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Effects of phenanthrene on seed germination and some physiological activities of wheat seedling

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are one of the highly persistent organic pollutants, and they are toxic to plants and other living organisms, including human beings. To analyze the response of higher plant to PAHs, we investigated the effects of phenanthrene (PHE) on seed germination and various physiological changes of wheat seedlings. Specifically, we investigated growth, chlorophyll content, lipid peroxidation (LPO), activities of antioxidant enzymes and H_2O_2 accumulation. The results showed that PHE inhibited seed germination, affected the growth and chlorophyll level of wheat seedlings. Furthermore, PHE elevated the levels of LPO and induced H_2O_2 accumulation in leaf tissues in a dose-dependent manner, accompanied by the changes in the antioxidant status. The activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), displayed a decreasing trend with the increasing of PHE concentration. The results indicated that PHE could exert oxidative damages in the early development stage of wheat and the harmfulness occurred mainly in samples with higher concentrations of PHE.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are one of persistent organic pollutants (POPs) with carcinogenic and mutagenic properties [1,2]. PAHs are mainly produced from incomplete combustion of organic materials and fossil fuel, and they exist in the different environmental matrices, including soil, water, and sediment [3–6]. They are persistent in various environmental media and enter the plants through their leaf and/or root systems. Subsequently, they are transferred to the food chains and threaten human health [5,7]. Therefore, an improved understanding of PAHs toxic mechanism is essential for the assessment of the risk of PAHs exposure.

Recently, the toxic effects of PAHs on plants have been extensively documented [8–12]. It has been reported that PAHs inhibited the growth of plants, caused a decrease in the photosynthesis of antioxidant enzymes, and even led to lipid peroxidation, DNA damage in plants [13–15]. The degree of toxicity varies with the kind of PAHs and the species of plants [12,16]. Liu et al. [14] suggested that excessive production of ROS in plants was a biochemical response to PAHs. In general, oxidation of PAHs results in the generation of ROS, which in turn causes oxidative stress and subsequent damage to plant cells. However, plants have enzymatic systems, including free radical scavengers, like superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) [17]. When the elevated levels of ROS exceed the levels that the antioxidant systems can handle, the damage may occur. Thus, it is important to

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understand the responses of plant system to environmental stress by determining the occurrence of oxidative damages and the changes in antioxidant enzymes' activities in plant.

Although many studies have been published on PHE's phytotoxicity against different plants, less attention has been paid to seed germination and growth of wheat seedlings. Wheat is one of the main grains in China. Its quality directly affects people's life and food security [18]. Thus, to get a better insight into the mechanism of PAHs toxicology on wheat is essential to an effective environmental management. The objective of this study was to elucidate the effects of PHE on seed germination and growth of wheat seedlings. Variations of the activities of SOD, CAT, glutathione peroxidase (GPX), the content of chlorophyll, and malondiadehyde (MDA) in wheat seedlings were detected, and the probable mechanisms were discussed.

2. Materials and methods

2.1. Plant materials and growth conditions

Authentic wheat seeds were obtained from the Institute of Agriculture Science in Shanxi Province, Taiyuan, China. The wheat (Yunmai 494) seeds were surface sterilized with 0.1% $HgCl_2$ for 10 min, and soaked in water for 12 h. Then, the seeds were randomly divided into four groups, with each group consisting of at least 100 seeds and transplanted in 30-cm diameter Petri dishes with two filter papers. Seeds were exposed to PHE in three different concentrations. The negative control group was exposed to distilled water for the same time period. The treatment was replicated three times. All of the test groups were maintained in an incubator at 25 ± 1 °C under a dark/light cycle (14/10 h). Plants were fertilized daily with water and Hoagland's nutrient solution. The germination rate would be counted after seven days. Seedlings were harvested after 15 days for biochemical and physiological studies, and the leaf samples were immediately frozen in liquid nitrogen and stored at −80 °C.

2.2. PHE preparation and treatment application

PHE (Sigma, 98% purity) was initially dissolved in acetone and then diluted with MilliQ water to get final concentrations of 0.05, 0.1, 0.2 mg/mL (acetone:water, v:v = 1:1000). Then, three groups of seeds were sprayed with each concentration of freshly prepared PHE. Control seeds were sprayed with MilliQ water containing equal concentrations of acetone. The concentration of acetone used in the current experiment was lower than 0.5%, which had been reported as having no negative effects on tomato seedlings [15].

2.3. Germination energy, germination rate and length of root and stem

The number of germinated seeds on the seventh day after initiation was defined as the germination rate. After fifteen days of growth, the roots and stem lengths of 100 seedlings in each group were randomly measured. Three replicates were conducted from each experimental unit.

2.4. Chlorophyll content measurements

A fresh leaf sample (0.2 g) was pulverized with distilled water and the homogenate was extracted using 80% acetone. Chlorophyll a (chl *a*) and chlorophyll b (chl *b*) were determined by spectrophotometry according to the procedure of Sang et al. [19].

2.5. Determination of enzyme activity

For the extraction of enzymes, 0.5 g of the leaf tissues was homogenized in 5 mL of an ice-cooled phosphate buffered solution (0.05 M, pH 7.8) containing 0.2 M EDTA and 2% polyvinylpyrrolidone (w/v). The homogenate was centrifuged at 12,000 rpm and 4 °C for 20 min. The supernatant was used immediately to determine the enzyme activity and the contents of MDA and H_2O_2.

SOD activity was measured by nitroblue tetrazolium (NBT) spectrophotometry [20]. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of reduction of NBT as monitored at 560 nm and the enzymes activity was expressed as $U \cdot (g\ FW)^{-1}$.

CAT activity was tested through the absorbance decrease at 240 nm using the ultraviolet absorbance method [21]. Results were expressed as $U\ (min\ g\ FW)^{-1}$.

GPX activity was determined according to the method of Lawrence and Burk [22]. The reaction mixture contained the enzyme extract, 0.2 mM NADPH, 1 mM sodium azide (pH 7.0), 1 mM GSH, 1 U glutathione reductase and 2 mM H_2O_2. The reaction was initiated by adding H_2O_2 and the absorbance was measured at 340 nm. One unit of GPX activity was defined as the amount of enzyme catalyzing the oxidation of 1 μmol of NADPH per minute [23].

2.6. Measurement of H_2O_2

The formation of H_2O_2 was measured colorimetrically as described by Ishida et al. [24]. The homogenate supernatant was incubated in 2 mL (24 h) or 5 mL (48 h) of the reaction mixture containing 50 mM Na-acetate buffer (pH 6.5), 1 mM 4-aminoantipyrine, 1 mM 2,4-dichlorophenol, 50 mM $MnCl_2$, and 0.2 mM NADH. The increase in absorbance was measured at 510 nm.

2.7. Determination of the MDA content

The MDA content was determined according to the method of Draper and Hadley [25]. Then, 0.2 g stems were homogenized in trichloroacetic acid (TCA) and centrifuged at 3000 *g* for 10 min. An amount of 200 μL of homogenate supernatant were mixed with 0.8 mL of 0.5% (w/v) thiobarbituric acid (TBA) and 20% TCA, then put in a boiling water-bath for 30 min. The absorbance was measured at 532 nm. The value for non-specific absorbance at 600 nm was subtracted.