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A preliminary analysis of the effects of bisphenol A on the plant root growth via changes in endogenous plant hormones

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ABSTRACT

Bisphenol A (BPA) is ubiquitous in the environment worldwide, affecting plant growth and development. Endogenous plant hormones serve as switches that regulate plant growth and development. However, plants have different physiological requirements and environmental adaptive capacities during the different growth stages. Here, we investigated the effects of BPA on soybean (*Glycine max* L.) root growth at the three growth stages and analyzed the mechanisms underlying the effects of BPA on the root growth by assessing changes in endogenous hormone. The results showed that low concentration of BPA (1.5 mg L^{-1}) improved root growth (except at the seed-filling stage), increased indole-3-acetic acid (IAA) content at the first two growth stages, and increased zeatin (ZT) content and decreased gibberellic acid (GA_3) content at the seedling stage. But low concentration of BPA caused decreased ethylene (ETH) contents and constant abscisic acid (ABA) content at all three stages. However, BPA at moderate and high concentrations (6.0 and 12.0 mg L^{-1}) inhibited root growth, causing the decreased IAA, GA_3 and ETH contents and increased ABA content at all three growth stages. The change degrees of above indices were weakened with prolonging the growth stages. After BPA withdrawal, both the root growth and the hormone contents recovered (with the exception of ZT and ETH), and the recovery degrees had negative correlation with the BPA exposure concentration and had positive correlation with the growth stage. Changes in residual BPA content in the roots were also observed at different BPA concentrations and different growth stages. Our results demonstrated the effects of BPA on root growth were related to BPA-induced changes in hormone, which performed differently at various growth stages.

1. Introduction

Bisphenol A [BPA; 2,2-bis(4-hydroxyphenyl) propane] is a raw material widely used in the industrial synthesis of polycarbonate, polysulfone resin and epoxy resin and is thus applied in the production of many commodities, such as hard disks, solid dental sealants, baby bottles and food packaging (Geens et al., 2011; Huang et al., 2012). The large production volume and broad application of BPA has resulted in it being ubiquitous in the environment (Arditsoglou and Voutsa, 2010; Da et al., 2016; Navarro-Ortega and Barceló, 2010). Investigations have shown that the average BPA content in the air is 0.51 ng m^{-3} with a maximum value of 208 ng m^{-3} in Osaka, Japan (Vandenberg et al., 2007). BPA content has been found to be 800 ng g^{-1} in soil in Denver, USA (Burkhardt et al., 2005), and 3.92 μg L^{-1} in the estuary of Pearl River, Guangzhou, China (Dong et al., 2009). In Japan, BPA contents have been recorded as high as 370 μg L^{-1} and 17.2 mg L^{-1} in paper mill effluent and landfill leachate, respectively (Yamamoto et al., 2001).

BPA has also been identified as being a typical environmental endocrine disruptor; toxicological studies on animals have shown that BPA can disturb the physiological functions and the endocrine system, particularly the genital system of animals, including humans (Crain et al., 2007; Liu et al., 2011; Takagi et al., 2004). Plant roots come into directly contact and absorb BPA in soil, which may provide a route for BPA to enter the food chain through crops, thus leading to food safety issues (Flint et al., 2012). At present, several studies about the effects of

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BPA on terrestrial plants have been reported (Adamakis et al., 2016; Ali et al., 2015; Chang et al., 2015; Ferrara et al., 2006; Jiao et al., 2015; Loffredo et al., 2010; Nie et al., 2015; Pan et al., 2013; Qiu et al., 2013; Rąpała et al., 2017; Saiyood et al., 2010; Speranza et al., 2011; Staples et al., 2010; Sun et al., 2013a, 2013b; Terauchi et al., 2010; Wang et al., 2015a, 2015b; Xia et al., 2017; Zhang et al., 2016, 2015). A study has found that 1.15 mg L^{-1} BPA is a threshold to inhibit seed germination of *Arabidopsis thaliana* (Pan et al., 2013); a treatment with 46 mg L^{-1} BPA has been found to suppress the root lengths of perennial ryegrass (*Lolium perenne*) and radish (*Raphanus sativus*) (Loffredo et al., 2010). Moreover, treatments with BPA at concentrations higher than 17.2 mg L^{-1} have been shown to inhibit root growth in soybeans (Wang et al., 2015b), and BPA concentrations ranging from 120 to 320 mg L^{-1} decreased stem dry weight of corn (*Zea mays*), cabbages (*Brassica oleracea*), and oats (*Avena sativa*) (Staples et al., 2010). BPA concentrations ranging from 10 to 50 mg L^{-1} were identified to inhibit pollen tube formation and elongation in kiwis (*Actinidia deliciosa*) in a manner directly related to the BPA concentration (Speranza et al., 2011). In addition, studies investigating the mechanisms underlying the effects of BPA on plant growth have shown that BPA can alter physiological processes, such as respiration (Nie et al., 2015), photosynthesis (Jiao et al., 2015; Qiu et al., 2013; Zhang et al., 2015), the antioxidant system (Ali et al., 2015; Wang et al., 2015a; Zhang et al., 2016), and mineral nutrition (Sun et al., 2013a, 2013b).

Plant roots hold plants in place, absorb water and nutrition, and synthesize hormones etc. (Hodge et al., 2009), which result in them being easily affected by BPA. Therefore, investigating the effects of BPA on root growth will provide results that are closer to reality. In addition to the aforementioned physiological processes, there might be other regulatory pathways through which BPA affects root growth. It is essential to further elucidate the effects of BPA on plant growth. Endogenous plant hormones [mainly including indole-3-acetic acid (IAA), Zeatin (ZT), gibberellic acid (GA_3), abscisic acid (ABA) and ethylene (ETH)] are “switches” that regulate plant growth, directly controlling the growth and morphogenesis of plants (Vanstraelen and Benková, 2012), and improving the adaptation of plants to their environment to complete their life cycles. It is therefore important to investigate if a relationship exists between the effects of BPA on plant root growth and the changes in endogenous hormone, and to examine what differences exist between the effects of BPA on hormone at different growth stages. In this study, therefore, we examined the effects of BPA on plant root growth at the three stages (the seedling stage, the flowering and podding stage, and the seed-filling stage), and investigated the mechanisms underlying root growth changes through an analysis of endogenous hormone at different growth stages. Soybean, a plant recommended by the United States Environmental Protection Agency for toxicological studies (Raney, 2006), was chosen as the experimental material. This study sought to gain a comprehensive understanding of the pathways through which BPA affects plant growth and development, results of which aim to provide a reference for objective and accurate evaluation of the potential ecological risks of BPA.

2. Materials and methods

2.1. Preparation of BPA solution

BPA with a purity of 99% was provided by Sigma-Aldrich Co. Ltd. (Shanghai, China). In accordance with current levels of BPA detected in the environment, and the increasing trend of BPA in soils, we designed four treatments: a control treatment (0.0 mg L^{-1} BPA), a low-concentration treatment (1.5 mg L^{-1} BPA), a moderate-concentration treatment (6.0 mg L^{-1} BPA) and a high-concentration treatment (12.0 mg L^{-1} BPA). 1.5 mg L^{-1} represents the upper concentration limit for drinking water according to the U.S. Environmental Protection Agency (Geens et al., 2011), and BPA concentrations of 6.0 and 12.0 mg L^{-1} have been observed in soil, river sediment and landfill leachate (Huang et al., 2012; Sun et al., 2005; Yamamoto et al., 2001). These three concentrations were usually selected to study the effects of BPA on plants (Ali et al., 2015; Nie et al., 2015; Zhang et al., 2016). The BPA solutions were prepared by dissolving the corresponding quantities of BPA in half-strength Hoagland's nutrient solution (pH 7.0).

2.2. Plant cultivation and treatment with BPA

Soybean seeds (*Glycine max* L.) (Zhonghuang 25, Wuxi seed Co., Ltd., China) were germinated and the seedlings were cultured according to previous reports (Jiao et al., 2015). When the seedlings reached approximately 4 weeks of age (at the seedling stage), they were transplanted into the BPA solutions with the different concentrations. Simultaneously, some plants were cultivated in half-strength Hoagland's solution without BPA to serve as the control group. Each treatment was repeated five times. Plant roots were exposed to the corresponding BPA solution for 7 d and then transplanted into the half-strength Hoagland's solution without BPA for another 7 d. All of the solutions were renewed every 3 d. The roots were collected for measurements after BPA exposure for 7 d and after BPA withdrawal for 7 d. As root samples used to determine hormones and BPA contents must be rapidly preserved, liquid nitrogen was used to freeze the samples before they were stored in an ultra-low temperature freezer (− 80 °C) until analysis.

When plants with both the flowers and the young pods (approximately 2 cm in length) accounted for 50% of all plants (the flowering and podding stage), soybean plants with the same growth status were treated with BPA. When plants with clearly bulging pods accounted for 50% of all plants (the seed-filling stage), these were then treated with BPA. The treatment methods used were the same as those used for plants at the seedling stage.

2.3. Determination of the root growth indices

Root morphological indices (length, surface area and volume) were determined using root automatism scan apparatus (Perfection V700 Photo, Seiko Epson Corp, Japan) with WinRHIZO software (version 2009a, Regent Instruments, Quebec, Canada). Harvested fresh roots were washed and dried using absorbent paper before being promptly weighed using an electronic balance. The roots were then dried in a drying over at 80 °C until a constant weight was recorded.

2.4. Determination of the endogenous hormone contents in the roots

The pretreatment of the root samples (3.00 ± 0.01 g) before hormone analysis included extraction, evaporation and purification, which were performed according to the previously described procedures by Yang et al. (2014). Finally, the hormone samples were re-dissolved in 2 mL of methanol for high performance liquid chromatography (HPLC) analysis. The Varian 9050 HPLC system, coupled with a UV detector, Varian 9010 pumps and a Nukleosil C_{18} column (4.6 mm × 150.0 mm), was used for the analysis. The mobile phases were 100% methanol (A) and 0.75% acetic acid solution (B), and the wavelength was set to 254 nm. The detailed elution procedure and other operating parameters were set in accordance with the above literature (Yang et al., 2014).

Ethylene evolved from soybean roots was determined according to a previously mentioned method with some modifications (Yang et al., 2006). Fresh roots (2.00 ± 0.01 g) were placed on moist paper for 30 min at 27 °C in darkness to allow wound-induced ethylene release, then transferred to 20-mL glass vials and rapidly sealed. Ethylene was gradually released by keeping the vials in a dark place for 12 h at 22 ± 1 °C. Ethylene was assayed using a gas chromatograph (Hewlett Packard 5890 Series II) equipped with a Porapak Q column (3.175 mm od × 1.2 m) fueled with activated alumina and a flame ionization detector (FID). The temperatures for the injection port, column and detector were kept constant at 90, 90 and 200 °C, respectively.