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Photosynthetic and antioxidant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the soil

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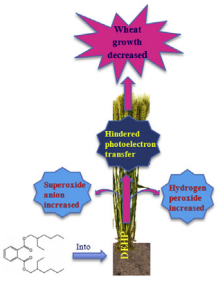
HIGHLIGHTS

- DEHP seems to have hindered the photoelectron transfer process in wheat growing stage.
- Superoxide anion and hydrogen peroxide content increased in wheat plant under DEHP stress.
- The toxic effects of DEHP on root tissues were more serious both seedling and jointing stages.
- The extent of damage to the roots, stems, and leaves was depended on DEHP concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

Di(2-ethylhexyl) phthalate (DEHP) is a commonly used, artificially-synthesized, industrial chemical that can be released into the soil. However, to date, there is no comprehensive study on the effects of DEHP on photosynthesis, induction of reactive oxygen species, and response of the antioxidant defense system in wheat plants growing in DEHP contaminated soil. This study was conducted to address this gap in knowledge. Our results showed that after application of 10, 20, and 40 mg/kg DEHP, photosynthetic parameters, fluorescence parameters, and chlorophyll content of wheat leaves at seedling, jointing, and booting stages decreased, while the intercellular carbon dioxide concentration increased. This indicates that the observed decrease in net photosynthetic rate in wheat leaves was due to a non-stomatal limitation, wherein DEHP seems to have hindered the photoelectron transfer process. Both superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) content increased in the roots, stems, and leaves in plant under DEHP treatment compared with those in the control plants. Antioxidant enzyme activity increased with increasing DEHP stress, except under the 40 mg/kg treatment at the seedling stage. The antioxidant system had a certain protective effect on wheat, but DEHP still caused peroxidation of cell membrane lipids. The extent of DEHP damage to the roots, stems, and leaves was concentration dependent. Furthermore, enzymatic activity tolerance increased with metabolism, and long-term effects of DEHP gradually decreased with plant growth. Finally, the toxic effects of DEHP on root tissues were more serious at the seedling and jointing stages.

1. Introduction

Phthalate acid esters (PAEs) are common industrial chemicals

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that are widely used in different kinds of manufacturing activities. For example, they are used as adhesives and plasticizers for polyvinyl chloride (PVC) materials to improve flexibility and processability (Gomez-Hens and Aguilar-Caballos, 2003). DEHP is one of the most common PAEs; indeed, it represents over 40% of the total PAEs manufactured in the world. Since DEHP does not chemically bond with PVC resin, DEHP can actually leak into the environment during production and the use of PVC products, thereby polluting a range of environmental components such as water, air, sediment, soil, and plant, as well as food and beverage (Wang et al., 2012, 2013, 2014; Wu et al., 2013; Liu et al., 2014; Sakhi et al., 2014). Xu et al. (2008) reported that the concentration of DEHP ranged from 0.01 to 1.11 mg/kg in agricultural soils of the Jiangsu Province, along the Yangtze River region (Jiangsu, China). Similarly, Xu et al. (2008) reported a DEHP content of around 1.15–7.99 mg/kg (average 4.858 mg/kg) in Handan soils and 0.49–4.20 mg/kg in Harbin soils (average 2.35 mg/kg).

Like many other persistent organic pollutants, DEHP is highly teratogenic, mutagenic, and carcinogenic. A previous study has found that DEHP can retard antral follicle growth, induce atresia, and inhibit steroid hormone production in mouse (Hannon et al., 2015). Moreover, DEHP can change the expression of peroxisome proliferator-activated receptors in mammals, and decrease the ability to remove reactive oxygen species (ROS) (Mathieu-Denoncourt et al., 2015). In addition, some researchers have reported that DEHP can be absorbed by plants from contaminated soils. Thus, Yin et al. (2003) found that vitamin C and capsaicin content in *Capsicum* fruits decreased with increasing soil DEHP content, implying that the quality of capsicum fruits was affected by the concentration of DEHP in the soil (Yin et al., 2003). Additionally, DEHP can influence the growth of Chinese flowering cabbage and significant differences in DEHP tolerance exist among cultivars (Zhao et al., 2015).

Photosynthesis is an oxygenic, sunlight-driven process. Being photoautotrophic organisms, plants assimilate atmospheric carbon dioxide (CO_2) into organic molecules for their own use. Three different photosynthetic pathways exist in different terrestrial plant species. They are named C_3, C_4, and crassulacean acid metabolism (CAM) pathways. The C_3 photosynthesis pathway is the most widespread among land plants, such as wheat (*Triticum aestivum*). Photosynthesis is influenced by many factors, including stomatal conductance (G_s), transpiration rate (Tr), C_i, chlorophyll content, and chlorophyll fluorescence. Leaf photosynthetic pigments capture light energy; thereby, climbing to an excited energy state, which is at the base of the photosynthetic capacity of the plant (Qiu et al., 2013). The most important among these pigments is chlorophyll. In addition, a strictly light-driven electron transport chain is one of the key elements in the light reactions of photosynthesis (Nanda and Agrawal, 2016). Along with photoelectron transfer, ROS are generated. Previous studies have found that excess ROS generation can have a negative effect on plants, as shown in soybean (*Glycine max*) photosynthesis (Xu et al., 1999). As environmental signals, PAEs can obstruct normal physiological and biochemical plant metabolic activities. However, higher plants can turn on antioxidant activities to defend against stress conditions, such as salinity, irradiation, low temperature, organic pollutants, and heavy metals (Ma et al., 2013). Several antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and ascorbate peroxidase (APX) are used to regulate the presence of ROS in plant tissues. These enzymes protect plant tissues under various abiotic stress conditions. Malondialdehyde (MDA) is a common indicator used to determine the level of oxidative damage in tissues. Thus, photosynthetic parameters, ROS, and antioxidant enzymes may help in understanding how DEHP affects plant growth.

In recent years, there has been growing concern about DEHP toxicity in different plants (Yin et al., 2003; Liu et al., 2016). Our own previous studies showed that DBP and DEHP induced changes in photosynthesis, ROS, and antioxidant enzymes in wheat seedlings grown hydroponically (Gao et al., 2016, 2017). However, less attention has been paid to understanding the response mechanism of growing wheat to PAEs. Therefore, our aim here was, (1) to understand the defense response of the photosynthetic machinery and antioxidant enzymes in growing wheat plants against DEHP, and (2) to elucidate the mechanism of DEHP toxicity in wheat.

2. Materials and methods

2.1. Materials

2.1.1. Reagents

Standard DEHP reagent (96.8% purity, CAS: 117-81-7) was purchased from Lark Technology Co., Ltd. (Beijing, China). Methanol was obtained from Thermo Fisher Scientific (Chinese) Co. Ltd. (China, Shanghai), and Sodium hypochlorite was purchased from Tianjin Branch Miou Chemical Reagent Co., Ltd. (Tianjin, China). Assay kits for SOD, CAT, GSH-Px, APX, H_2O_2, MDA, and total protein (analytical purity) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Other reagents were obtained from Tianjin Guangfu Fine Chemical Engineering Co., Ltd. (Tianjin, China). Distilled water was prepared by a Barnstead E-Pure water purification system (Thermo Scientific, Dubuque, IA).

2.1.2. Soil samples

Cinnamon soils (0–30 cm layer) were collected from Shanxi, China. Soil samples were air-dried in the shade and sieved through a 3 mm sieve. Soil alkali-N (AN), Olsen-P (OP), avail-K (AK), total N (TN), total P (TP), total (TK), and organic matter (OM) were determined according to Lu (1999). The results of soil analysis are summarized in Table 1.

2.2. DEHP exposure experiments

2.2.1. Soil contamination

Three different volumes (0.15 mL, 0.3 mL, and 0.6 mL) of DEHP standard solution were diluted in 1000 mL methanol. The concentrations of stock solutions were 150 mg/l, 300 mg/L, and 600 mg/L, respectively. Soils were polluted using the gradient dilution method. The different DEHP concentrations were added into 1.5 kg of soil. Soils were thoroughly mixed, and then, 13.5 kg of clean soil were added gradually. After thorough mixing, DEHP concentrations in the prepared soils were 10, 20, and 40 mg/kg (dry weight). DEHP treated soils were vigorously stirred for 1 h to evaporate methanol completely. Subsequently, soil moisture was adjusted to about 60% of field capacity by adding deionized water and allowing 5 days for attaining equilibrium. Methanol-treated plants and controls were prepared. The methanol group was treated with 2% of co-solvent methanol. Three replications per treatment were prepared.

2.2.2. Wheat cultivation

Wheat seeds ('Jingqiang 8') were obtained from the Agro-Environmental Protection Institute, Ministry Agriculture (Tianjin, China). Seeds were sterilized in 0.3% sodium hypochlorite for 30 min and washed with distilled water before seeding. Polypropylene pots (length × width × height = 18 cm × 16 cm × 22 cm) were filled with the prepared soil samples. Next, urea, potassium dihydrogen phosphate, and potassium chloride were applied as base fertilizers. Finally, N, P, and K content were determined to be 0.73, 1.03, and 24.3 g/kg of soil, respectively. Distilled water (300 mL) was added to the soil to adjust soil moisture to about 60%