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Effect of octylphenol on physiologic features during growth in *Arabidopsis thaliana*

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HIGHLIGHTS

- The damage of octylphenol (OP) on *Arabidopsis thaliana* has been studied for the first time.
- The plants showed worse physiologic features and germination with increased OP concentration.
- OP caused levels of chlorophyll decreased and proline accumulated in leaves.
- OP caused oxidative stress on *A. thaliana.*
- APX, CAT, CuZnSOD activity was significantly increased with OP treatment.

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ABSTRACT

Alkylphenol ethoxylates are widely used as detergents, emulsifiers, solubilizers, wetting agents and dispersants. Octylphenol (OP) ethoxylates, one of alkylphenol ethoxylates, represent 15–20% of the market, and their metabolic residues may be discharged to surface waters, sediments and soils as a persistent and ubiquitous pollutant. We tested the response of *Arabidopsis thaliana* to different concentrations of OP. OP affected the germination percentage and mean germination period. 10 d treatment with OP, especially high concentration (10 and 50 mg L^{-1}), decreased shoot and root biomass and root length of 30 d-old *A. thaliana*. Content of chlorophyll was decreased but that of proline was increased in leaves with OP treatment. OP caused oxidative stress in leaves; malondialdehyde content was increased, and the activities of ascorbate peroxidase, catalase and superoxide dismutase were induced. OP affects the physiologic and morphologic features of *A. thaliana* during growth. Because plants might be exposed to OP for a long time in the surroundings, more attention needs to be paid to the effect of OP on plants.

1. Introduction

Alkylphenol ethoxylates (APEs) are widely used as detergents, emulsifiers, solubilizers, wetting agents and dispersants. The most used APEs derived from nonylphenol (NP) are called nonylphenol ethoxylates (NPEs) and represent about 80% of the total market volume and octylphenol (OP), called octylphenol ethoxylates (OPEs), represent about l5–20% of the total APE market (Staples et al., 1999). Due to the harmful effects of the degradation products of NPEs in the environment, the use and production of such compounds have been banned in EU (Soaresa et al., 2008).

Because of the wide use of OPEs, their metabolic residues may be discharged to surface waters, sediments and soils (Bennie et al., 1997; Ferguson et al., 2001). In recent years, OP was found a persistent and ubiquitous environmental pollutant with endocrine-disrupting properties and caused secreting vitellogenin in rainbow trout (Jobling and Sumpter, 1993). However, few studies have investigated OP toxicity in plants. OP can be taken up by crops and then enter the food chain by (1) OP-containing irrigation water, (2) pesticide adjuvants, and (3) accumulation in agricultural soils due to industrial sewage in agricultural fields (Krogh et al., 2003; Pedersen et al., 2005; Sánchez-Avila et al., 2009). More attention needs to be paid to the effect of OP on plants.

Arabidopsis thaliana is a well-known model plant. It has been widely investigated under environmental stresses such as ozone, extreme temperatures and polychlorinated biphenyl exposure (Asai et al., 2002; Tamaoki et al., 2004; Miles et al., 2005). *Arabidopsis thaliana* was the first plant to have its genome sequenced by The Arabidopsis Genome Initiative (2000), and is a popular tool for understanding the molecular biology of many plant traits. Therefore, in this study, we researched the behavior of *A. thaliana* under organic stress.

In this study, we aimed to explore the effects of the organic-pollutant OP on the growth, physiological performance, and

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antioxidative defense systems of *A. thaliana* under hydroponic cultivation.

2. Materials and methods

2.1. Chemicals and reagents

OP (99.1% purity, CAS No. 68152-92-1) was from Chemservice Co. (USA). Other chemicals were from Sigma Chemical Co. (USA). Individual OP stock was dissolved in methanol at 10 000 mg L^{-1} before use.

2.2. Plant preparation for germination percentage, mean germination period

Seeds were placed in an eppendorf tube with 1 mL of 75% EtOH and vertexed for 1 min. After removing the EtOH, we added ddH_2O (1–2% NaOCl + 0.1% tween80) and vertexed for 15 min. Clean the seeds by sterile water after removing above ddH_2O avoid to disturbing germination. We germinated 50 sterilized seeds of *A. thaliana* ecotype Columbia in 1/2 MS solid medium. OP was added at 0 (control), 0.1, 1, 10 and 50 mg L^{-1}. Tween80, 0.1%, was applied to distribute the OP uniformly. In order to confirm the effect of OP and find out that tween 80 in the mix with OP does not affect the tests, tween80 treatment was set up as a vehicle control. After 2 d jarovization at 4 °C, transfer the plants into plant growth chambers, the growth conditions were 12 h light/12 h dark at 25 °C and relative humidity 60%. All experiments were performed in quadruplicate.

The germination of seeds was observed at days 0, 3, 6, 9, 12. Germination percentage was calculated as follows:

$$\text{Germination percentage (G)} = 100 * N/S$$

$$\text{Mean germination period} = 1/[\Sigma(D * n)]/\Sigma n$$

where N is number of seeds for germination, S number of seeds, D the record rate for seed germination, and n is the number of seeds for germination at time D.

2.3. Incubation experiment of A. thaliana with OP

Seeds of *A. thaliana* (ecotype Columbia) were cultured in pots containing 320 mL water culture solution modified from Murashige and Skoog solution (Murashige and Skoog, 1962). The pH value of the water culture solution was adjusted to 5.7 with HCl. After 7 d incubation, each germinated seed was grown in one pot under 12 h light/12 h dark at 25 °C and relative humidity 60%. All experiments were performed in quadruplicate. We totally prepared 48 plants, 24 plants of them were used for antioxidant enzymes test, and the remaining plants were used for other tests.

The germinated seeds were incubated for 30 d after 7 d of their pre-incubation, OP was added to the water culture at 0 (control), 0.1, 1, 10 and 50 mg L^{-1}. Tween80 at 0.1% was applied to distribute OP uniformly. Tween80 treatment was set up as vehicle control. The appearance and morphologic features of plants as well as leaf damage were recorded. After 10 d of OP treatment, plants were harvested and all leaves were excised. Root length and fresh and dry weight were measured.

2.4. Analysis of chlorophyll (a + b) concentration

An amount of 0.1 g fresh leaves was homogenized with 2 mL sodium phosphate buffer (pH 6.8). Chlorophyll was extracted with ethanol (100%), and solution rested in the dark for 30 min. After centrifugation at 1000*g* for 10 min, level of chlorophyll ($a + b$) was measured by spectrophotometry (Wintermans and Mots, 1965). The content was calculated as follows:

$$\text{Chlorophyll } (a+b) \text{ level } (\mu\text{g Chl mL}^{-1}\text{ FW}) = 6.1 * A_{665} + 20.04 * A_{649}$$

2.5. Analysis of protein concentration

An amount of 0.1 g leaves was ground in liquid nitrogen and 5 mL sodium phosphate buffer (50 mM, pH 6.8); 100 μL homogenized solution was placed in a 1.5 mL tube and 20 μL supernatant was transferred to a new tube after centrifugation at 12 000*g* for 20 min. An amount of 1 mL dye solution (Coomassie Brilliant Blue G-250, 0.01%; ETOH, 4.45%; phosphoric acid, 8.5%) was added, and the tube was shaken. After 10 min, protein level was measured at 595 nm by spectrophotometry (Hitachi U-1100) (Peterson, 1983). A standard curve was created by detecting 0–2.0 mg mL^{-1} standard protein.

2.6. Analysis of proline concentration

An amount of 0.1 g fresh leaves was homogenized in liquid nitrogen and 1 mL sulfosalicylic acid (3%, w/v); 0.7 mL homogenized solution was transferred to a 1.5 mL tube. After centrifugation at 10 000*g* for 20 min, 0.5 mL of supernatant was transferred to a glass tube with 100 °C water bath for 60 min after the addition of 1 mL chromogenic solution (0.25 g ninhydrin in 10 mL acetic acid), then the glass tube was immediately placed in an ice bath to finish the reaction. An amount of 2 mL toluene was added to the tube for vortexing for 15 s, then the tube rested for 10 min. The absorption of the toluene layer was measured at 520 nm by spectrophotometry (Bates et al., 1973). A standard curve was created by detecting 0–0.04 mg mL^{-1} L-proline in the toluene.

2.7. Analysis of malondialdehyde (MDA) concentration

An amount of 0.1 g fresh leaves was homogenized in liquid nitrogen and 5 mL sodium phosphate buffer (50 mM, pH 6.8); 1 mL homogenized solution was transferred in a 1.5 mL tube, and 0.5 mL trichloroacetic acid (9%, w/v) was added. After centrifugation at 10 000*g* for 5 min, 1 mL supernatant was transferred to a glass tube. An amount of 4 mL 2-thiobarbituric acid solution [(0.5 g 2-thiobarbituric acid dissolved in 100 mL trichloroacetic acid (20%, w/v)] was added into each glass tube, and tubes were placed in a 95 °C water bath for 30 min, then reacted with an ice bath. After de-gassing by use of an ultrasonic cleaner, the tube was centrifuged at 3000*g* for 10 min. The samples were measured at 532 nm and 600 nm by spectrophotometry (Heath and Packer, 1968). MDA content was calculated as follows:

$$\text{MDA content } (\text{nmol g}^{-1}) = (A_{532} - A_{600}) \div 155\ (\text{mM}^{-1}\ \text{cm}^{-1}) * 5\ (\text{mL}) * 5 * 1.5 * 1000 \div \text{FW (g)}$$

2.8. Extraction and determination of antioxidant enzymes

Leaf tissue was homogenized with liquid nitrogen and 0.1 M sodium phosphate buffer (pH 6.8). After centrifugation at 12 000*g* for 20 min, the supernatant was used for determining enzyme activity and protein content. Ascorbate peroxidase (APX) activity was determined as described (Nakano and Asada, 1981). One unit of catalase (CAT) enzymatic activity was defined as the amount of enzyme that degraded 1 μmol H_2O_2 per minute (Kato and Shimizu, 1987). Glutathione reductase (GR) activity was determined as described (Foster and Hess, 1980). One unit of SOD was defined