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Metabolomic analysis of two rice (*Oryza sativa*) varieties exposed to 2, 2′, 4, 4′-tetrabromodiphenyl ether☆

Jie Chen [a, b], Kelun Li [a, b], X. Chris Le [c], Lizhong Zhu [a, b, *]

[a] Department of Environmental Science, Zhejiang University, Hangzhou, Zhejiang, 310058, China
[b] Zhejiang Provincial Key Laboratory of Organic Pollution Process and Control, Hangzhou, Zhejiang, 310058, China
[c] Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, T6G 2G3, Canada

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are toxic chemicals widely distributed in the environment, but few studies are available on their potential toxicity to rice at metabolic level. Therefore we exposed ten rice (*Oryza sativa*) varieties to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), a predominant congener of PBDEs, in hydroponic solutions with different concentrations. Two varieties that showed different biological effects to BDE-47, YY-9 and LJ-7, were screened as sensitive and tolerant varieties according to changes of morphological and physiological indicators. Metabolic research was then conducted using gas chromatography–mass spectrometry combined with diverse analyses. Results showed that LJ-7 was more active in metabolite profiles and adopted more effective antioxidant defense machinery to protect itself against oxidative damages induced by BDE-47 than YY-9. For LJ-7, the contents of 13 amino acids and 24 organic acids, especially L-glutamic acid, beta-alanine, glycolic acid and glyceric acid were up-regulated significantly which contributed to scavenging reactive oxygen species. In the treatment of 500 μg/L BDE-47, the contents of these four metabolites increased by 33.6-, 19.3-, 10.6- and 10.2-fold, respectively. The levels of most saccharides (such as D-glucose, lactulose, maltose, sucrose and D-cellobiose) also increased by 1.7–12.4 fold which promoted saccharide-related biosynthesis metabolism. Elevation of tricarboxylic acid cycle and glyoxylate and dicarboxylate metabolism enhanced energy-producing processes. Besides, the contents of secondary metabolites, chiefly polyols and glycosides increased significantly to act on defending oxidative stress induced by BDE-47. In contrast, the levels of most metabolites decreased significantly for YY-9, especially those of 13 amino acids (by 0.9%–67.1%) and 19 organic acids (by 7.8%–70.0%). The positive metabolic responses implied LJ-7 was tolerant to BDE-47, while the down-regulation of most metabolites indicated the susceptible nature of YY-9. Since metabolic change might affect the yield and quality of rice, this study can provide useful reference for rice cultivation in PBDEs-polluted areas.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) (Tagliaferri et al., 2010) are manufactured as a class of additive brominated flame retardants and have been broadly used in a wide array of materials, including electronic, equipment, plastics, textiles and building materials since the 1970s (Wu et al., 2008; Xu et al., 2015). Previous studies reported that PBDEs were widely distributed in various environmental media, and even had been detected in animals (Ben Ameur et al., 2011; Luo et al., 2007), humans (Qu et al., 2007) and terrestrial plants (Jin et al., 2008). Due to their persistence, bioaccumulation and toxicity to human and wildlife (Ben Ameur et al., 2011), some PBDEs have been listed as persistent organic pollutants (Mohr et al., 2014). Among them, 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) has drawn extensive attention as one of the most potent congeners (Jin et al., 2008; Kawashiro et al., 2009) which can induce multiple toxicities to humans (An et al., 2011; Kawashiro et al., 2009; Lema et al., 2008; Song et al., 2009; Zhong et al., 2011).

Reactive oxygen species (Spicher et al., 2017) have been demonstrated as one of the potential factors inducing toxicity after organisms were exposed to many organic contaminants (Fernie

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* Corresponding author. Department of Environmental Science, Zhejiang University, Hangzhou, Zhejiang, 310058, China.
E-mail address: zlz@zju.edu.cn (L. Zhu).

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et al., 2005; Riaz et al., 2017; Xu et al., 2015; Zhang et al., 2007). Previous studies have reported that BDE-47 played a critical part in neurodegenerative processes due to the production of ROS. As a result, motor neuron injuries, cell apoptosis, DNA damages and genotoxicity have been observed in human and rat cells (An et al., 2011; Pellacani et al., 2012; Tagliaferri et al., 2010; Zhong et al., 2011). Researches on the phytotoxicity of BDE-47 on terrestrial plants also found that ROS posed an adverse effect in plant development (Xie et al., 2013; Xu et al., 2015). Xu et al. found BDE-47 could inhibit seed germination and seedling development, decrease the biomass, cause lipid peroxidation, and damage DNA under the overproduction of ROS in maize (Xu et al., 2015). They also detected the rise in contents of antioxidant defense enzymes, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). These enzymes are always stimulated in cells to scavenge ROS, therefore can be used to characterize the oxidative stress response induced by contamination.

In addition to these physiological indicators, metabolic responses to BDE-47 have also been investigated in previous studies (Ji et al., 2013a, 2013b; Wu et al., 2015). Ji et al. revealed that BDE-47 disturbed the energy metabolism and osmotic regulation attributed to the alteration of amino acids, betaine, glucose, maltose, ATP and succinate in male mussel gills and earthworm *Eisenia fetida* (Ji et al., 2013a, 2013b). Wu et al. reported that BDE-47 could trigger metabolic responses in human cells by motivating the levels of ethanol, glutathione, creatine, aspartate, UDP-glucose and NAD+, and increased lactate/alanine ratios were also detected to cope with the overproduction of ROS(Wu et al., 2015). These studies implied BDE-47 could induce metabolic responses and perturb energy metabolism in animal and human cells. Nevertheless, few studies focused on the response induced by PBDEs at metabolite levels in crops.

As a powerful and sensitive approach, metabolomics has been used to elucidate biological mechanisms, including toxicity and possible changes in chemical composition of crops (Obata and Fernie, 2012). Faced with various environmental stressors, plants adjusted the structures of some crucial molecular to adapt to detrimental living processes (Krasensky and Jonak, 2012; Pidatala et al., 2016; Zhao et al., 2016a), including primary metabolites (amino acids, saccharides and organic acids, etc) and various secondary metabolites (phenols, polyols, glycosides and nitrogen compounds, etc). Furthermore, these metabolites tend to be related to nutritional quality (sugar, starch, maltose, protein, and fatty acid contents) which is associated with quality of food crops (Rico et al., 2013). Previous studies indicated that pollutants can act as environmental stressors that can affect crop metabolism and thus impact its yield and quality (Rico et al., 2013; Vecerova et al., 2016). Rico et al. revealed rice grains from $n\mathrm{CeO_2}$-treated plants had less prolamin, glutelin, lauric acids, starch and total antioxidant capacity was reduced (Rico et al., 2013). As the environmental release of PBDEs is a concern, it is imperative to investigate their effects on the metabolite profiles of major agricultural crops to provide reference for the research on quality of crop grains.

Rice is a vital staple food crop around the world, especially in Asian countries (Peng et al., 2009). Varieties of rice originated from a range of geographical areas which could bring the differences in morphological development, physiological reaction and adaptive responses to toxicants (Wang et al., 2016). It is well documented that rice varieties displayed different biological effects under various abiotic stresses, such as drought, salt, low temperature, nutrition deficiency, etc (Krasensky and Jonak, 2012; Morsy et al., 2007; Zhao et al., 2014). For the stress-tolerant rice varieties, they have developed effective mechanisms for adaptation by over-expressing specific genes to enhance their tolerance (Gill and Tuteja, 2010). Thus there likely exists a difference in endurance when exposed to PBDEs among rice varieties and screening PBDEs-tolerant rice varieties will provide an important reference for its cultivation in areas heavily polluted by PBDEs.

Hence, the basic hypothesis of the study was that the different tolerance of rice varieties to BDE-47 might due to their different metabolic responses. Therefore, the changes of morphological and physiological indicators of ten rice varieties were firstly detected to screen out the sensitive and tolerant varieties, and then determined the metabolite profile changes of the two varieties using GC-MS and the results were analyzed using diverse methods (Rico et al., 2011). This research made an explanation for the tolerance mechanism of rice to BDE-47 at metabolic level and contributed to elucidating the potential influence of PBDEs on the quality of rice grain. Ultimately, metabolic identification of BDE-47-related responses of rice will provide biomarkers for selection in breeding programs which is helpful for rice cultivation in the PBDEs-polluted areas (Alamgir et al., 2016).

2. Materials and methods

2.1. Chemicals and reagents

Standard stock solution of BDE-47 was obtained from AccuStandard (AccuStandard, USA). *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), methoxyamine hydrochloride, and pyridine were all acquired from J&K (Beijing, China).

2.2. Seeds and germination

Ten rice varieties, including Lianjing-7 (LJ-7), Jinzao-47 (JZ-47), Zhongjiazao-17 (ZJZ-17), Xiushui-134 (XS-134), Yongyou-1540 (YY-1540), Zhongzheyou-1 (ZZY-1), Jiayou-5 (JY-5), Zhejing-88 (ZJ-88), Yongyou-9 (YY-9) and Y-linagyou-1 (Y-LY-1) from three subspecies (indica, japonica and indica japonica hybrid) were selected to investigate the phytotoxicity of BDE-47. All varieties were widely cultivated in the Yangtze River Delta and the seeds were obtained from Zhejiang University, College of Agriculture (Hangzhou, China). Seeds were sterilized in 3% (v/v) H_2O_2 for 30 min and then washed with deionized water before germination. After that, 30 seeds were germinated on culture dishes with 20 mL of test solution. The test solutions were prepared by dissolving stock standards of BDE-47 in acetone and then diluted with deionized water. The concentrations of the test solutions were from 10, 20, 50, 100–500 μg/L for each varieties and concentration of the residual acetone in the test solutions was about 0.1% (v/v). Controls were obtained with 20 mL of deionized water containing 0.1% (v/v) acetone. The culture dishes were covered with lids and placed darkly at $27 \pm 0.5\,^{\circ}\mathrm{C}$. Renewing the test solutions every day. After 7 days, the number of germinated seeds (30 seeds per dish, 3 dishes per treatment) was counted (Xu et al., 2015).

2.3. Growth inhibition of seedlings

The impact of BDE-47 on rice seedling growth was tested after germination. Six uniformly germinated seedlings were sterilized as described above, and then transferred to colored vitreous pots with 120 mL Hoagland nutrient solution containing BDE-47 (as per concentration described above). Controls exposed to Hoagland nutrient solution containing 0.1% (v/v) acetone were included. The test solutions were renewed every three days. After 14 days of exposure, the roots and leaves of the whole plants and the hydroponic solutions of all groups were sampled separately. Plant tissues (leaves and roots) were used to measure the elongation firstly (6 plants per pot, 4 pots per treatment) and then were stored at $-80\,^{\circ}\mathrm{C}$ for further treatment.