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Acceleration of the herbicide isoproturon degradation in wheat by glycosyltransferases and salicylic acid

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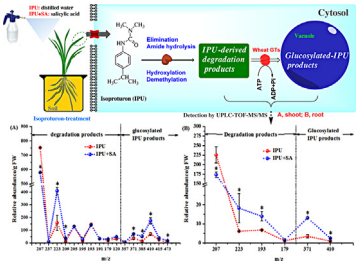
HIGHLIGHTS

- We identified residues of IPU and its degradation products in wheat.
- Most detected IPU derivatives in wheat were sugar-conjugated.
- Production and glycosylation of IPU-derivatives in wheat were enhanced by applying SA.
- All structures of IPU-derivatives were characterized by UPLC-TOF-MS/MS.
- A pathway for the IPU degradation and glucosylation was provided.

GRAPHICAL ABSTRACT



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ABSTRACT

Isoproturon (IPU) is a herbicide widely used to prevent weeds in cereal production. Due to its extensive use in agriculture, residues of IPU are often detected in soils and crops. Overload of IPU to crops is associated with human health risks. Hence, there is an urgent need to develop an approach to mitigate its accumulation in crops. In this study, the IPU residues and its degradation products in wheat were characterized using ultra performance liquid chromatography-time of fight tandem-mass spectrometer/mass spectrometer (UPLC-TOF-MS/MS). Most detected IPU-derivatives were sugar-conjugated. Degradation and glycosylation of IPU-derivatives could be enhanced by applying salicylic acid (SA). While more sugar-conjugated IPU-derivatives were identified in wheat with SA application, lower levels of IPU were detected, indicating that SA is able to accelerate intracellular IPU catabolism. All structures of IPU-derivatives and sugar-conjugated products were characterized. Comparative data were provided with specific activities and gene expression of certain glucosyltransferases. A pathway with IPU degradation and glucosylation was discussed. Our work indicates that SA-accelerated degradation is practically useful for wheat crops growing in IPU-contaminated soils because such crops with SA application can potentially lower or minimize IPU accumulation in levels below the threshold for adverse effects.

1. Introduction

Pesticide (herbicide) is widely used in agricultural managements. While appropriate use of pesticides brings economic benefits to crop production, continuous application of pesticide often leads to adverse effects on crop growth. Furthermore, application of pesticides brings about a series of environmental problems [1]. Crop contamination with pesticides not only affects the quality

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of cereals, but also serves as a food chain pollutant and threats to human health. Hence, there is a need to lower the human intake of these toxins.

Isoproturon [IPU, 3-(4-isopropylphenyl)-1, 1-dimethylurea] belongs to the phenylurea herbicide family widely used to prevent pre- and post-emergence weeds in winter cereals (*e.g.* wheat and barley). Due to its extensive use in China and other developing countries, the residues of IPU are often detected in soils and ground or surface water [2,3]. Field and lab research have shown its harmful effects on freshwater algae, soil microbial communities and higher plants [4–6]. IPU is considered to be genotoxic as it induced a significant frequency of aberrations [7,8] and has been classified as a possible carcinogen in human beings by the annex X of the Water Framework Directive [9]. IPU is water soluble, moderately hydrophobic and weakly absorbed by soils [10]. These characteristics suggest that IPU residues are easy to be absorbed by crops [6]. Genetic differences of plants in pesticide uptake were observed, and intake and translocation of pesticides in plants vary considerably not only among plant species but also in cultivars within the same species [11,12]. This makes it possible that some genotypes of crops growing in the IPU-contaminated soils may accumulate IPU at a very low level or below the threshold for adverse health effects [13]. Although microbial-based remediation is beneficial to reduce the organic hazards, some technical difficulties (*e.g.* how to keep microbes active in an open soil environment) remain to be solved [14].

Recent phytodegradation research about the molecular biology mechanism of contaminant uptake, root-to-shoot translocation and degradation provides a clue that allows to develop a new strategy by exploring low pesticide-accumulating genotypes (or cultivars) of crops. This approach is based on certain cultivars that grow in the hazard-contaminated environment without apparent toxic symptoms but with low levels of accumulation [15]. The molecular mechanism underlying the process is largely unknown, but these crops may possess a highly efficient detoxification and catabolism system. Recently, it has been demonstrated that some plant species and green algae do have remarkable potentials to degrade certain herbicides inside cells [11,16–18]. Interestingly, degradation of two herbicides napropamide and isoproturon is shown to be promoted by salicylic acid (SA) [6,18]. This suggests that exploration of natural and low price additives such as SA is also a promising approach to minimize toxic compounds in crops.

Higher plants have developed sophisticated strategies to cope with absorbed toxic compounds through multi-degradation or detoxification pathways [19]. One of them is the glycosyltransferases (GTs; EC 2.4.x.y)-dependent detoxification mechanism (Phase II metabolism) [14,20]. GTs comprise a large group of enzymes that form glycosidic bonds through the transfer of sugars from activated donor molecules to acceptor molecules. Based on functional groups (–OH, –NH or –SH), plant glucosyltransferases are classified as *O*-glucosyl-glucosyltransferase (*O*-GTs), *N*-glucosyl-glucosyltransferase (*N*-GTs) and *S*-glucosyl-glucosyltransferase (*S*-GTs) subfamilies [18]. Currently, nearly 452 GT genes in Arabidopsis and 609 potential GT loci from rice (*Oryza sativa*) have been located on their corresponding genome [21]. To date, 59 novel rice GTs genes in response to atrazine have been identified using high-throughput sequencing technology [22,23]. In wheat, only a few of GTs have been reported in response to hazards [24]. In this study, we characterized degradation of IPU in a previously selected wheat cultivar using multiple methods, such as analytical chemistry and molecular biology approaches. Our study has shown that IPU was degraded to various secondary products, which could be further glucosylated. Structures and abundance of all degraded products were specified by ultra performance liquid chromatography-double mass spectrometer (UPLC-MS/MS). Importantly, the catabolic processes and glucosylation of IPU-derivatives have been found to be enhanced by applying SA. Thus, the objective of this study was (1) to obtain information on the possible IPU catabolic pathway by characterizing IPU-derivatives and their glucosylated products in wheat and (2) to identify whether SA-promoted lower IPU accumulation in wheat is through IPU degradation linked to glucosylation.

2. Materials and methods

2.1. Treatments

Isoproturon (IPU) technical material[1] was obtained from Institute of Pesticide Science, Academy of Agricultural Sciences in Jiang Su, Nanjing, China, with a purity of 96.9%. SA used was analytical grade (Sinopharm Chemical Reagent Co.). The chemical characteristics of isoproturon (IPU) were described in SI Table S1. Uncontaminated soil (Eutric gleysols) was air-dried, ground, and passed through 3 mm sieves for wheat planting. Wheat seeds (*Triticum aestivum*, *cv.* Yangmai 13) were surface-sterilized in a 5% sodium hypochlorite and germinated. After 24 h, uniformly germinated seeds (20 plants per pot) were transferred to a plastic pot (1 L) containing 1120 g prepared soil mixed with IPU at 4 mg kg^{-1} dry soil. The method for SA treatment was based on the method described previously [6]. Briefly, wheat leaves were sprayed with 5 mg L^{-1} SA after 4 days of IPU exposure (4 mg kg^{-1}). The SA treatment was then applied once per day. For each time, the leaves sprayed with SA were fully wetted. The IPU and/or SA treated seedlings were harvested at the time interval of 2, 4 and 6 days. Seedlings were grown in a growth chamber (PGX-350D, SAFE Co) under a light intensity of 300 μmol m^{-2} s^{-1} with a light/dark cycle of 14/10 h at 25/20 °C and watered to keep 70% relative water content in soils [6].

2.2. Extraction of IPU-transformed products from wheat tissues

IPU and its metabolites (or derivatives) in wheat were analyzed based on the method of Liang et al. [6]. Fresh tissues (4 g shoot and 3 g root) were ground to fine powder with liquid nitrogen. The sample was ultrasonically extracted three times with mixed acetone–water (3:1, v/v) (10 mL of solution per extraction for 30 min), followed by centrifugation at 5000 × g for 15 min. The supernatant was 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 column. The eluent was discarded. The column was washed with 1 mL methanol, which was collected for analysis with UPLC-TOF-MS/MS. The external standard and relative abundance were used to quantify IPU and its metabolites, respectively. The spiked recovery and RSD of IPU extraction and detection limit from wheat tissues were showed in SI Table S2.

2.3. Analysis of IPU-derivatives and IPU-glucosylated products

Liquid chromatography/mass spectrometer (LC/MS) analysis of plant extracts was performed with an UPLC apparatus equipped with a Waters Acquity PDA detector (Milford, MA, USA) connected to a WATERS SYNAPT Q-TOF Mass Spectrometer (Milford, MA, USA) (UPLC-TOF-MS/MS). Chromatographic operation was set under conditions as following: XBridge™ C_{18} columns (2.1 × 100 mm, granulation of 3.5 μm, Waters); injection of 4 μL of the sample solution to the LC/MS system; mobile phase with two solvents: A (100% acetonitrile) and B (99% H_2O/0.1% formic acid, v/v) at a 0.3 mL min^{-1}

[1] See Materials and methods section provided in the online supplemental material for the methods for assessment of (1) glucosyltransferase activity, (2) membrane permeability and (3) transcript analysis by real-time PCR.