on the Growth of Wheat Seedlings. The structure and some chemical properties are listed in **Figure 1**. The wheat growth was sensitive to the isoproturon exposure. As shown in **Figure 2A**, treatment with isoproturon at 2, 3.5, 5, 10, and 20 mg/kg progressively inhibited the shoot growth, as expressed by dry weight. However, the significant inhibition occurred at 10−20 mg/kg. The root was in much less response to the isoproturon exposure. In contrast, the elongation of roots was rather sensitive to isoproturon (**Figure 2B**). Treatment with 20 mg/kg isoproturon decreased the root length to 44% of the control. The similar inhibition of shoot elongation was observed with a 32% decrease as compared to the control.

3.2. Effect of Isoproturon on Metabolite Accumulation. To examine the responses of plants to isoproturon, we measured chlorophyll in wheat leaves. The content of chlorophyll significantly decreased after the exposure to isoproturon (**Figure 3A**). Even at 2 mg/kg of isoproturon, the chlorophyll content decreased by 11% as compared to the control. Exposure of wheat plants to isoproturon led to lipid peroxidation in roots and leaves. The level of lipid peroxides, expressed as TBARS, was elevated with the increasing isoproturon (**Figure 3B**). The maximum accumulation in the above ground was observed at 10 mg/kg isoproturon, where the leaves with isoprotron accumulated more than 2-fold higher TBARS than the control. Interestingly, the root did not appear to be affected by isoproturon exposure.

3.3. Effect of Isoproturon on Enzyme Activities. Activities of antioxidant enzymes in wheat plants showed a substantial change compared to the control, when subjected to the isoproturon exposure. Activities of SOD in roots and leaves progressively increased with isoproturon concentrations at 2−10 mg/kg. However, further application of isoproturon up to 20 mg/kg led to the decreased activity (**Figure 4A**). CAT activities in roots increased with the isoproturon concentrations within 2−5 mg/kg (**Figure 4B**). When the isoproturon concentrations were raised to 10−20 mg/kg, the CAT activity decreased. CAT activity in leaves showed a constant reduction with the

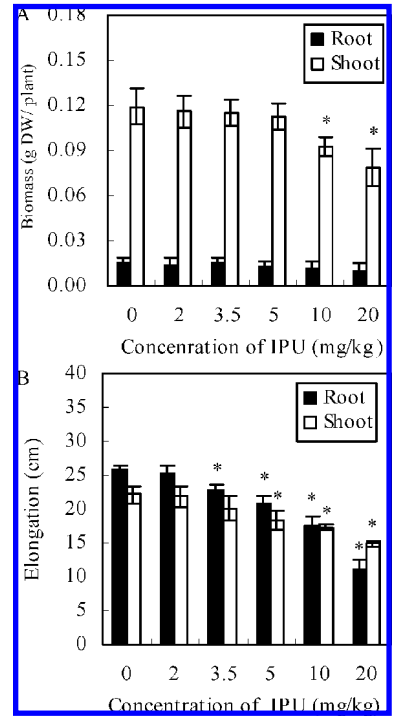


Figure 2. Effect of isoproturon on the wheat growth. Wheat seedlings were cultured in soils containing isoproturon at 0, 2, 3.5, 5, 10, and 20 mg/kg for 10 days. Then, the biomass (A) and elongation (B) of roots and leaves were measured, respectively. Values are the means ± standard deviation (SD) (n = 3). Asterisks indicate the significant differences between the treatments and the control ($p < 0.05$).

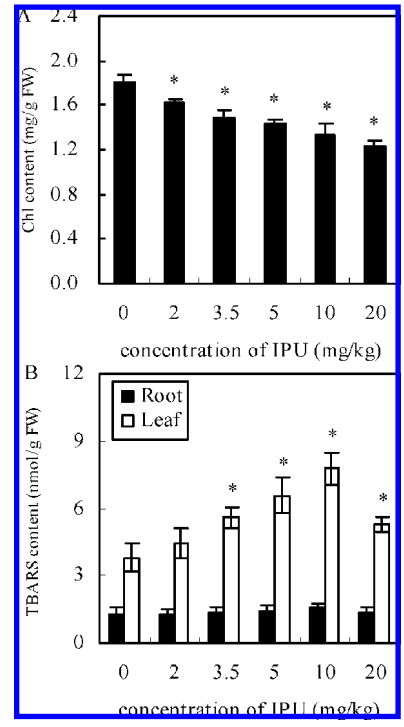


Figure 3. Effects of isoproturon on the content of chlorophyll (A) and TBARS (B) in wheat plants. Wheat seedlings were cultured in soils containing isoproturon at 0, 2, 3.5, 5, 10, and 20 mg/kg for 10 days. Then, the content of chlorophyll and TBARS were measured. Values are the means ± SD (n = 3). Asterisks indicate significant differences between the treatments and the control ($p < 0.05$).

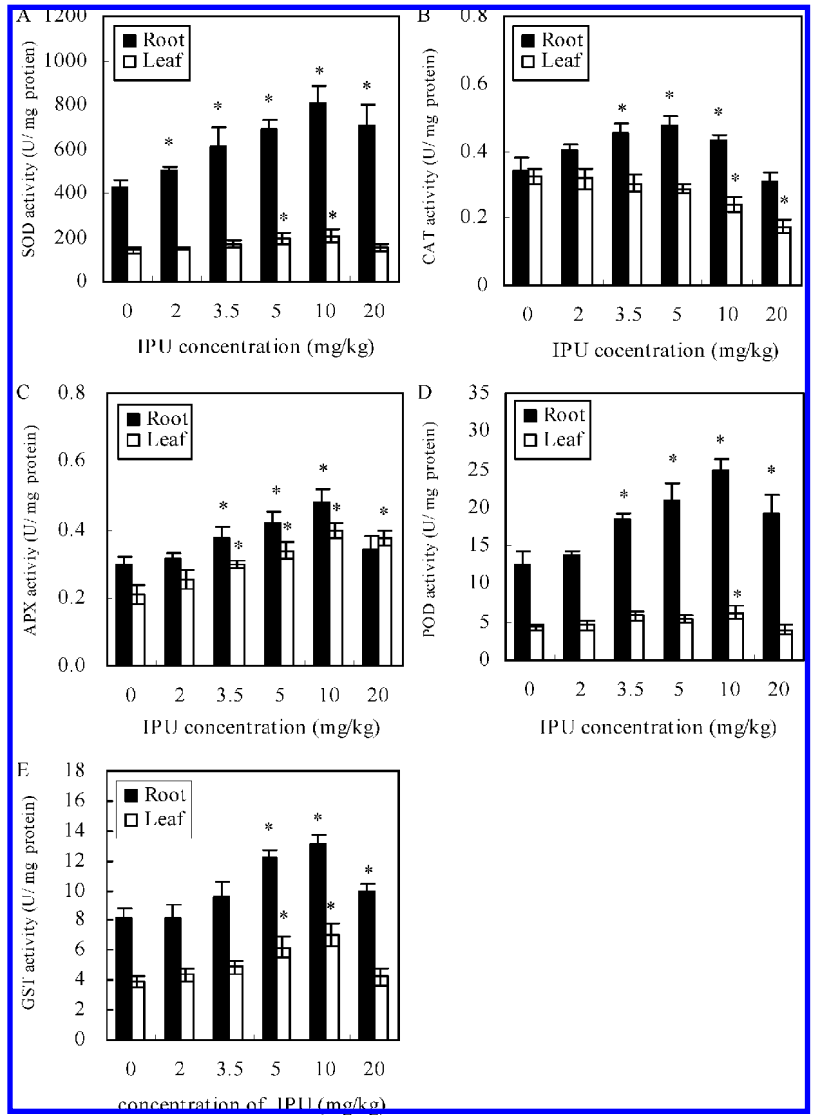


Figure 4. Effect of isoproturon on the activities of SOD (A), CAT (B), APX (C), POD (D), and GST (E) in wheat plants. Seedlings were cultured in soils containing isoproturon at 0, 2, 3.5, 5, 10, and 20 mg/kg for 10 days. Then, the enzyme activities were measured. Values are the means ± SD (n = 3). Asterisks indicate that mean values are significantly different between the treatments and the control ($p < 0.05$).

concentrations of isoproturon applied. However, the significant decrease was observed at 10−20 mg/kg. The pattern of APX activities was similar in root and leaf, showing a typical "low−high−low" change (**Figure 4C**). Within 5−10 mg/kg of isoproturon, the APX activity was significantly higher than the control; while the concentration of isoproturon reached to the top (20 mg/kg), the APX activity decreased.

Activities of POD exhibited a pattern similar to APX, with an exception of a small change in root POD activity (**Figure 4D**). GSTs are believed to be typical detoxifying enzymes (*28*). The change of its activity was consistent with that of the other antioxidative enzymes (**Figure 4E**). The above results indicate the involvement of all of these enzymes in the similar response to the herbicide.

To obtain an insight into the effect of isoproturon on the enzymes, a nondenaturing polyacrylamide gel electrophoresis (PAGE) for SOD, POD, and CAT activities was performed. Under the conditions of this study, only two isoforms of SOD were detected. The patterns of these isoforms were similar in response to isoproturon exposure. Within the concentrations of isoproturon from 2 to 10 mg/kg, the activities of SOD increased in both roots and shoots and a further increase in isoproturon concentration up to 20 and 10 mg/kg resulted in a decrease in the SOD activities, respectively (**Figure 5A**).

At least four bands of POD isoforms in the wheat root were detected (**Figure 5B**). All isoforms of POD in roots showed increasing activities at 3.5−10 mg/kg of isoproturon. Six bands were detected in leaves (**Figure 5B**). However, with regard to isoforms I−IV, no difference of the activities between the treatments and control was observed. For isoform VI, only a slight increase in activity of POD was found relative to the control.

We also performed native PAGE for catalase. Only one isoenzyme of CAT in the wheat was detected (**Figure 5C**). The activities of CAT in roots showed a slight increase with the concentrations of isoproturon ranging from 2 to 5 mg/kg but a decrease at high concentrations of isoproturon (10−20 mg/kg). Activities of CAT in leaves showed the progressive decrease in response to isoproturon.

4. DISCUSSION

Several lines of evidence have demonstrated that organic pollutants, such as pesticide/herbicide and their residues in soil, cause a variety of physiological stresses in plants (*13, 16, 17, 20*).

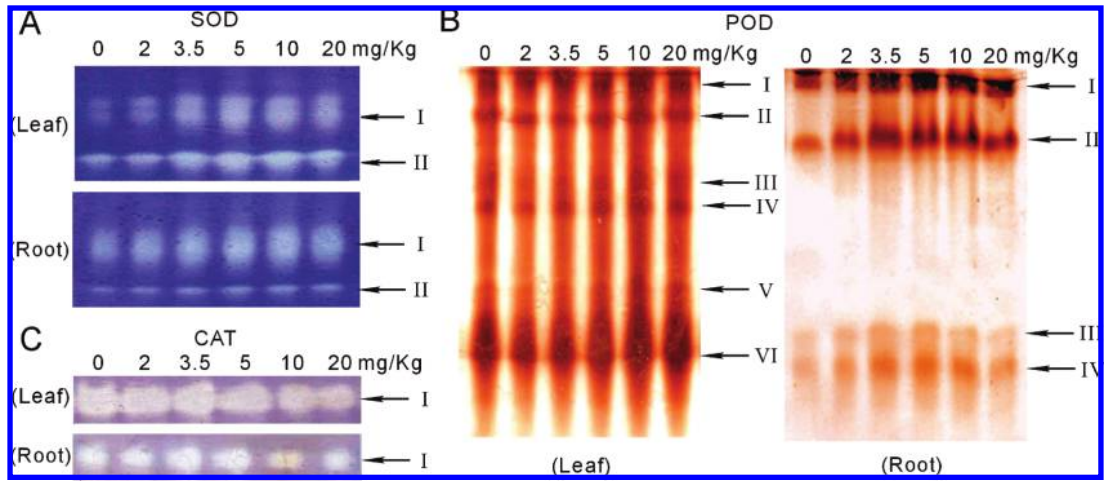


Figure 5. Nondenaturing PAGE patterns of SOD (A), POD (B), and CAT (C) isoforms present in the roots and leaves of wheat grown for 10 days in soils supplemented with isoproturon at 0−20 mg/kg. Lane 1, 0 mg/kg; lane 2, 2 mg/kg; lane 3, 3.5 mg/kg; lane 4, 5 mg/kg; lane 5, 10 mg/kg; and lane 6, 20 mg/kg.

Within the stresses, production of ROS in plants is a major response (*13, 18, 20*). We tested isoproturon, one of the most prevalent herbicides (*2*) and observed that wheat plants exposed to isoproturon showed a general inhibition of growth in terms of elongation and biomass of roots and shoots (**Figure 2**). The toxic symptoms of these growth parameters represented severe stress caused by high level of isoproturon. However, it is insufficient to evaluate the soil quality based on the changes in these parameters, because the growth rates of both roots and shoots were slightly inhibited at 2 mg/kg isoproturon (**Figure 2A**). For a better understanding of isoproturon effects on plants, sensitive parameters related to the biochemical parameters might be required.

Previous studies with agrochemicals or pesticides demonstrated that the reduction of chlorophyll content could be used as a visible symptom to monitor the damage to the growth and development of plants (*13, 20, 29*). The current results were consistent with the previous data. It has been shown that chlorophyll was rather sensitive to isoproturon, because even at 2 mg/kg isoproturon, the chlorophyll formation was suppressed significantly (**Figure 3A**). These results suggested that isoproturon as phenylurea herbicide might be active in the chloroplast electron-transport system and may disturb the photosynthesis of the target plant.

Exposure of wheat plants to isoproturon led to lipid peroxidation in leaves. The level of lipid peroxides was elevated with the isoproturon applied (**Figure 3B**). The wheat leaves showed a great response to the isoproturon exposure because they accumulated more peroxidation products. It appeared that exposure of isoproturon did not induce high levels of TBARS in wheat roots. However, it can not be concluded that no oxidative stress occurred in the roots. In fact, TBARS is not the only oxidative products caused by ROS, and there are much more other peroxides in response to the oxidative damage (*12, 21*). Therefore, the TBARS is not a good candidate used for an indicator of isoproturon-induced stresses in wheat.

To prevent the ROS-induced oxidative damage, plant cells have to develop multiple defense systems, including enzymatic antioxidants, such as SOD, CAT, APX, and POD. SOD is the first line of defense against oxidation, catalyzing the dismutation of $O_2^{\bullet}$ to H_2O_2 and O_2. It is composed of a family of metalloenzymes that occur in different isoforms as Cu−Zn−SOD, Mn−SOD, and Fe−SOD (*40*). Our analysis revealed that the protective enzymes SOD in the isoproturon-treated seedlings were generally activated (**Figure 4A**). This was supported by the data of nondenaturing PAGE assay and indicated that two isoforms (I and II) in roots were progressively stimulated by high levels of isoproturon (**Figure 5A**). Increased SOD activity might be attributed to the elevated production of superoxides, thus resulting in the activation of existing enzyme pools or upregulated expression of the genes (*41, 42*). On the other hand, raising the isoproturon concentration up to 20 mg/kg decreased the SOD activities, which might be due to enzyme protein damage by excessive H_2O_2 or isoproturon. The elevated activities of SOD were correlated with the increased degree of oxidative stress; thus, the activation of SOD might represent circumstantial evidence for the occurrence of oxidative stress in wheat (*13*). Because the production of free-radical responses to pollutants is contrary to the trends in growth parameters, this may also suggest that the peroxides might be the cause of the inhibition of root elongation and reduction of root biomass.

Hydrogen peroxide (H_2O_2) generated by the SOD-mediated reaction is highly toxic and must be tightly controlled at low levels in cells. In plants, a number of enzymes regulate the levels of intracellular H_2O_2. POD, APX, and CAT are considered the most dominant enzymes that catalyze H_2O_2 to H_2O or the other nontoxic products (*43*). APX is the major H_2O_2-scavenging enzyme that catalyzes the removal of H_2O_2 (*44*). Under the isoproturon stress, the activity of APX was stimulated in both roots and leaves (**Figure 4C**). It is noted that the peak activity was found at 10 mg/kg isoproturon and increasing the isoproturon concentrations depressed the activities. It was possible that the enzyme might be impaired at the high level of isoproturon. POD is another indicator of oxidative stress in higher plants (*20, 22*). Within the various enzymes involved in the elimination of active oxygen species, POD can be considered one of the key enzymes, because both of its extra- and intracellular forms are participating in the breakdown of H_2O_2 and lignin biosynthesis in the presence of H_2O_2 (*45*). POD can use a wide range of electron donors, including NAD(P)H, but the substrate for commonly assayed total POD activity is guaiacol, which represents the nonspecific activity (*45, 46*). In this study, the activity of POD was enhanced at 3.5−10 mg/kg isoproturon (**Figures 4D** and **5B**), suggesting an increased degree of oxidative stress. CAT is located in peroxisomes, glyoxysomes, and mitochondria and shown to remove most of the photorespiratory and respiratory H_2O_2 (*46*). However, the CAT activities in leaves were suppressed under isoproturon

exposure (**Figure 4B**). It was possible that CAT in this section would be rather sensitive to excess isoproturon. Another possibility can be interpreted as the adjustment of ROS production. Any change in the activities of antioxidant enzymes tends to upset the redox state, leading to cellular oxidative stress. CAT, in a contrast to APX, does not require reducing power to break down H_2O_2 to H_2O and O_2 and has a high reaction rate but low affinity for H_2O_2, whereas APX has a high affinity for H_2O_2 and is able to detoxify low concentrations of H_2O_2 (*36, 37*).

GST (EC 2.5.1.18) is found in most aerobic organisms, catalyzing the nucleophilic addition of GSH to electrophilic centers in organic molecules (*47*). GST belongs to a large family and can be grouped into six distinct classes: α, μ, π, θ, δ, ζ, and β (*48*). It plays an important role in the plant protection against oxidative damage. Various reactive electrophiles, formed by oxidation of cellular metabolites (e.g., unsaturated fatty acids and DNA constituents), can be the substrates for GST. Additionally, GST can function as GSH peroxidase, able to reduce fatty acid hydroperoxides or thymidine hydroperoxides to the corresponding hydroxy derivatives with the correlative production of GSSG (*49, 50*). The intracellular level of GST is increased by the product of uncontrolled oxidative reactions or xenobiotics, such as herbicides (*28, 51*). Our results showed that the activity of GST was activated in response to isoproturon at relatively high levels (**Figure 4E**), suggesting that this enzyme was most likely playing a role in the detoxification of isoproturon in wheat and that the GSH system constituted a sensitive biochemical indicator of contamination by isoproturon for wheat.

Taken together, the tested herbicide slightly affected the wheat growth at a relatively low dose but exerted a remarkable effect at high levels. Relative to the growth parameters, the physiological process in wheat seedlings was more sensitive and significantly affected by isoproturon exposure. To deal with the isoproturon-induced oxidative stress, wheat plants activated a variety of antioxidative enzymes, such as SOD, CAT, APX, and POD, to diminish the ROS. Additionally, the activity of GST, one of typical detoxifying enzymes, was elevated in response to isoproturon. These biochemical responses can be interpreted as an internal tolerant mechanism and may allow us to develop strategies for reducing the risks of the herbicide contamination to crop production.

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