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Jasmonic Acids Facilitate the Degradation and Detoxification of Herbicide Isoproturon Residues in Wheat Crops (*Triticum aestivum*)

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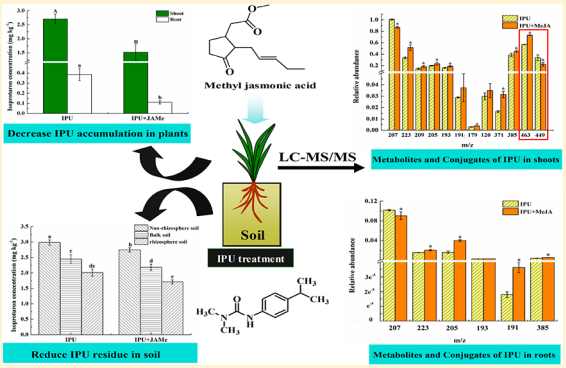
Supporting Information

ABSTRACT: Jasmonic acid (JA) [or methyl-jasmonic acid (MeJA)] is one of the important regulators of plant growth, development, and defense with respect to environmental stresses, but how JA is involved in mediation of pesticide accumulation and degradation in plants is largely unknown. This study investigated the contribution of MeJA to detoxification and degradation of isoproturon (IPU) residues in wheat (*Triticum aestivum*). Wheat plants were exposed to 4 mg of isoproturon kg^{-1} (environmentally realistic concentration). The level of growth and chlorophyll concentration were reduced, while the electrolyte permeability in plants was enhanced. When plants were sprayed with 0.1 μM MeJA, the phytotoxicity induced by isoproturon was significantly assuaged, which was manifested by an increased chlorophyll concentration and a reduced level of cellular damage in wheat. Activities of several stress marker enzymes with isoproturon were repressed in the presence of MeJA. We measured accumulation of isoproturon in wheat and its residues in soil by high-performance liquid chromatography. The concentration of isoproturon in wheat and soils with MeJA was drastically reduced. Using ultraperformance liquid chromatography−tandem mass spectrometry, 12 isoproturon derivatives (eight metabolites and four conjugates) in wheat were characterized. We further provided evidence that the concentration of endogenous MeJA was significantly increased in IPU-exposed plants. These results suggest that MeJA was able to detoxify or degrade isoproturon in wheat when grown in a realistic environmental isoproturon-polluted soil.



INTRODUCTION

Isoproturon, 3-(4-isopropylphenyl)-1,1-dimethylurea, belongs to the phenylurea herbicide family that is widely used for weeding in the field of cereal, cotton, and fruit production.[1] Recently, there has been growing concern about accumulation of isoproturon residues in the environment, which potentially impacts crop production and food safety.[2,3] The leaking of the residual isoproturon from the site of use into the adjacent ecosystems through the surface and groundwater expands the areas of contamination.[4] Because isoproturon is moderately persistent, water-soluble, and weakly absorbed by soils,[5] its residues in environments exert adverse effects on the growth of aquatic organisms and soil microbial communities, leading to high risks to human health.[6−9] As most crops exposed to the isoproturon-contaminated environments can freely absorb isoproturon, some crops such as wheat and rice have become major targets of the food chain in areas where people rely on the grains as staple foods.[5,10] Thus, it is imperative to investigate the toxic impact of isoproturon on plant−soil systems and understand the detoxifying or degradative mechanism in the crops so that we can formulate corresponding countermeasures to lower the levels of food chain contamination of the herbicide.

Jasmonic acid (JA) belongs to one of the cyclopentanone compounds synthesized from linolenic acid and serves as a lipid-derived phytohormone necessary for plant growth and development as well as defense against various biotic and abiotic stresses.[11−13] Methyl jasmonate (MeJA) is the methyl ester form of JA, which is transformed by JA carboxyl methyltransferase and has a role similar to that of JA in plants.[14] JA serves as an elicitor involved in the anti-inflammatory properties of the essential oil of lettuce leaf basil (*Ocimum basilicum* L.) by affecting the yield, chemical composition, and antioxidant.[15] A supply of exogenous JA induces oxidative stress tolerance in tobacco (*Nicotiana tabacum*) exposed to imazapic.[16] Several reports indicated that JA also improves defense against metal stress.[12,17,18] The

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stress-relieving effects of JA imply the enhancement of detoxification of toxicants.[19] Until now, no report about the effect of JA on the degradation of toxic compounds, including pesticides in crops, has been available. Furthermore, the mechanism of JA mediating the degradation and detoxification of toxic herbicides is unknown.

Soil is a nonrenewable resource that performs multiple functions to ensure sustainable agriculture and ecosystems.[20] Soil is also an important matrix providing a platform for degradation of many pollutants. As reported, the processes of sediment- and microbe-based transformation of herbicides can individually or coordinately influence the degradation of residual herbicides.[21] Organic contaminants often degrade more rapidly from planted soils than from the soils without plants.[22,23] Wheat is one of the most important crops, providing an enormous number of calories for humans worldwide. Understanding the mechanism for the isoproturon residues in crops like wheat is critically important to ensure crop production and food safety.[24] In this study, we investigated the detoxification and degradation of isoproturon in wheat crops in the presence of MeJA. Metabolites and degraded products of isoproturon were characterized using ultraperformance liquid chromatography−tandem mass spectrometry (UPLC−MS/MS). The purpose of the study is (1) to understand whether and how MeJA promoted the degradation of isoproturon in wheat and (2) to determine the mechanism for the specific pathway of isoproturon transformation and degradation in wheat as well as in soil.

MATERIALS AND METHODS

Materials and Treatments. Isoproturon (96.9% pure) was provided by the Institute of Pesticide Science, Academy of Agricultural Sciences in Jiang Su, Nanjing, China. Methyl-jasmonic acid was of analytical grade. Isoproturon-free soil collected from the 0−20 cm surface layer at the Experimental Station of Nanjing Agricultural University was manually crumbled, air-dried, ground, and sieved through a 3 mm sieve before use. The major chemical properties were as follows: organic carbon, 2.13%; total N, 1.26 g kg^{-1}; available P, 34.3 mg kg^{-1}; available K, 91.5 mg kg^{-1}; pH 7.6.

Wheat seeds (*Triticum aestivum*, cv. Yangmai 20) were surface-sterilized with a 3% solution of H_2O_2, rinsed, and germinated on moist filter paper for 24 h. After germination, seedlings (20 per pot) were transferred to and grew on the soil in a plastic container (1 L) with 1120 g of dried soils mixed with 4 mg of isoproturon kg^{-1}. To prepare the 100 μM MeJA stock solution, a small amount of ethyl alcohol was added to dissolve the MeJA. When used, a certain amount of the stock solution (100 μM MeJA) was diluted with water. As the working solution contained ethyl alcohol (e.g., 0.1 μM MeJA with 0.1% ethyl alcohol), the control solution was prepared with the corresponding amount of ethyl alcohol only.

When the third true leaf (~1 week) was well-developed, the aerial parts of the plant were sprayed with 0.1 μM MeJA once a day. Meanwhile, control plants were sprayed with a 0.1% water solution of ethyl alcohol. The treatment was repeated daily for 6 days. Seedlings were grown in a chamber under controlled conditions (temperature, 25/20 °C; light/dark cycle, 14/10 h; light intensity, 300 μmol of photons m^{-2} s^{-1}) and watered each day to retain 70% soil moisture. When harvested, shoots and roots of plants were individually sampled and immediately analyzed. Soils that adhered tightly to the root system were collected as rhizosphere soil (RS). Soils shaken off from the roots were collected as nonrhizosphere soil (NRS). All collected rhizosphere and nonrhizosphere soils were considered bulk soils (BS).[25,26]

Determination of Physiological Responses. Elongation of roots and leaves was measured with a ruler. To determine the dry mass, tissue samples were oven-dried at 70 °C for 72 h and weighed. The chlorophyll content in tissues was quantified according to the method of Porra et al.[27] Fresh leaves (0.1 g) were extracted in 8 mL of 80% acetone (pH 7.8), followed by centrifugation at 5000*g* for 10 min. The chlorophyll content was spectrophotometrically measured. To determine the membrane permeability, 0.1 g fresh shoots or roots were cut into a 5 mm length, placed in 10 mL of deionized water, and incubated at 32 °C for 2 h. An electrical conductivity meter (Mettler Toledo FE30-FiveEasy) was used to measure the sample medium (EC_1). After that, the sample was boiled at 100 °C for 20 min and cooled to 25 °C immediately. The second conductivity of the dead tissue extracts (EC_2) was measured again. The electrolyte leakage (EL) was calculated by the formula $EL = EC_1/EC_2 \times 100$.[10]

Thiobarbituric acid reactive substances (TBARS) were assessed following the method described previously.[28] Briefly, 0.5 g fresh tissues were ground and dissolved in 3 mL of a 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 added with 2 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA. After being heated in boiling water for 30 min, the mixture was cooled on an ice bath and centrifuged at 15000 *g* for 5 min. The amount of TBARS was calculated from the difference in absorbance of the supernatant at 532 and 600 nm using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Assays of Enzyme Activities in Plant Tissues. Fresh tissues (shoot or root, 0.3 g) were ground and homogenized in 1.5 mL of ice-cold sodium phosphate buffer (50 mM, pH 7.8) that consisted of 1.0% (w/v) polyvinylpyrrolidone and 0.1 mM EDTA. The homogenate was centrifuged at 12000*g* and 4 °C for 20 min. The supernatant was taken as the crude extract used to assay of enzyme activities.[21]

Catalase (CAT, EC 1.11.1.6) activity was determined with the method of Yin et al.[29] The reaction mixture contained potassium phosphate buffer (100 mM, pH 7.0), H_2O_2 (15 mM), and enzyme extract (50 μL). Activity was calculated using an extinction coefficient of 0.036 mM^{-1} cm^{-1} by the consumption of H_2O_2 at 240 nm. Peroxidase (POD, EC 1.11.1.7) activity was assayed by the change in absorbance at 470 nm because of guaiacol oxidation. The reaction solution included potassium phosphate buffer (100 mM, pH 7.0), guaiacol (20 mM), H_2O_2 (10 mM), and enzyme extract (50 μL).[30] Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by monitoring the decrease in the absorbance at 290 nm of ascorbate.[29] The reaction mixture contained sodium phosphate buffer (50 mM, pH 7.6), ascorbic acid (0.25 mM), H_2O_2 (0.1 mM), and enzyme extract (100 μL). APX activity was calculated by using the extinction coefficient (2.8 mM^{-1} cm^{-1}). Glutathione *S*-transferase (GST, EC 2.5.1.18) activity was measured by monitoring the increase in absorbance at 340 nm. The mixture included potassium phosphate buffer (100 mM, pH 7.4), 1-chloro-2,4-dinitrobenzene (30 mM), glutathione (GSH) (3.3 mM), and enzyme extract (100 μL). GST activity was calculated by using the extinction coefficient (10 mM^{-1} cm^{-1}).[31] Polyphenol oxidase (PPO, EC 1.10.3.l) activity was estimated with the following mixture: potassium phosphate buffer (100 mM, pH 6.5) containing 10 mM L-3,4-dihydroxyphenylalanine and enzyme extract (300 μL). One unit of PPO activity was defined using the extinction coefficient (1 mg^{-1} cm^{-1}) at 475 nm.[32]

Analysis of Isoproturon and Its Derivatives. Isoproturon and its metabolites (or derivatives) in the wheat tissues were analyzed with the following method.[5] After being ground using liquid nitrogen, the sample powder (4 g shoot or root) and 10 g of soil were extracted ultrasonically in 10 mL of an acetone/water mixture [3:1 (v/v)] for 30 min and centrifuged at 4000*g* for 8 min. The extraction procedure was repeated in triplicate. The supernatant was collected in a flask and concentrated to remove acetone in a vacuum rotary evaporator at 40 °C. The residual water was loaded onto an LC-C_{18} solid phase extraction (SPE) column. The elute was discarded. The column was washed with 2 mL of methanol and collected for high-performance liquid chromatography (HPLC) analysis and UPLC-TOF−MS/MS. The spiked recoveries of IPU from the soil and plant are listed in Table S1. The content of isoproturon (IPU) was determined by HPLC (Waters 515, Waters Technologies Co. Ltd.) with an ultraviolet detector under the following conditions: Hypersil reversed

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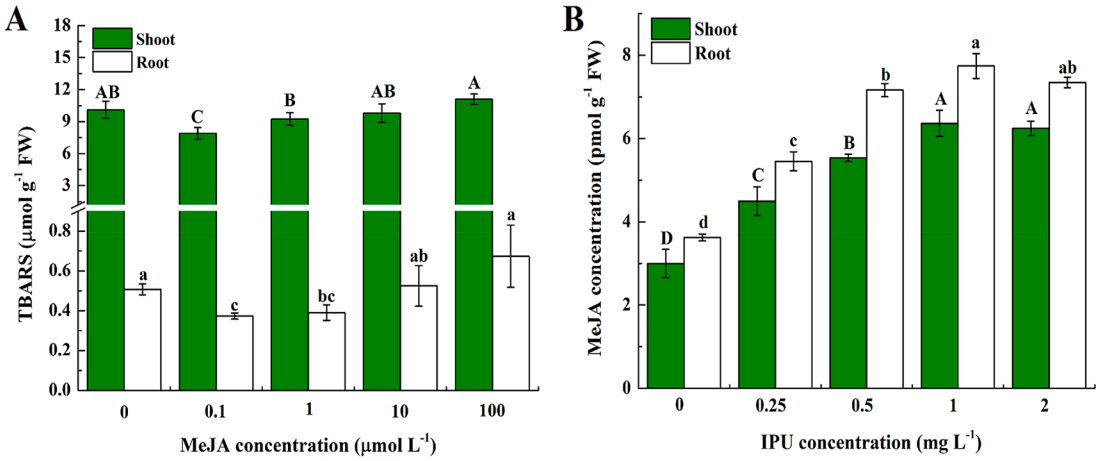

Figure 1. Effect of MeJA on TBARS concentrations and IPU on MeJA concentrations in wheat. (A) One-week-old wheat growing in the soil with 4 mg of isoproturon kg^{-1} was treated with MeJA (0, 0.1, 1, 10, and 100 μM MeJA spray) for 6 days. (B) One-week-old wheat growing in a 0, 0.25, 0.5, 1, and 2 mg L^{-1} nutrient solution for 72 h. Roots and shoots of plants were separately harvested and analyzed. Values are means ± standard deviations (n = 3). Data denoted with different letters are significantly different (p < 0.05).

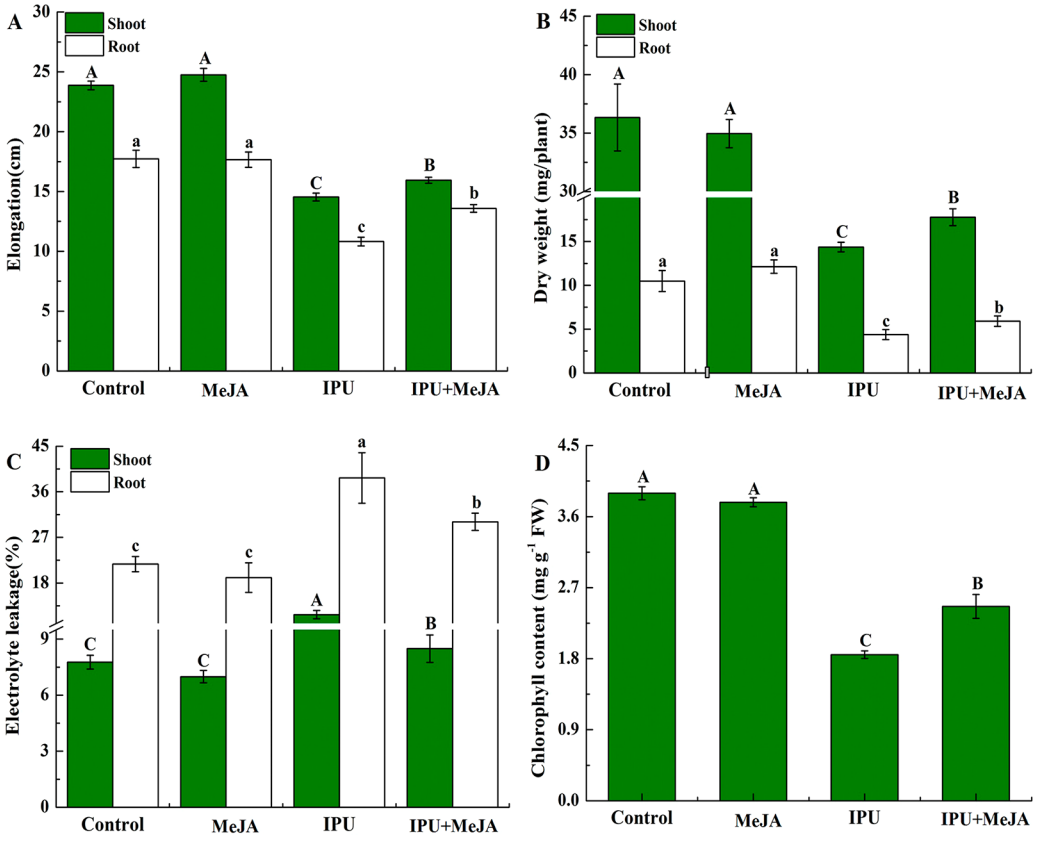

Figure 2. Effects of IPU and/or MeJA on (A) elongation, (B) dry mass, (C) electrolyte content, and (D) chlorophyll content of wheat. Seedlings grew in soils with IPU (4 mg kg^{-1}) and without IPU (CK, control) for 4 days. After that, the leaves were sprayed with 0.1 μmol L^{-1} MeJA once a day for 6 days. Values are means ± standard deviations (n = 3). Data denoted with different letters are significantly different (p < 0.05).

phase C18 column [Thermo, 250 mm × 4.6 mm (inside diameter)]; mobile phase, methanol/water [68:32 (v/v)]; wavelength, 241 nm; flow rate, 0.6 mL/min; injection volume, 20 μL.

Characterization of IPU Derivatives and IPU-Conjugated Products. Liquid chromatography−mass spectrometry (LC−MS) analysis of plant extracts was performed on a Shimadzu LC 20ADXR LC system in-line with an AB SCIEX Triple TOF 5600 mass spectrometer-equipped Accelerator TOF Analyzer and electrospray ionization source. The autosampler temperature was set at 4 °C. The injection volume was set as 20 μL. Separations were performed on a Poroshell 120 EC-C18 column (50 mm × 2.1 mm, 2.7 μm, Thermo Fisher Scientific). The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), and the flow rate was 0.3 mL/min. The elution steps were set as follows: (1) 5% B for 1 min, 5 to 15% B from 1 to 5 min, 15 to 35% B from 5 to 20 min, 35 to 100% B from 20 to 22 min, and 100% B for 3 min, (2) back to the initial conditions, and (3) equilibration for 1 min before the next sample injection. TOF-MS parameters were as follows: ion source gas 1, 65 psi; ion source gas 2, 65 psi; curtain gas, 30 psi; source temperature, 550 °C; ion spray voltage floating, 5500 V.

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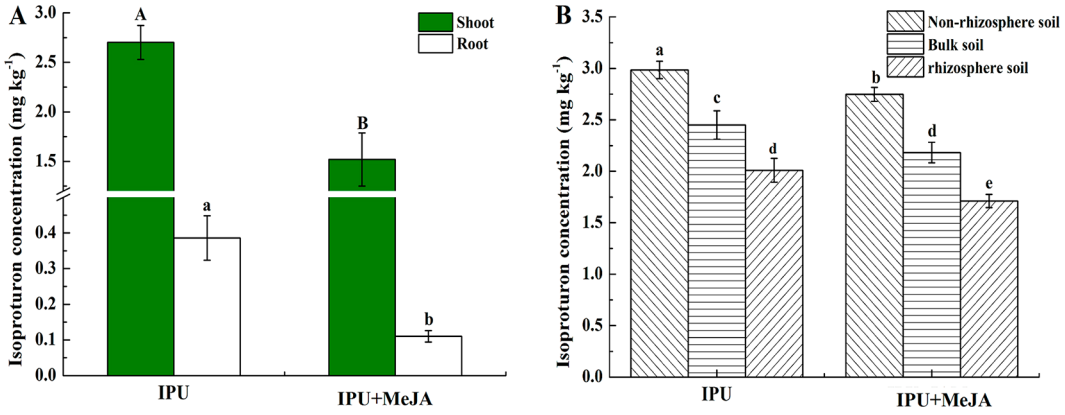

Figure 3. Accumulation of IPU in shoots and roots of wheat (A) and rhizosphere soil (RS), bulk soil (BS, whole soil including rhizosphere and nonrhizosphere), and nonrhizosphere soil (NRS) (B) in different treatments. Seedlings grew in soils with IPU (4 mg kg^{-1}) and without IPU (CK, control) for 4 days. After that, the leaves were sprayed with 0.1 μmol L^{-1} MeJA once a day for 6 days. Values are means ± standard deviations (n = 3). Data denoted with different letters are significantly different ($p < 0.05$).

The mass spectrometer was operated in positive product ion mode. External mass calibration with the AB SCIEX Triple TOF systems was performed once a week to ensure a working mass accuracy of <5 ppm. Data were processed with PeakView software and MultiQuant software.

Determination of Endogenous MeJA in Plants. The assay of internal MeJM was conducted using the specific methyl jasmonate (meja) enzyme-linked immunoassay (ELISA) kit with the double antibody sandwich method (http://www.jonln.com/). Briefly, the MeJA extract from plants captures the antibody and encapsulates the antibody onto the micropore plate to make the solid phase antibody. Then, the sample MeJA was added to the encapsulated micropore and combined with the labeled antibody to form the antibody antigen−enzyme-labeled antibody complex. After a thorough washing, the substrate TMB was added and colored. The color is positively correlated with the content of MeJA. The absorbance was determined at 450 nm, and the content of MeJA was calculated with a standard curve.

Statistical Analysis. Each result shown in the figures is the mean of three biological replicates. The values are expressed as means ± the standard deviation. The data between differently treated groups were compared statistically by analysis of variance (ANOVA) followed by the least significant difference (LSD) test if the ANOVA result is significant at the $p < 0.05$ level.[33] All data were calculated using the model procedure in SPSS 20.

RESULTS AND DISCUSSION

MeJA Improved the Growth of Wheat under Isoproturon Stress. To examine whether MeJA had a role in reducing the toxicity of isoproturon to wheat, we first determined the toxic response of plants by quantifying lipid peroxidation, in terms of TBARS, a biomarker widely used to evaluate the degree of injury in plants.[29,34,35] Wheat plants were grown in the soil with 4 mg of isoproturon kg^{-1}. MeJA at 0, 0.1, 1, 10, and 100 μM was sprayed on the aerial parts of wheat. The treated samples were analyzed. As shown in Figure 1A, compared to the control, the levels of TBARS in the roots and shoots were significantly lower with 0.1−1 and 0.1−10 μM MeJA, respectively. A higher level of MeJA supply had no effect on the generation of TBARS, indicating that 0.1−1 μM MeJA was effective in alleviating isoproturon-induced toxicity in wheats. Because 0.1 μM MeJA was sufficient to reduce the stress, that concentration of MeJA was employed in the following studies.

If MeJA would work against IPU-induced toxicity, there might be a physiological interaction between MeJA and IPU. To address the question, we examined the effect of IPU on endogenous MeJA in wheat. One-week-old plants were exposed to IPU at concentrations of 0, 0.25, 0.5, 1, and 2 mg L^{-1} for 72 h. Root and shoot tissues were harvested for MeJA quantification using an ELISA. Our analysis showed that the concentrations of endogenous MeJA were progressively enhanced when wheat plants were exposed to 0.25−2 mg L^{-1} IPU (Figure 1B). At 1 mg L^{-1} IPU, the roots and shoots had MeJA concentrations 2-fold higher than that of the control. A similar result was detected in plants exposed to IPU for 24 and 48 h (Figure S1).

We then examined the effect of isoproturon on growth and physiological responses such as biomass, chlorophyll content, and electrolyte permeability of wheat in the presence of MeJA. Compared to the control (without isoproturon or MeJA), treatment of wheat with isoproturon reduced the elongation of shoots and roots by 38.6 and 39.0%, respectively (Figure 2A). While under the same condition the dry weight and chlorophyll concentration of wheat significantly decreased, the electrolyte permeability drastically increased (Figure 2B−D). Supplying MeJA in the absence of isoproturon affected neither the growth nor the electrolyte permeability of wheat. However, when exposed to isoproturon in the presence of MeJA, the wheat plants showed improved growth but inhibited electrolyte permeability. For example, isoproturon-inhibited shoot and root elongation improved by 9.71 and 16.33%, respectively (Figure 2A). Similarly, the chlorophyll concentration with isoproturon was decreased compared to that of the control but increased 33.0% when plants were concomitantly treated with MeJA (Figure 2C). For electrolyte leakage, treatment with a MeJA spray attenuated the isoproturon-induced stress by 27.80 and 22.34% in shoots and roots, respectively (Figure 2D).

MeJA Reduced the Level of Accumulation of Isoproturon in Wheat and Soil. To investigate how MeJA alleviated the toxicity of isoproturon in wheat, the residual isoproturon in wheat with or without MeJA application was assessed. In the absence of MeJA, 6.5-fold higher levels of isoproturon residues accumulated in wheat shoots than in roots (Figure 3A). Such a model of isoproturon location in wheat plants could be governed by genetic traits of wheat such as its transport efficiency, physiological statue, and degrading capacity as well as the properties of herbicides.[5] The level of accumulation of isoproturon in wheat was significantly

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decreased by addition of MeJA (Figure 3A), suggesting that MeJA could mediate the accumulation of isoproturon in the plant.

The bioconcentration factor (BCF) is the ratio of pesticide concentration in plants to the bulk soil and is frequently used to evaluate the ability of a pesticide to be transported from the soil to roots or other plant tissues.[36] Our analysis showed that BCFs of shoots and roots were reduced from 1.11 to 0.70 and from 0.15 to 0.05, respectively, following MeJA foliar supply (Table 1), indicating that MeJA was able to prevent isoproturon from moving from the soil to roots and shoots of wheat. The translocation factor (TF), expressed as the pesticide concentration ratio of the shoot BCF to the root BCF, is another parameter of interest reflecting pesticide translocation in plants.[37] The TF values increased in the following order: isoproturon treatment alone < isoproturon with MeJA (Table 1). Addition of MeJA obviously enhanced the capacity to translocate isoproturon from roots to shoots. The possible mechanism responsible for the lower level of JA-mediated accumulation of isoproturon in wheat might be the translocation of the herbicide from roots to shoots. Because the uptake and accumulation of isoproturon in wheat were closely associated with the availability of isoproturon in soil, the residues of isoproturon in RS, BS, and NRS were determined by chromatography. The rhizosphere soil always contained fewer isoproturon residues than the bulk soil and nonrhizosphere soil did (Figure 3B). The levels of residues of isoproturon in RS, BS, and NRS in the presence of MeJA were lower than those in wheat without MeJA treatment. Supplying MeJA could contribute to the removal of isoproturon in the soil where wheat was planted. Most of plants or crops benefit from the ability to remove herbicides by absorbing them from soil through roots and detoxifying them via complicated metabolic pathways.[22] They may clean up herbicide residues by coordinating microorganisms.[38,39] In this regard, microbial activities play a pivotal role in degrading the herbicide residues in the rhizosphere.[26] Our analysis showed that the rhizosphere had lower levels of residues of the herbicide than the nonrhizosphere, suggesting that wheat plants would contribute to the uptake of isoproturon into plants and degradation in the soil. These observations allowed us to assume that the enhanced removal of isoproturon by MeJA may be involved in the following processes. (a) The rhizosphere was close to plant roots where many more microorganisms were activated.[40] Plant roots secrete metabolites such as low-molecular weight organic acids, amino acids, enzymes, and aliphatics, all of which would flourish in specific microbial populations or communities.[41] (b) Wheat cultivation possibly fostered the microbial population and led to isoproturon degradation more efficiently in the rhizosphere over the nonplant cultivation. However, further investigation will be required to clarify the assumptions.

Table 1. Bioconcentration Factors (BCFs) and Translocation Factors (TFs) for IPU in Shoots and Roots of Wheat[a]

	BCF[b]		
treatment	shoot	root	TF[c]
IPU	1.11 ± 0.098	0.15 ± 0.031	7.40 ± 0.745
IPU with MeJA	0.70 ± 0.047*	0.05 ± 0.001*	13.82 ± 0.965*

[a]Concentrations of IPU were determined in shoots and roots. Seedlings grew in soils with IPU (4 mg kg^{-1}) and without IPU (control) for 4 days. After that, the leaves were sprayed with 0.1 μmol L^{-1} MeJA once a day for 6 days. Values are means ± standard deviations (n = 3). Asterisks indicate significant differences between treatments ($p < 0.05$). [b]BCF is the fresh weight ratio of IPU in plants to the bulk soil. [c]TF is the ratio of shoot BCF to root BCF.

MeJA-Regulated Activities of Stress Marker Enzymes under Isoproturon Stress. Previous studies have shown that xenobiotics can trigger a burst of reactive oxygen species (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) and consequently resulted in oxidative stress.[2,29,42] The abundance of ROS is mediated by a group of antioxidative enzymes.[43] In this study, several enzymes, including peroxidase (POD, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.1), glutathione *S*-transferase (GST, EC 2.5.1.18), and polyphenol oxidase (PPO, EC 1.10.3.1), were assessed in the presence of isoproturon and/or MeJA. POD, APX, and CAT catalyze the conversion of H_2O_2 to H_2O and O_2 using different substrates and thus remove H_2O_2. For example, APX uses ascorbate as an electron donor to remove H_2O_2.[43] GST catalyzes the conjugation of toxicants and glutathione (GSH) through nucleophilic addition of GSH to electrophilic centers in the organic molecules and can help compartmentalize the complexes into subcellular organelles like vacuoles for deep degradation. It is therefore believed to be one of the potent detoxifying enzymes.[44] PPO basically catalyzes O-hydroxylation of monophenol molecules in which the benzene ring carries a single hydroxyl substituent to *O*-diphenols (phenol molecules containing two hydroxyl substituents) and catalyzes the oxidation of *O*-diphenols to produce *O*-quinones, as well. Compared to the control (IPU-free), treatment with IPU induced activities of the enzymes, although their activities were changed differently (Figure 4). In the presence of MeJA, the activities of all enzymes with isoproturon were shown to be lower (Figure 4). Treatment with MeJA reduced the GST activities in roots and shoots by 22.7 and 75.1%, respectively, relative to the control (isoproturon treatment only). The MeJA-mediated decrease in enzyme activity suggests that the isoproturon-responsive stress was attenuated.

Analysis of Isoproturon Metabolites and Conjugates in Wheat. To investigate the putative pathways of isoproturon degradation in wheat, the metabolites and conjugates of isoproturon in plants exposed to isoproturon and/or MeJA were characterized using an ultraperformance liquid chromatography AB SCIEX Triple TOF 5600 mass spectrometer (UPLC-Triple TOF 5600). The isoproturon-free treatment or treatment with MeJA alone was set as a control. The mass spectrometric data of isoproturon derivatives, including metabolites and conjugates, in wheat are summarized in Table 2. The structures of isoproturon derivatives were determined by analyzing MS^2 data generated from collision-induced dissociation (CID) fragmentation patterns (Figures S2 and S3).

The extracted MS^2 spectra of IPU (m/z 207) occurred with a retention time of 14.99 min (Table 2). Fragmentation of IPU led to a main product ion at m/z 134 that was generated by the loss of two methyl groups and a -N$(CH_3)_2$ group, and further loss of an acylamino generated the fragment ion at m/z 91 (Figure S2A and Table 2). The $[M + H]^+$ ion at m/z 193 with a retention time of 13.33 min generated the fragment ion at m/z 94 via the cleavage of isopropyl and CH_3-NH-CO-, which was identified as monodemethyl-IPU, the N-demethylation

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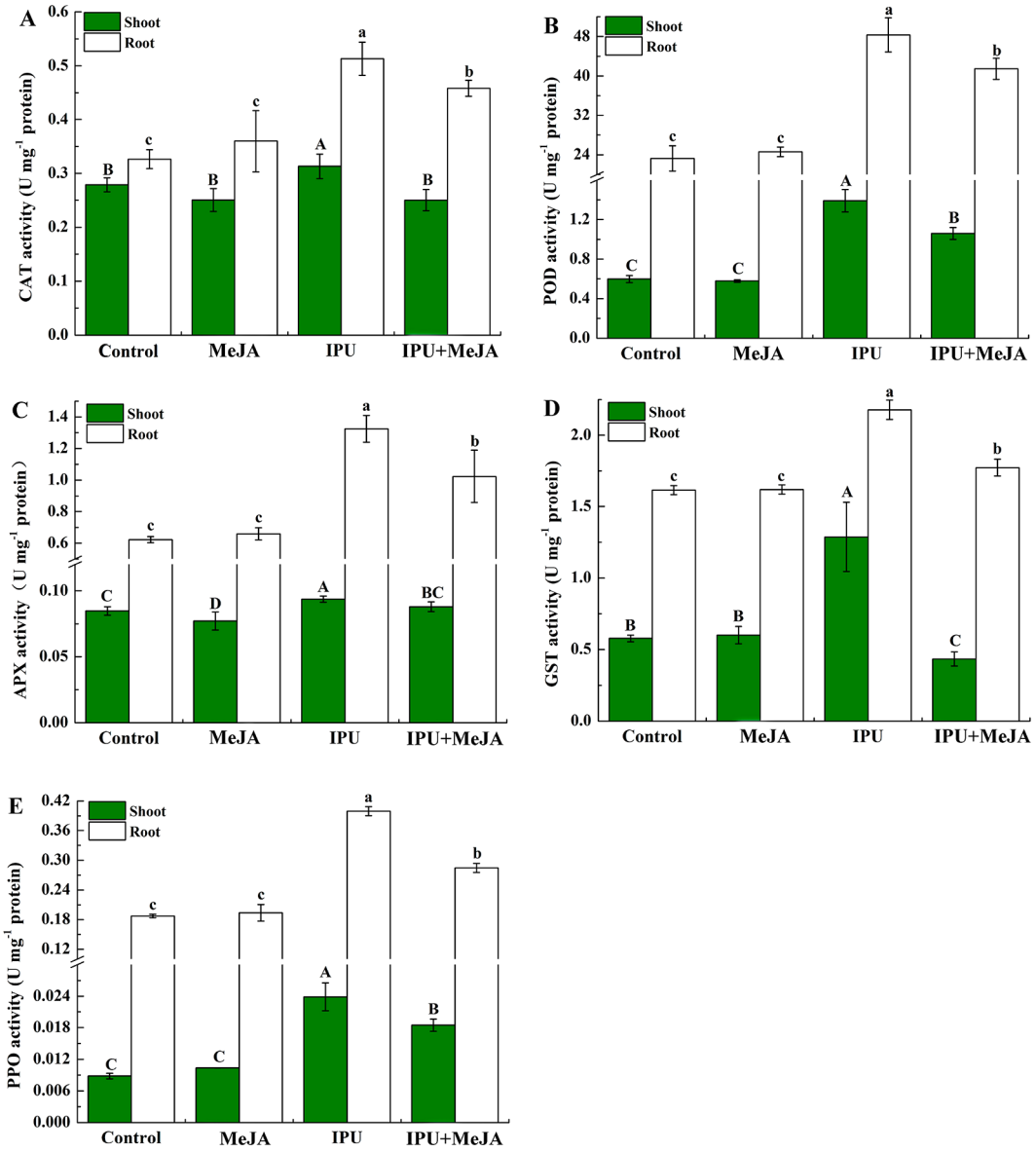

Figure 4. Effect of IPU and/or MeJA on (A) catalase (CAT), (B) peroxidase (POD), (C) ascorbate peroxidase (APX), (D) glutathione *S*-transferase (GST), and (E) polyphenol oxidase (PPO) activities in shoots and roots of wheat. Seedlings grew in soils with IPU (4 mg kg^{-1}) and without IPU (CK, control) for 4 days. After that, the leaves were sprayed with 0.1 μmol L^{-1} MeJA once a day for 6 days. Values are means ± standard deviations (n = 3). Data denoted with different letters are significantly different (p < 0.05).

product (Figure S2B and Table 2). The precursor ion at m/z 179 peaking at 11.71 min with the fragment ions at m/z 94 and 136, which were generated by the elimination of acylamino and further loss of -C_3H_7, respectively, was identified as didemethyl-IPU (Figure S2C and Table 2).

The precursor ion at m/z 223 (t_R = 5.94 min) yielded characteristic fragment ions at m/z 72 and 59, which were $C_3H_6NCO^+$ and $C_3H_7O^+$, respectively, indicating that this metabolite was 2-OH-isopropyl-IPU (Figure S2D and Table 2). Another hydroxyled metabolite peaked at 4.56 min. Its precursor ion at m/z 209 generated the characteristic fragment ions at m/z 94 and 59, which were positively charged aniline and $C_3H_7O^+$, respectively, indicating that the metabolite was 2-OH-monodemethyl-IPU (Figure S2E and Table 2).

The precursor ion at m/z 205 (t_R = 13.59 min) generated two main fragment ions at m/z 105 and 72, which were positively charged ethylbenzene and $C_3H_6NCO^+$, respectively. Thus, this metabolite was identified as isopropenyl-IPU (Figure S2F and Table 2). The precursor ion at m/z 191 (t_R = 11.98 min) formed a fragment ion at m/z 77, being positively charged benzene, and another main fragment ion at m/z 106 was produced by the breakage of the double bond and the loss of C_3H_6NCO-. Thus, the metabolite pointed to demethyl-isopropenyl-IPU (Figure S2G and Table 2). There was a low-molecular weight metabolite from the degraded products with a precursor ion at m/z 120, giving ions at m/z 91 (positively charged benzyl) and m/z 77 (positively charged benzene). On the basis of the analysis, the final IPU-degraded product was 4-vinylaniline (Figure S2H and Table 2).

Four isoproturon conjugates, including two GSH *S*-conjugates and two glycosylated conjugates, were identified (Figure S3 and Table 2). The precursor ion at m/z 371 (t_R = 4.58 min) generated main fragment ions at m/z 103, 134, and 162. The ions at m/z 103 and 134 were formed by the loss of a

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Table 2. Summary of All Mass Spectrometer Data for Metabolites and Conjugates of IPU in Wheat

	metabolite or IPU derivative	t_R[a] (min)	calcd m/z[b]	exptl m/z[b]	chemical formula	error (ppm)	location[c]	MS^2 m/z precursor ion	MS^2 m/z main fragment ions
1	2-OH-isopropyl-IPU	5.94	223.1402	223.1400	$C_{12}H_{18}N_2O_2$	−0.860	shoot, root	223	59, 72, 132
2	2-OH-monodemethyl-IPU	4.56	209.1245	209.1247	$C_{11}H_{16}N_2O_2$	0.929	shoot	209	59, 77, 94
3	isopropenyl-IPU	13.59	205.1296	205.1295	$C_{12}H_{16}N_2O$	−0.435	shoot, root	205	72, 105
4	monodemethyl-IPU	13.33	193.1296	193.1298	$C_{11}H_{16}N_2O$	1.036	shoot, root	193	58, 77, 94
5	demethyl-isopropenyl-IPU	11.98	191.1140	191.1139	$C_{11}H_{14}N_2O$	−0.208	shoot, root	191	58, 77, 94, 106
6	didesmethyl-IPU	11.71	179.1140	179.1142	$C_{10}H_{14}N_2O$	0.919	shoot	179	77, 94, 136
7	4-vinylaniline	0.88	120.0769	120.0768	C_8H_9N	−0.875	shoot	120	77, 91
8	isoproturon (IPU)	14.99	207.1453	207.1454	$C_{12}H_{18}N_2O$	0.561	shoot, root	207	77, 91, 134
1	2-OH-monodemethyl-IPU *O*-glucoside	4.58	371.1774	371.1783	$C_{17}H_{26}N_2O_7$	2.625	shoot	371	60, 103, 134, 162
2	2-OH-IPU *O*-glucoside	5.72	385.1930	385.1931	$C_{18}H_{28}N_2O_7$	0.088	shoot, root	385	72, 205, 223
3[d]	GSH *S*-deisopropyl-didesmethyl-IPU	5.87	449.1107	449.1106	$C_{17}H_{22}N_4O_7S$	−0.089	shoot	449 (+Na)	299, 329
4[d]	GSH *S*-deisopropyl-monodemethyl-IPU	7.27	463.1264	463.1265	$C_{18}H_{24}N_4O_7S$	0.147	shoot	463 (+Na)	298, 313, 343

[a]Retention time. [b]Ion modes are $[M + H]^+$ or $[M + Na]^+$. [c]Distribution of metabolites of isoproturon in the plant. [d]Compounds that have been reported for the first time.

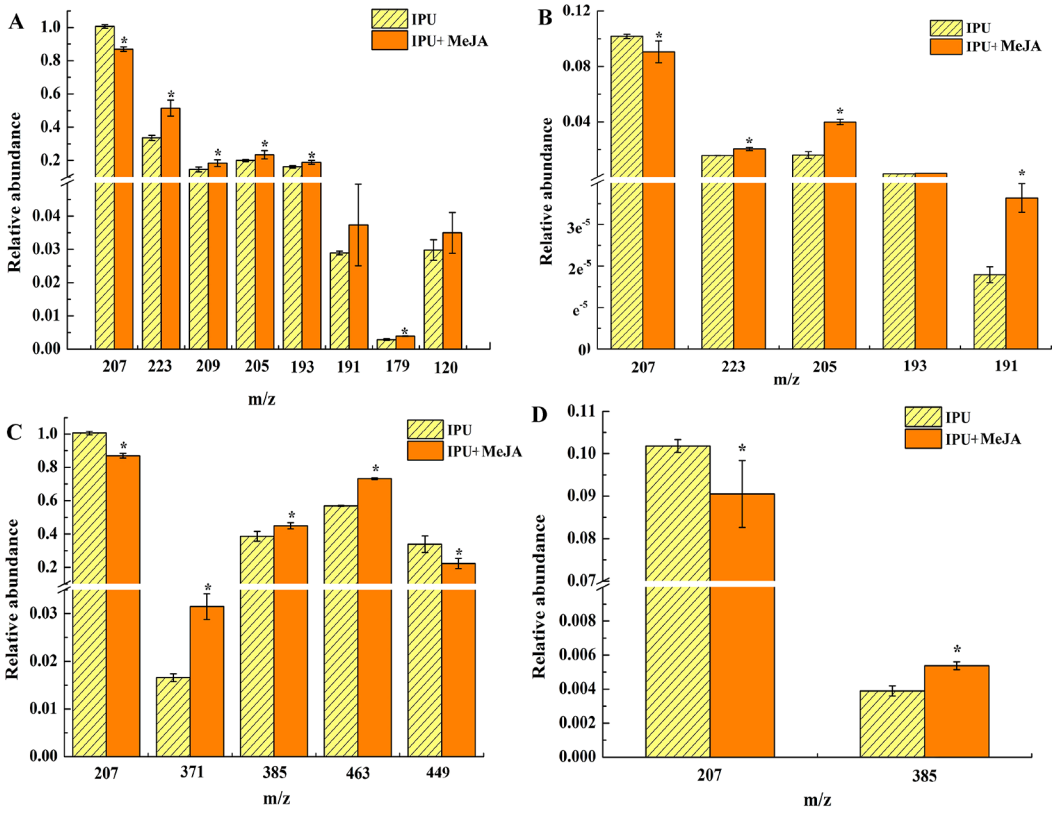

Figure 5. IPU-derived degradation products in (A) shoots and (B) roots and conjugates of IPU in (C) shoots and (D) roots extracted from samples from wheat treated with IPU for 10 days under MeJA treatment or no treatment. Seedlings grew in soils with IPU (4 mg kg^{-1}) and without IPU (CK, control) for 4 days. After that, the leaves were sprayed with 0.1 μmol L^{-1} MeJA once a day for 6 days. Values are means ± standard deviations (n = 3). Asterisks indicate the significant difference between the treatments with IPU and MeJA and treatments with IPU ($p < 0.05$).

glucose moiety, and the ion at m/z 162 was produced by the loss of glucose and two methyl groups. It turned out to be 2-OH-monodemethyl-IPU *O*-glucoside (Figure S3A and Table 2). The precursor ion at m/z 385 (t_R = 5.72 min) formed the fragment ions at m/z 72, 205, and 223. The ion at m/z 72 was produced by the elimination of -$CON(CH_3)_2$, and the ion at m/z 223 was formed by the loss of glucose and isopropyl. Thus, it was assigned to 2-OH-IPU *O*-glucoside (Figure S3B and Table 2). Two GSH *S*-conjugates peaked at 5.87 and 7.27 min with $[M + Na]^+$ ions at m/z 449 and 463, respectively (Figure S3C,D and Table 2). The precursor ion at m/z 449 was identified by detecting the fragment ion at m/z 329 formed by the loss of an amino and a carboxyl, indicating this metabolite was GSH-*S*-deisopropyl-didesmethyl-IPU (Figure S3C and Table 2). The other GSH *S*-conjugate with a $[M + Na]^+$ ion at m/z 463 was identified by fragment ions at m/z 294 and 343. The ion at m/z 294 was due to the removal of an amino, while the ion at m/z 343 was generated by the loss of

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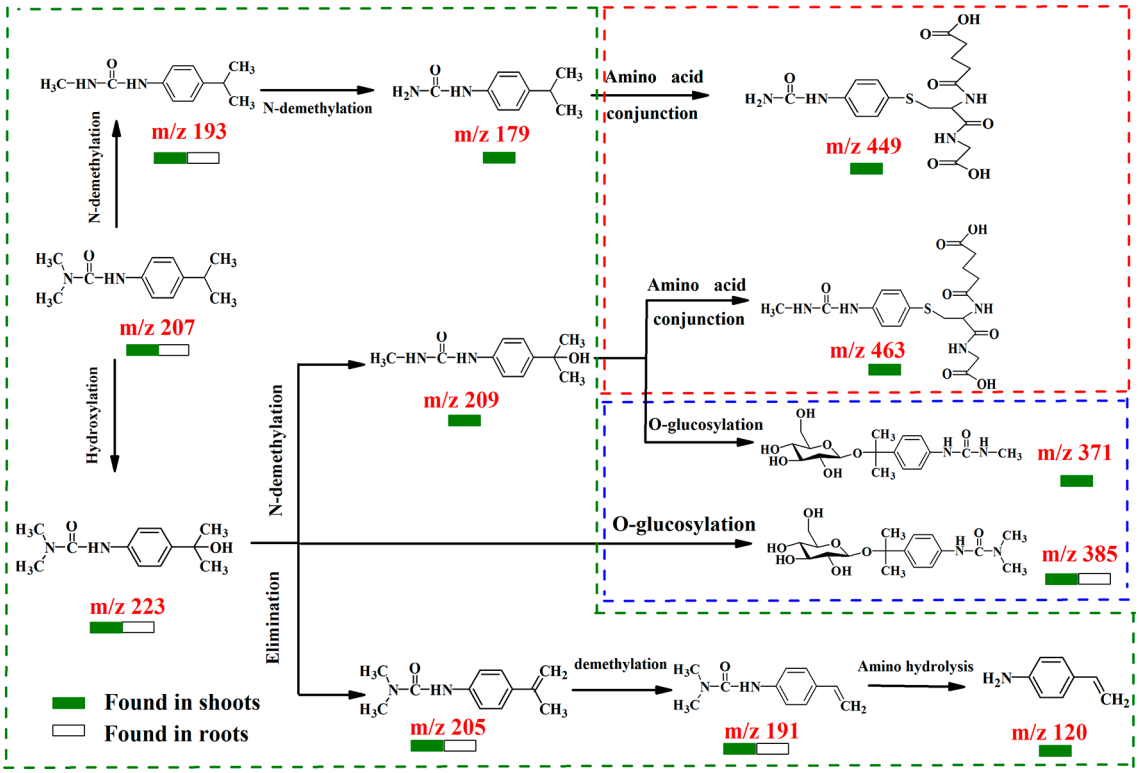

Figure 6. Proposed metabolic pathway of IPU in wheat exposed to IPU and/or MeJA. The contents in the green box are metabolized from IPU. The substances in the red box are conjugates conjugated with an amino acid of IPU. The contents in the blue box are conjugates conjugated via glycosylation of IPU.

-CONHCH$_3$ and -COOH (Figure S3D and Table 2). The two GSH *S*-conjugates were reported for the first time.

Effect of MeJA on Degradation of Isoproturon in Wheat. To identify the role of MeJA in degradation and conjugation of isoproturon, the relative accumulation of isoproturon and its derivatives in terms of intensity was measured using UPLC−MS. All 12 derivatives were detected in shoots, while six derivatives were examined in roots (Figure 5A,B). The isoproturon concentration in tissues after MeJA spraying was significantly decreased compared to that under non-MeJA treatment. With regard to metabolites, most of them had a significantly higher intensity in the presence of MeJA, except two metabolites in shoots (m/z 191 and 120) and one metabolite in roots (m/z 193). Nevertheless, all conjugations had significant differences between treatments with isoproturon with MeJA and treatments with isoproturon. For instance, the concentration of 2-OH-monodemethyl-IPU *O*-glucoside (m/z 371) was increased significantly under MeJA treatment in wheat shoots (Figure 5C). In addition, the concentration of 2-OH-IPU *O*-glucoside (m/z 385) was also found to be affected by MeJA significantly in wheat roots (Figure 5D). These results suggest that MeJA could foster IPU transformation to various derivatives and thereby contribute to the detoxification of IPU in wheat.

Plants (or crops) have developed sophisticated strategies for metabolizing a diverse variety of xenobiotics, including pesticides. Understanding the mechanism of isoproturon transformation during the course of degradation is critical. Mastering this topic would help in the development of strategies for minimizing the environmental risks of crop production and human health.[45] Herbicides can be detoxified through three different routes in plants.[46] One of the commonly observed routes of herbicide detoxification is transformation into other molecules through hydroxylation and N-demethylation. In this study, two dealkylated derivatives, two hydroxyled derivatives, and three dehydrated derivatives were characterized (Table 2). Another group of herbicides is detoxified through the conjugation of the activated herbicides with polar donor molecules, such as glutathione, sugars, and amino acids, which are mediated by glycosyltransferases and glutathione *S*-transferases.[10] Following conjugation, the modified products are transferred into certain organelles (e.g., vacuole or peroxisome) for further catabolism. Two derivatives were found to be of the GSH type, and two others are of the glycoside type in this study. These GSH *S*-conjugates were first reported in wheat.

To demonstrate the degrading pathways of isoproturon in wheat, we summarized the derivatives of isoproturon based on the LC−MS/MS data sets. As shown in Figure 6, didemethyl-IPU and monodemethyl-IPU were among the two products of N-demethylation, which was the main metabolic pathway of phenylurea herbicides in microbial systems reported previously.[24] Moreover, the transformed product 2-OH-isopropyl-IPU (m/z 223) was generated by hydroxylation of isoproturon. Among the derivatives, 2-OH-isopropyl-IPU seemed to be one of the most important metabolites, which generated 2-OH-monodemethyl-IPU (m/z 209) by N-demethylation and isopropenyl-IPU (m/z 205) by dehydration. Furthermore, the demethylation and subsequent amide hydrolysis of isopropenyl-IPU generated demethyl-isopropenyl-IPU (m/z 191) and 4-vinylaniline (m/z 120), respectively (Figure 6). Organic pollutants conjugated with xenobiotics, such as glucosyl, malonyl-glucosyl, and acetyl-glucosyl moieties and glutathione, are considered as a way to detoxify pollutants by

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increasing their solubility in cells.[47−49] In this study, the hydroxylated product 2-OH-isopropyl-IPU conjugated a glucose to generate 2-OH-IPU *O*-glucoside (*m*/*z* 385). Similarly, 2-OH-monodemethyl-IPU *O*-glucoside (*m*/*z* 371) was generated by glycosylation of 2-OH-monodemethyl-IPU. In addition, 2-OH-monodemethyl-IPU conjugated GSH to form GSH-*S*-deisopropyl-monodemethyl-IPU (*m*/*z* 463) along with the generation of isopropanol. GSH-*S*-deisopropyl-didemethyl-IPU, the other GSH *S*-conjugate, was generated by conjugation of didemethyl-IPU with GSH along with the loss of propane. Among the conjugates, only 2-OH-IPU *O*-glucoside was detected in both shoots and roots (Figure 6). From the analyses, the MeJA-inhibited isoproturon accumulation in wheat was not attributed to the prevention of isoproturon intake but rather to the simulative degradation by MeJA caused by more isoproturon derivatives being detected in wheat (Figure 5). Apparently, MeJA is able to facilitate the removal of isoproturon in wheat through a putative detoxification pathway to attenuate the isoproturon-induced cellular toxicity.

CONCLUSIONS

A foliar supply of MeJA was able to reduce the level of accumulation of isoproturon in wheat. Consistent with that finding, the growth responses, manifested in elongation, biomass, and total chlorophyll accumulation, were increased, whereas the membrane permeability was decreased by the MeJA treatment under IPU stress. The BCF of wheat shoots and roots showed a downward trend, whereas the TF values increased in the presence of MeJA, showing a translocation of isopuroturon from roots to shoots. Additionally, the activities of CAT, POD, APX, GST, and PPO with MeJA were relatively lower, indicating that the degree of oxidative stress was reduced. Moreover, a supply of MeJA led to a higher level of derivatives of isoproturon in wheat, suggesting that MeJA was involved in transformation and reduced the level of isoproturon accumulation. Overall, our analyses demonstrate that MeJA can exert a beneficial effect on enhancement of isoproturon degradation in wheat.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.8b00100.

Effect of different concentrations of IPU on the endogenous methyl jasmonate (MeJA) in wheat for 24 and 48 h (Figure S1), eight metabolites that were identified on the basis of accurate MS data and appropriate fragmentation patterns from MS^2 data (Figure S2), four conjugates identified on the basis of accurate MS data and appropriate fragmentation patterns from MS^2 data (Figure S3), and spiked recoveries of isoproturon in soil, wheat shoots, and wheat roots (Table S1) (PDF)

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Notes

The authors declare no competing financial interest.

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