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Chem. Res. Toxicol., **Just Accepted Manuscript** • DOI: 10.1021/acs.chemrestox.8b00100 • Publication Date (Web): 03 Jul 2018

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Jasmonic acids facilitate the degradation and detoxification of herbicide isoproturon residues in wheat crops (*Triticum aestivum*)

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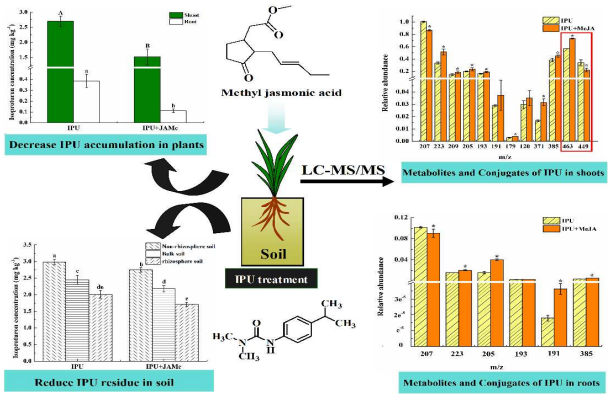
Methyl jasmonic acid
Decrease IPU accumulation in plants
LC-MS/MS
Metabolites and Conjugates of IPU in shoots
Soil
IPU treatment
Reduce IPU residue in soil
Metabolites and Conjugates of IPU in roots

ABSTRACT

Jasmonic acid (JA) (or methyl-jasmonic acid, MeJA) is one of the important regulators for plant growth, development and defense 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 residues in wheat (*Triticum aestivum*). Wheat plants were exposed to 4 mg kg^{-1} isoproturon (environmental realistic concentration). The 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 increased chlorophyll concentration and reduced 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 (HPLC). The concentration of isoproturon in wheat and soils with MeJA was drastically reduced. By ultra performance liquid chromatography-double mass spectrometer (UPLC-MS/MS), twelve isoproturon derivatives (eight metabolites and four conjugates) in wheat were characterized. We further provided evidence that the concentration of the 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 the realistic environmental isoproturon-polluted soil.

KEYWORDS: Isoproturon; wheat; methyl jasmonic acid; detoxication; degradation

INTRODUCTION

Isoproturon, 3-(4-isopropylphenyl)-1, 1-dimethylurea belongs to the phenylurea herbicide family widely used for weeding in the field of cereal, cotton and fruit production.[1] Recently, a growing concern has been raised about accumulation of isoproturon residues in environments, which potentially impacts on crop production and food safety.[2,3] The leaking of the residual isoproturon from the side of the use into the adjacent ecosystems through surface and ground water expand the areas of contamination.[4] Since 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 of crops exposed to the isoproturon-contaminated environments can freely absorb isoproturon, some crops such as wheat and rice become a major target of food chain in the areas where people rely on the grains as the staple food.[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 as to formulate corresponding countermeasures to lower 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 to 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 similar role of JA in plants.[14] JA serves as an elicitor involved in anti-inflammatory properties of essential oil of lettuce leaf basil (*Ocimum basilicum* L.) by

affecting yield, chemical composition and antioxidant.[15] Supply with exogenous JA induces oxidative stress tolerance in tobacco (*Nicotiana tabacum*) exposed to imazapic.[16] Several reports indicated that JA is also improving the capability against the metal stress.[12,17-18] The relieving stressful effects by JA imply the enhancement of detoxification of toxicants.[19] Up to now, no report is available about the effect of JA on the degradation of toxic compounds including pesticides in crops. Furthermore, the mechanism for JA mediating the degradation and detoxification of toxic herbicides is unknown.

Soil is a non-renewable resource which performs multi-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 microbial-based transformation of herbicides can individually or coordinately influence the degradation of residual herbicides.[21] Organic contaminants often are degraded more rapidly from planted soils than from the soils without planting.[22-23] Wheat is one of the most important crops, providing the major calories for mankind worldwide. Understanding the mechanism for the isoproturon residues in crops like wheat is critically important to ensure the crop production and food safety.[24] In this study, we investigated the detoxification and degradation of isoproturon in wheat crop in the presence of MeJA. Metabolites and degraded products of isoproturon were characterized using 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 figure out 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 of 96.9% purity was provided by the Institute of Pesticide Science, Academy of Agricultural Sciences in Jiang Su, Nanjing, China. Methyl jasmonic acid used 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 analyzed as organic carbon, 2.13%; total N, 1.26 g kg^{-1}; available P, 34.3 mg kg^{-1} and available K, 91.5 mg kg^{-1} with pH 7.6.

Wheat seeds (*Triticum aestivum*, cv. Yangmai 20) were surface-sterilized with 3% solution of H_2O_2, rinsed, and germinated on moist filter paper for 24 h. After germination, seedlings (twenty per pot) were transferred to and grew on the soil in a plastic container (1 L) with 1120 g dried soils mixed with 4 mg kg^{-1} isoproturon. For preparing the 100 μM MeJA stock solution, a small amount of ethyl alcohol was added to make MeJA dissolved. When used, a certain amount of the stock solution (100 μM MeJA) was diluted to 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 (around one 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 0.1% water solution of ethyl alcohol. The treatment was repeated daily for 6 days. Seedlings were grown in a chamber under the controlled condition (temperature, 25/20 °C; light/dark cycle, 14/10 h; light intensity, 300 μmol photons m^{-2} s^{-1}) and watered each day to

keep 70 % soil moisture. When harvested, shoots and roots of plants were individually sampled and immediately analyzed. Soils adhered tightly to the root system were collected as rhizosphere soil (RS). Soils shaken off from the roots were taken as non-rhizosphere soil (NRS). All collected rhizosphere and non-rhizosphere soils were considered as bulk soils (BS).[25,26]

Determination of physiological responses. Elongation of roots and leaves was measured with a ruler. For determination of dry mass, tissue samples were oven-dried at 70 °C for 72 h and weighted. The chlorophyll content in tissues was quantified according to the method of Porra et al.[27] Fresh leaves (0.1 g) was extracted in 8 mL of 80% acetone (pH 7.8), followed by centrifugation at 5,000×*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 5 mm length, placed in 10 mL deionized water and incubated at 32 °C for 2 h. An electrical conductivity meters (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 $EL=EC_1/EC_2\times100$.[10]

Thiobarbituric acid reactive substances (TBARS) was determined following the method described previously.[28] Briefly, 0.5 g fresh tissues were ground and dissolved in 3 mL of 0.1% (w/v) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 10,000×*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 heating in boiling water for 30 min, the mixture was cooled on ice bath, and centrifuged at 15000×*g* for 5 min. The amount of TBA-reactive substance (TBARS) was calculated from the difference in absorbance of the supernatant at 532 and at 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 Na-phosphate buffer (50 mM, pH 7.8) consisted of 1.0% (w/v) poly-vinylpyrrolidone and 0.1 mM EDTA. The homogenate was centrifuged at 12,000×*g* at 4 °C for 20 min. The supernatant was taken as crude extract using to assay of enzyme activities.[21]

Catalase (CAT, E.C. 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), enzyme extract (50 μL). Activity was calculated using the extinction coefficient 0.036 mM^{-1} cm^{-1} by the consumption of H_2O_2 at 240 nm. Peroxidase (POD, EC1.11.1.7) activity was assayed by the change of 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 ascorbate decrease in absorbance at 290 nm[29]. The reactive 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) (3.3mM), enzyme extract (100 μL). GST activity was calculated by using the extinction coefficient (10 mM^{-1} cm^{-1}).[31] Polyphenol oxidases（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 y using the extinction coefficient (1 mg^{-1} cm^{-1}) at 475 nm.[32]

Isoproturon and derivative analysis. Isoproturon and its metabolites (or derivatives) in the wheat tissues were analyzed with following method.[5] After being grinded using liquid nitrogen, the sample powder (4 g shoot or root) and 10 g soil was extracted ultrasonically in 10 mL mixed acetone–water (3:1, v/v) for 30 min and centrifuged at 4,000×*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 ℃. 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 methanol and collected for HPLC analysis and UPLC-TOF-MS/MS. The spiked recoveries of IPU from the soil and plant are showed in Table S1. The content of isoproturon (IPU) was determined by HPLC (Waters 515; Waters Technologies Co. Ltd., USA) with UV detector under the condition: Hypersil reversedphase C18 column (Thermo, 250 mm × 4.6 mm i.d.); mobile phase, methanol:water (68:32, V:V); wavelength, 241 nm; flow rate, 0.6 mL/min; and injection volume, 20 μL.

Characterization of IPU-derivatives and IPU-Conjugated products. Liquid chromatography/mass spectrometer (LC/MS) analysis of plant extracts was performed with an 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 × 2.1 mm, 2.7 µm, Thermo Fisher Scientific). Mobile phase was consisted of 0.1 % formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) and the flow rate is 0.3 mL/min. The elution steps were set as follows: (1) 5% B for 1 min, 1-5 min from 5%-15% B, 5-20 min from 15%-35% B, 20-22 min from 35%-100% B, and 100% B for 3 min, (2) Back to the initial conditions and (3) equilibrating for 1 min before the next sample injection. TOF-MS parameters included ion source gas 1, 65 psi, ion source gas 2, 65 psi, curtain gas 30 psi, source temperature 550 °C and ion spray voltage floating 5500 V. The mass spectrometer was operated in the positive product ion mode. The external mass calibration with the AB SCIEX Triple TOF™ systems was performed once a week to ensure a working mass accuracy <5 ppm. Data was processed by 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 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, sample MeJA was added to the encapsulated

micropore and combined with the labeled antibody to form the antibody antigen-enzyme labeled antibody complex. After thorough washing, the substrate TMB were 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 by standard curve.

Statistic analysis. Each result shown in the figures was the mean of three biological replicates. The values were expressed as means ± 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 $p<0.05$.[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 isoproturon toxicity to wheat, we first determine the toxic response of plants by quantifying lipid peroxidation, in term of thiobarbituric acid reactive substances (TBARS), a biomarker widely used for evaluating degree of injury in plants.[29,34,35] Wheat plants were grown in the soil with 4 mg kg^{-1} isoproturon. MeJA at 0, 0.1, 1, 10 and 100 μM was sprayed on the aerial parts of wheat. The treated samples were analyzed. As showed in Figure. 1A, compared to the control, the levels of TBARS in the roots and shoots were significantly lower with 0.1-1 μM and 0.1-10 μM MeJA, respectively. A higher level of MeJA supply had no effect on the TBARS generation, indicating that 0.1-1 μM MeJA was effective to alleviate the isoproturon-induced toxicity in wheats. Since sufficient 0.1 μM MeJA was

sufficient to reduce the stress, the concentration of MeJA was employed for 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 0, 0.25, 0.5, 1 and 2 mg L^{-1} for 72 h. Root and shoot tissues were harvested for MeJA quantification using ELISA. Our analysis showed that the concentrations of endogenous MeJA were progressively enhanced when wheat plants were exposed to IPU at 0.25-2 2 mg L^{-1} (Figure 1B). At 1 mg L^{-1} IPU, the roots and shoots had two-fold higher MeJA concentration than 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 and 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 were significantly decreased, the electrolyte permeability was drastically increased (Figure 2B-D). Supplying MeJA in the absence of isoproturon neither affected the growth nor the electrolyte permeability of wheat. However, when exposed to isoproturon and in the presence of MeJA, the wheat plants showed improved growth but inhibited electrolyte permeability. For example, the isoproturon-inhibited shoot and root elongation was improved by 9.71% and 16.33%, respectively (Figure 2A). Similarly, the chlorophyll concentration with isoproturon was decreased compared to the control, but increased 33.0% when concomitantly

treated with MeJA (Figure 2C). For electrolyte leakage, treatment with MeJA spray attenuated the isoproturon-induced stress by 27.80 % and 22.34 % in shoots and roots, respectively (Figure 2D).

MeJA reduced the accumulations 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, wheat shoots accumulated 6.5-fold higher levels of isoproturon residues than roots (Figure 3A). Such a model of isoproturon location in wheat plants could be governed by the genetic trait of wheat such as transport efficiency, physiological statue and degrading capacity as well as the properties of herbicides.[5] Accumulation of isoproturon in wheat was significantly lowered by addition of MeJA (Figure 3A), suggesting that MeJA could mediate the accumulation of isoproturon in the plant.

Bioconcentration factor (BCF) is the ratio of pesticide concentration in plants to the bulk soil which is frequently used for evaluation of the ability to accumulate pesticide from 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 soil to roots and shoots of wheat. 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 were shown in the following order of isoproturon treatment alone < isoproturon+MeJA (Table 1). Addition of MeJA obviously enhanced the capacity of

translocating isoproturon from roots to shoots. The possible mechanism for JA-mediated lower 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 was closely associated with the availability of isoproturon in soil, the residues of isoproturon in rhizosphere soil (RS), bulk soil (BS) and non-rhizosphere soil (NRS) were determined by chromatography. The rhizosphere soil always contained less isoproturon residues than the bulk soil and non-rhizosphere soil (Figure 3B). The residues of isoproturon in RS, BS, and NRS in the presence of MeJA were lower than those without MeJA treatment on wheat. Supplying MeJA could contribute to the removal of isoproturon in the soil where wheat was planted. Most of plants or crops are gifted with the ability to remove herbicides by taking them in from soil through roots and getting them detoxified 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 rhizosphere.[26] Our analysis showed that the rhizosphere had less residues of the herbicide than the non-rhizosphere, suggesting that wheat plants would contribute to 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 zone of rhizosphere was close to plant roots where much 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 specific microbial population or communities;[41] and (b) wheat cultivation possibly fostered the microbial population and led to

isoproturon degradation more efficiently in rhizosphere over the non-plant cultivation. However, further investigation will be required to clarify the assumptions.

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) and 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 determined 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 likes vacuoles for deep degradation. It is therefore believed as one of the potent detoxifying enzymes.[44] PPO basically catalyses 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 catalyze 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 showed 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 of the 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 ultra performance liquid chromatography-AB SCIEX Triple TOF 5600 mass spectrometer (UPLC-Triple TOF 5600). The isoproturon-free or MeJA treatment alone was set as a control. The mass spectrometric data of isoproturon derivatives including metabolites and conjugates in wheat were summarized in Table 2. The structures of isoproturon derivatives were determined by analyzing MS^2 data generated from collision-induced dissociation (CID) fragmentation patterns (Figure S2, 3).

The extracted MS^2 spectra of IPU (*m/z* 207) occurred at the retention time of 14.99 min (Table 2). Fragmentation of IPU led to a main product ion *m/z* 134 which was generated by the loss of two methyl groups and a $-N(CH_3)_2$ group and further loss of acylamino generated the fragment ion *m/z* 91 (Figure S2A and Table 2). The $[M + H]^+$ ion *m/z* 193 with a retention time of 13.33 min generated fragment ion *m/z* 94 by the cleavage of isopropyl and CH_3-NH-CO-, which was identified as monodemethyl-IPU, the *N*-demethylation product (Figure S2B and Table 2). The precursor ion *m/z* 179 peaking at 11.71 min with the fragment

ion 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 *m/z* 223 (t_R =5.94 min) yielded characteristic fragment ions *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 *m/z* 209 generated the characteristic fragment ions *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 *m/z* 205 (t_R =13.59 min) generated two main fragment ions *m/z* 105 and 72, which were positively charged ethylbenzene and $C_3H_6NCO^+$. Thus, this metabolite was identified as isopropenyl-IPU (Figure S2F and Table 2). The precursor ion *m/z* 191 (t_R =11.98 min) formed fragment ion *m/z* 77, being positively charged benzene, and another main fragment ion *m/z* 106 was produced by the breakage of 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 small molecular weight metabolite from the degraded products with the precursor ion *m/z* 120, giving *m/z* 91 (positively charged benzyl) and *m/z* 77 (positively charged benzene). Based on the analysis, the final IPU-degraded product was 4-vinylanline (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 *m/z* 103, 134 and 162. The ions *m/z* 103 and 134 were formed by the loss of a glucose moiety, and the ion *m/z* 162 was produced by the loss of

glucose and two methyl groups. It turned out 2-OH-monodemethyl-IPU-*O*-glucoside (Figure S3A and Table 2). The precursor ion *m/z* 385 (t_R =5.72 min) formed the fragment ions *m/z* 72, 205 and 223. The ion *m/z* 72 was produced due to the elimination of -$CON(CH_3)_2$, and the ion *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 $[M + Na]^+$ ion at *m/z* 449 and 463, respectively (Figure S3C, D and Table 2). The precursor ion at *m/z* 449 was identified by detecting fragment ion *m/z* 329 formed by the loss of amino and carboxyl, indicating this metabolite was GSH-*S*-deisopropyl-didesmethyl-IPU (Figure S3C and Table 2). The other GSH *S*-conjugate with $[M + Na]^+$ *m/z* 463 was identified by fragment ions *m/z* 294 and 343. The ion *m/z* 294 was due to the removal of amino, while the ion *m/z* 343 was generated by the loss of -$CONHCH_3$ 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 were measured using UPLC-MS. All twelve derivatives were detected in shoots, while six derivatives were examined in roots (Figure 5A,B). The isoproturon concentration in tissues after MeJA spray was significantly decreased comparing that under non-MeJA treatment. With regard to metabolites, most of them had a significantly higher level in intensity in the presence of MeJA, except two metabolites in shoots (*m/z* 191 and *m/z* 120) and one metabolite in roots (*m/z* 193). Nevertheless, all conjugations had

significant differences between treatments of isoproturon+MeJA and 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). Besides, 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 to metabolize a diverse variety of xenobiotics including pesticides. It is critical to understand the mechanism of isoproturon transformation during the course of degradation. Mastering the knowledge would help develop strategies to minimize 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 transformed into other molecular 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 GSH-type and two other belong to 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 datasets. As showed in Fig. 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. Amongst 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-vinylanline (*m/z* 120), respectively (Figure 6). Organic pollutants conjugated with xenobiotics, such as glucosyl, malonyl-glucosyl, acetyl-glucosyl moieties and glutathione are considered as a way to detoxify pollutants by 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. Besides, 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. Amongst 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 on account of more isoproturon-derivatives detected in wheat (Figure 5). Apparently, MeJA is able to fascinate the removal of isoproturon in wheat through a putative detoxification

pathway to attenuate the isoproturon-induced cellular toxicity.

CONCLUSIONS

Foliar supply with MeJA was able to reduce accumulation of isoproturon in wheat. Consistent with it, the growth responses, manifested by elongation, biomass and total chlorophyll accumulation were increased, whereas the membrane permeability was decreased by MeJA treatment under IPU stress. The BCF of wheat shoots and roots showed a downtrend, whereas the TF values increased in the presence of MeJA, showing a translocation of atrazine 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, supply MeJA led to a higher level of derivatives of isoproturon in wheat, suggesting that MeJA was involved in transformation and reduced isoproturon accumulation. Overall, our analyses demonstrate that MeJA can exert a beneficial effect on enhancement of isoproturon degradation in wheat.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at

Figure S1. Effect of different concentrations of IPU on the endogenous methyl jasmonate (MeJA) in wheat for 24 h (A) and 48 h (B).

Figure S2 Eight metabolites were identified based on the accurate MS data and the appropriate fragmentation patterns from MS^2 data

Figure S3 Four conjugates were identified based on the accurate MS data and the appropriate fragmentation patterns from MS^2 data

Table S1 Spiked recoveries of isoproturon in soil, wheat shoots and roots

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** L.Y. Ma, S.H. Zhang and J.J. Zhang made equal contributions to the study.

Funding

We acknowledge the financial support of the National Natural Science Foundation of China (No. 21577064).

Notes

The authors declare no competing financial interest.

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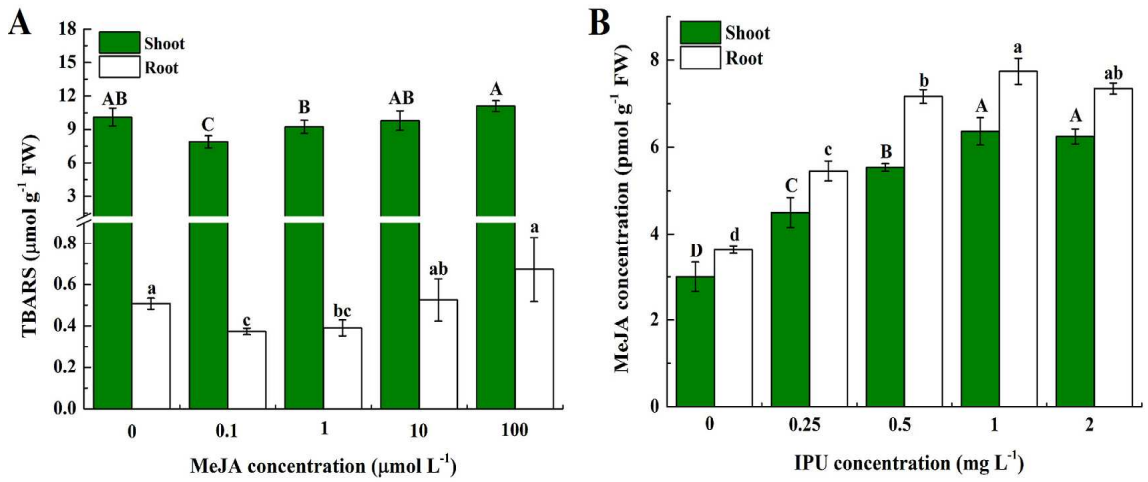

Figure 1. Effect of MeJA on TBARS concentrations and IPU on MeJA concentrations in wheat. (A) One week-old wheat growing in the soil with 4 mg kg^{-1} isoproturon was treated with MeJA (0, 0.1, 1, 10 and 100 μM MeJA spray) for 6 d. (B) One week-old wheat growing in nutrient solution with 0, 0.25, 0.5,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

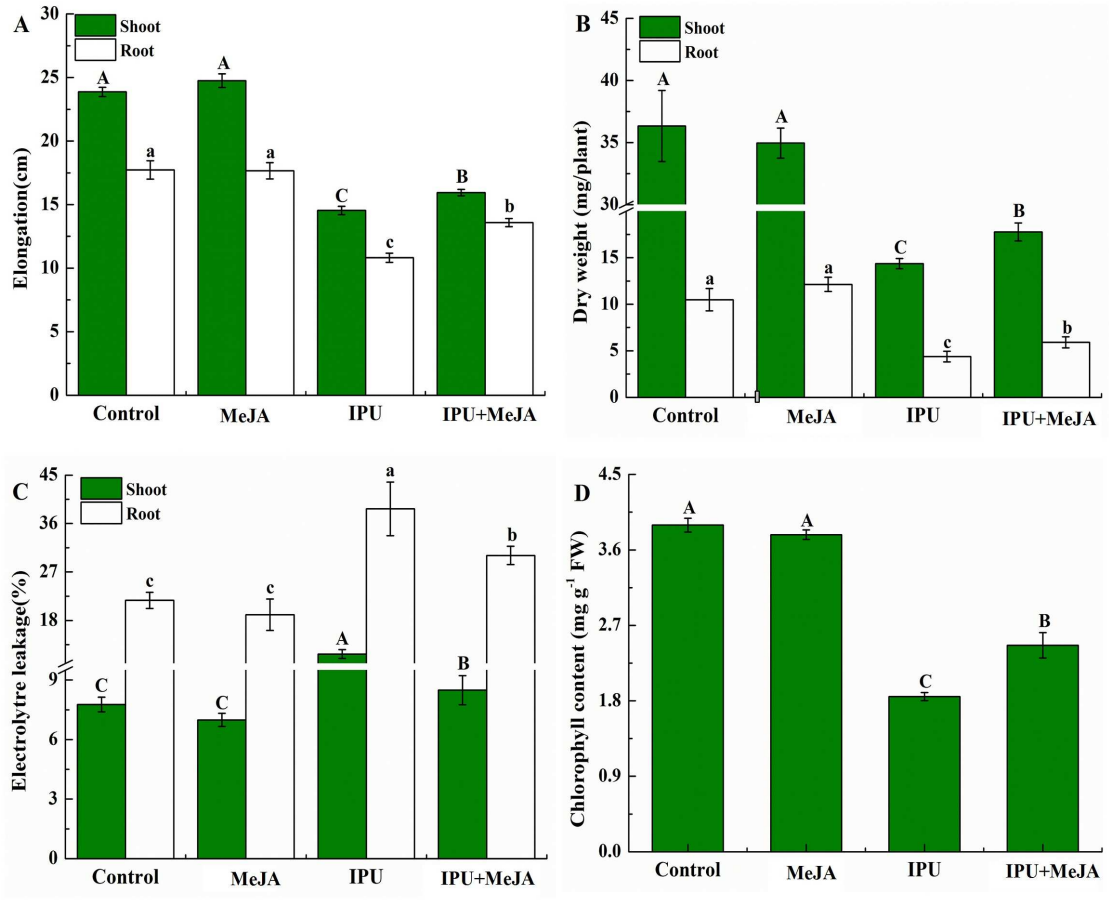

Figure 2. Effects of IPU and/or MeJA on elongation (A), dry mass (B), electrolyte (C) and chlorophyll (D) content of wheat. Seedlings grew in soils with IPU (4 mg kg^{-1}) and without IPU (CK, control) for 4 d. After that, the leaves were sprayed with 0.1 μmol L^{-1} MeJA once a day for 6 d. Values are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

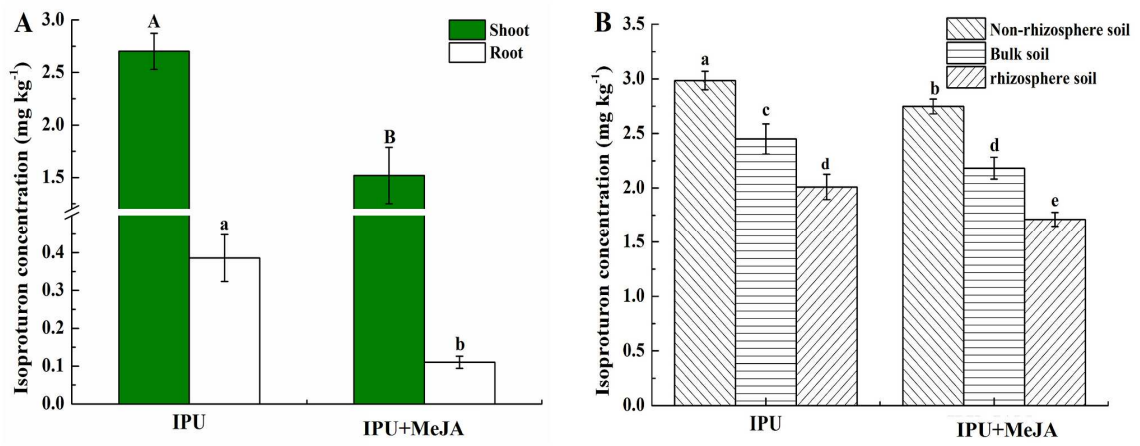

Figure 3. Accumulation of IPU in shoots and roots of wheat (A) and rhizosphere soil (RS), bulk soil (BS, whole soil including rhizosphere and non-rhizosphere), and non-rhizosphere soil (NRS) (B) in different treatment. Seedlings grew in soils with IPU (4 mg kg^{-1}) and without IPU (CK, control) for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

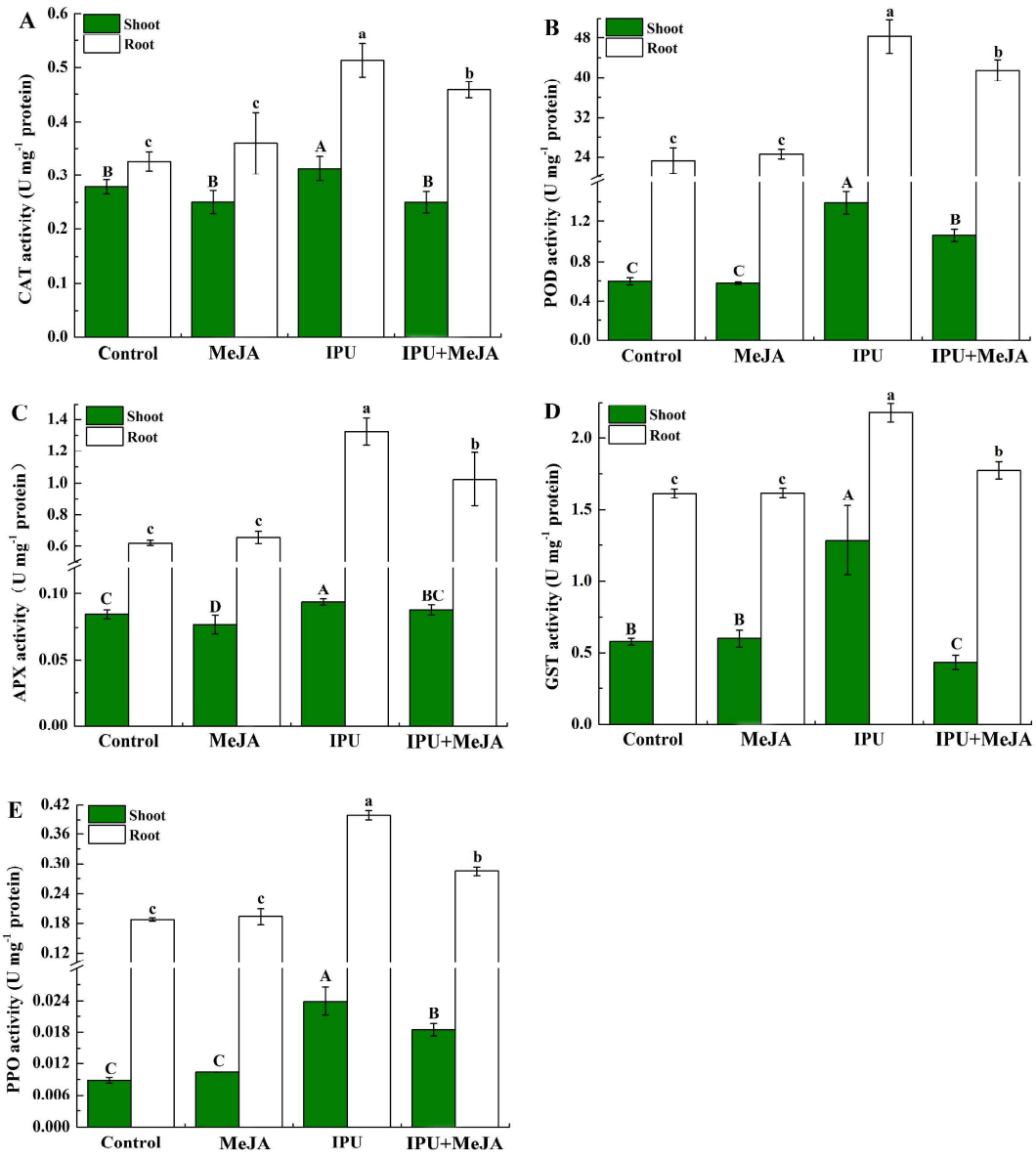

Figure 4. Effect of IPU and/or MeJA on catalase (CAT) (A), peroxidase (POD) (B), ascorbate peroxidase (APX) (C), glutathione S-transferase (GST) (D) and polyphenol oxidase (PPO) (E) activities in shoots and roots of wheat. Seedlings grew in soils with IPU (4 mg kg^{-1}) and without IPU (CK, control)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

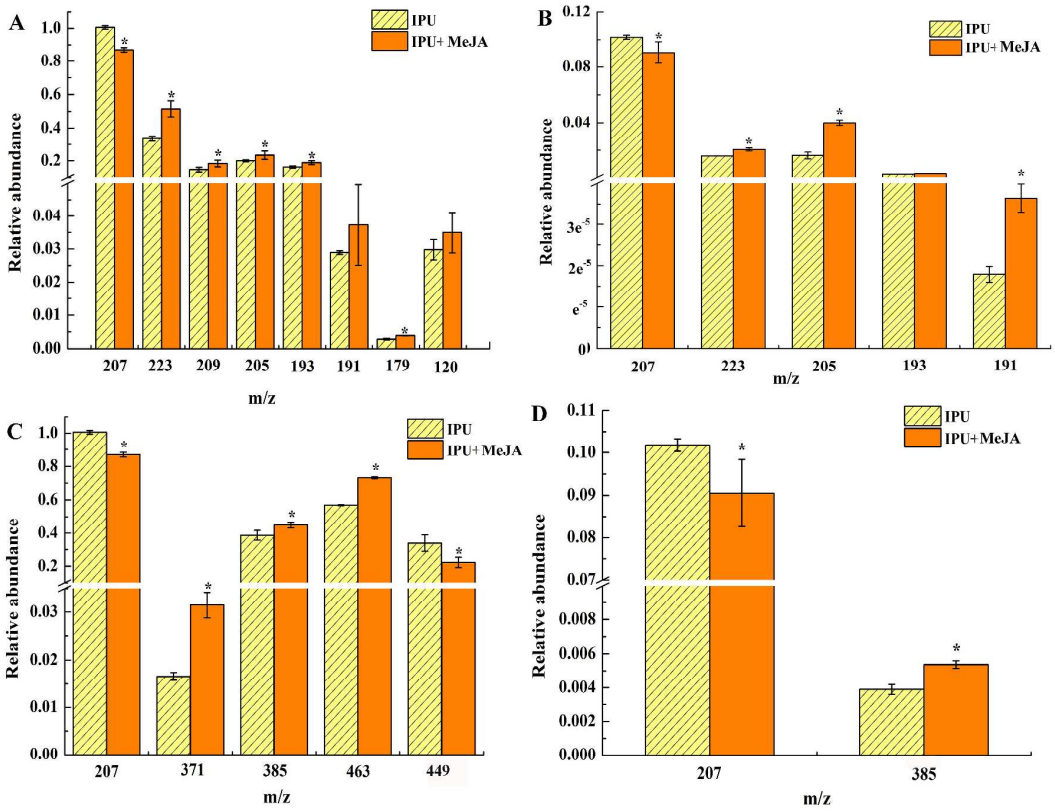

Figure 5. IPU-derived degradation products in shoots (A) and roots (B) and conjugates of IPU in shoots (C) and roots (D) extracted from IPU-treated in wheat for 10 d under MeJA treatment or untreatment. Seedlings grew in soils with IPU (4 mg kg^{-1}) and without IPU (CK, control) for 4 d. After that, the leaves were sprayed with 0.1 μmol L^{-1} MeJA once a day for 6 d. Values are the means ± standard deviations (n = 3). Asterisks indicate the significant difference between the treatments of IPU + MeJA and IPU ($p < 0.05$).

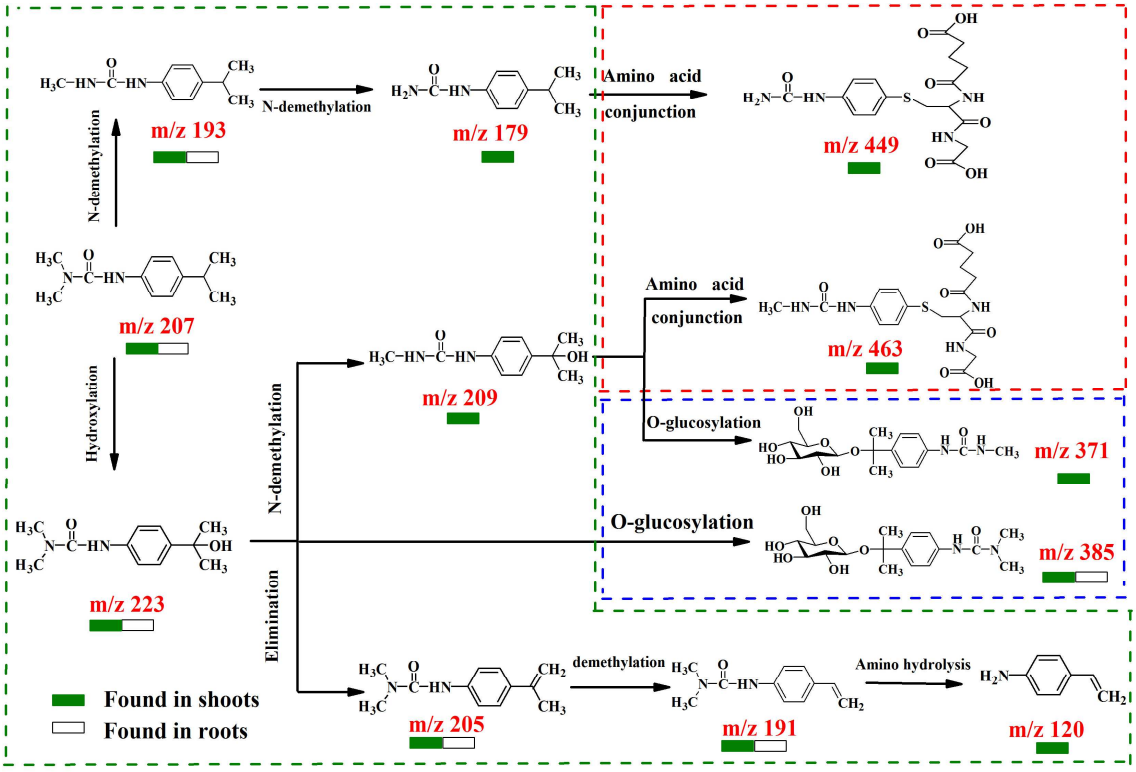

Figure 6. Proposed metabolic pathway of IPU in wheat exposed to IPU and/or MeJA. The contents in the green box are metabolized from IPU. The substances in the red box are conjugates conjugated with amino acid of IPU. The contents in the blue box are conjugates conjugated with glycosylation of IPU.

Table 1

Bioconcentration Factors (BCFs) and Translocation Factors (TFs) for IPU in Shoot and Root of wheat[a].

treatment	BCF[b]		TF[c]
	Shoot	Root	
IPU	1.11±0.098	0.15±0.031	7.40±0.745
IPU+MeJA	0.70±0.047*	0.05±0.001*	13.82±0.965*

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

Table 2

Summary of all mass spectrometer data for metabolites and conjugates of IPU in wheat.

No.	Metabolites	*Rt*[a] (min)	Calcd m/z[b]	exptl m/z[b]	Chemical formula	ppm error	Location[c]	MS^2 m/z[d]	
								Precursor ion	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-vinylanline	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
No.	IPU-derivatives								
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[e]	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[e]	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]icon modes are $[M+H]^+$ or $[M+Na]^+$.[c]Location: Distribution of metabolites of atrazine in plant. [d]MS^2 fragments: Base peak of MS^2 fragment ions are shown in bold. [e]Compounds that have been reported for the first time.