Environmental Pollution xxx (2017) 1–9

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Bisphenol A effects on the chlorophyll contents in soybean at different growth stages☆

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ARTICLE INFO

Article history:
Received 2 November 2016
Received in revised form
15 January 2017
Accepted 16 January 2017
Available online xxx

Keywords:
BPA
Chlorophyll
Intermediates
Key enzymes
Different growth stages

ABSTRACT

Bisphenol A (BPA), a suspected endocrine disruptor, can modify normal plant growth and development. Photosynthesis provides material and energy for the growth and development of plants, in which chlorophyll (Chl) plays a significant role. Many studies have shown that the growth and metabolism of plants vary at different growth stages. Thus the sensitivity of plant's responses to environmental pollution is correspondingly different. We studied the effects of BPA on the Chl contents of soybean (*Glycine Max* L.) at different growth stages (seedling, flowering and podding, seed-filling and maturation) by measuring the contents of essential intermediates (5-aminolevulinic acid, porphobilinogen, protoporphyrin IX, magnesium protoporphyrin and protochlorophyll) and the activities of key enzymes (5-aminolaevulinic acid dehydratase, porphobilinogen deaminase, uroporphyrinogen III synthase, magnesium chelatase) in chlorophyll synthesis. Low-dose (1.5 mg/L) BPA exposure increased the activities of key enzymes in addition to the contents of intermediates in Chl synthesis at different growth stages, resulting in increases in Chl contents and net photosynthetic rate. In contrast, medium and high-dose (17.2, 50.0 mg/L) BPA exposure produced inhibitory effects on the indices. Following the withdrawal of BPA exposure, the indices recovered to a degree that was related to the plant growth stage. The effect level (high to low) of BPA on these indices at different growth stages was: seedling stage > maturation stage > flowering and podding stage > seed-filling stage. The reverse effect was observed following the withdrawal of BPA exposure. The responses of key enzymes in plant Chl synthesis to BPA illustrate how BPA affects Chl contents. The effects of BPA show clear differences at different plant growth stages.

1. Introduction

Bisphenol A [BPA; 2, 2-bis (4-hydroxyphenyl)] is an important chemical typically used in the production of polycarbonate, epoxy resins, and fire retardant coatings. These products are widely used in the formulation of for commercial products and commodity items such as antioxidants, bottles, medical equipment, and thermal sensitive papers (Vandenberg et al., 2007). The global annual production of BPA may exceed 680 million tons, making it one of the most significant compounds currently being manufactured (Jandegian et al., 2015). Due to its massive production, wide use, and continuous release, BPA is ubiquitous in the environment (air, water, soil, indoor dust) (Flint et al., 2012; Huang et al., 2012). The existing literature shows that in the global water environment, the content of BPA ranges from 4.4 to 756 μg/L (Liu et al., 2017; Muñoz et al., 2009; Singh et al., 2010). The range of BPA content in irrigation water is approximately 0.265–4.67 μg/L (Chen et al., 2011; Crain et al., 2007; Qin et al., 2015). Moreover, the BPA contents in soil and agricultural soil are in the ranges of 0.1–21.3 μg/g (Huang et al., 2014; Qin et al., 2015) and 0.08–0.147 μg/g (Gibson et al., 2010; Kinney et al., 2008; Michałowicz, 2014), respectively. Furthermore, the BPA content in the hazardous landfill leachates in

☆ This paper has been recommended for acceptance by B. Nowack.

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http://dx.doi.org/10.1016/j.envpol.2017.01.042
0269-7491/

Japan ranges from 1.3 to 17.2 mg/L (Yamamoto et al., 2001). BPA has become a major environmental pollutant and chemical of public concern. It has been reported that BPA has adverse impacts on animals and humans, including damage to reproductive function, immune function, and genetic materials (Gies and Soto, 2012; Rahman et al., 2015; Wong and Cheng, 2011). In contrast, the effects of BPA on plants are poorly known. The European Union has carried out a comprehensive environmental risk assessment based on the toxicity of BPA. The draft required studies to focus on the acute toxicity and the latent ecological risk of BPA to plants (Commision, 2008).

Terrestrial plants can absorb BPA from soil and transfer it to aboveground parts where it may influence growth and development (Nakajima et al., 2002). Previous studies on the effects of BPA on plants have mainly focused on three areas (Ferrara et al., 2006; Nakajima et al., 2002; Qiu et al., 2013). The first area involves BPA effects on plant growth and development. Low doses (0.01 and 1.5 mg/L) of BPA promoted growth of carrot (*Daucus carota* L.) and soybean (*Glycine max* L.) (Qiu et al., 2013; Sun et al., 2013; Terouchi et al., 2004). In contrast, high doses (10.0, 17.2 and 50.0 mg/L) of BPA significantly suppressed growth of broad bean (*Vicia faba* L.), lettuce (*Lactuca sativa* L.), wheat (*Triticum durum* Desf.), soybean, and tomato (*Lycopersicon esculentum* Mill.) (Ferrara et al., 2006; Qiu et al., 2013; Salazar-Parra et al., 2015; Sun et al., 2013). Moreover, 10.0 mg/L and higher dose of BPA inhibited the kiwifruit [*Actinidia deliciosa* (A. Chev.) C. F. Liang et A. R. Ferguson] pollen germination and tube elongation (Speranza et al., 2011). Meanwhile, 100 mg/L BPA inhibited the germination of chickpea (*Cicer arietinum* L.) seeds (Dogan et al., 2010). The second area involves the mechanism of BPA on plant growth with a focus on nitrogen metabolism (Sun et al., 2013), photosynthesis (Jiao et al., 2015; Qiu et al., 2013), hormones (Wang et al., 2015b), and the antioxidant system (Wang et al., 2015a). The third area of BPA studies includes the mechanisms by which plants absorb, transfer and degrade BPA (Nakajima et al., 2007). For example, tobacco (*Ipomoea aquatic* Forsk.) seedlings can absorb and metabolize BPA to glucoside (Noureddin et al., 2004). Potato (*Solanum tuberosum* L.), mushroom [*Agaricus bisporus* (lang.) Sing], and eggplant (*Solanum melongena* L.) and Yacon (*Samallanthus sonchifolius*) can degrade BPA by oxidation (Yoshida et al., 2002).

Photosynthesis is the foundation for plant growth and development. The photosynthesis process is complicated and includes primary reactions, electron transport, photophosphorylation, and CO_2 assimilation. Chlorophyll (Chl), is a critical component of the primary photosynthetic reaction and it has dual functions. It captures light and also serves as the medium for the separation and transport of electrons, laying the foundation for synthesis of subsequent photosynthetic products (Rüdiger, 2009). Therefore, studying how BPA affects Chl is essential. Some studies have demonstrated that BPA affects Chl and other physiological process in plants (Jiao et al., 2015; Qiu et al., 2013). However, plants at different growth stages have different physiological needs and different responses to environmental stresses. We therefore asked the following questions: Does BPA affect the Chl of plants at different growth stages? Are BPA effects distinct at different growth stages, and, if so, what causes these differences? What is the mode of action and pathways by which BPA affects plant Chl? What is the relevance of BPA-initiated plant effects in assessing the potential ecological risk of BPA? To address these questions, we assessed the effects of BPA on Chl contents and net photosynthetic rate (P_n) by studying Chl synthesis [essential intermediates: 5-aminolevulinic acid (ALA), porphobilinogen (PBG), protoporphyrin IX (Proto IX), magnesium protoporphyrin (Mg-Proto IX) and protochlorophyll (Pchl); key enzymes: 5-aminolaevulinic acid dehydratase (ALAD), porphobilinogen deaminase (PBGD), uroporphyrinogen III synthase (UROS), magnesium chelatase (MgCH)] of soybean at different growth stages. Our experimental results indicate probable plant responses to environmental BPA and provide a basis for evaluation of the ecological risks of BPA.

2. Materials and methods

2.1. Solution preparation

We considered the following factors in selecting BPA concentrations for this study: (1) BPA concentrations used in previous plant and animal research (Ferrara et al., 2006; Mandich et al., 2007; Mihaich et al., 2009; Saiyood et al., 2013); (2) current BPA global pollution status, especially BPA pollution in developing countries; (3) high environmental levels of BPA resulting from accidental contamination events (such as spills). Three representative concentrations (1.5, 17.2, 50.0 mg/L) were selected. The 1.5 mg/L concentration is the upper limit of BPA concentration for drinking water safety, established by the United States Environmental Protection Agency (Geens et al., 2011), and is a concentration commonly used to study BPA effects on plants (Dogan et al., 2010; Saiyood et al., 2013; Terouchi et al., 2004). The 17.2 mg/L concentration is the BPA concentration often found in landfill leachates (Yamamoto et al., 2001). The 50.0 mg/L concentration is a BPA concentration that could result from a major environmental contamination event (Ferrara et al., 2006; Saiyood et al., 2013). In addition, the 50 mg/L concentration is frequently reported as being harmful to plants and animals (Jiao et al., 2015; Noureddin et al., 2004; Rüdiger, 2009; Salazar-Parra et al., 2015). The BPA solutions (1.5, 17.2 and 50.0 mg/L) were made by adding technical BPA (Sinopharm Chemical Reagent Co., Ltd, China) to the one-half strength Hoagland's solution and sonicating at 25 °C. At this temperature, the water solubility of BPA is within the range of 120 to 300 mg/L and has low volatility (Ferrara et al., 2006; Mihaich et al., 2009).

2.2. Plant culture and treatment

Soybean seeds (Zhonghuang 25, Wuxi Seed Ltd., China) were disinfected in $HgCl_2$ (0.1%) solution for 5 min and then rinsed. A dish lined with layers of gauze was used to move the seeds. Then an incubator was used to germinate the seeds at 25 ± 1 °C. When the radicle lengths were approximately 1 cm, the seedlings were held using foam boards (polyethylene foam, with similar size and number of holes) and then transplanted into 10.5 L plastic pots (35 × 25 × 12 cm, six individuals per pot) filled with distilled water. As soon as the second true leaves appeared (around 10 d following germination), the plants were cultivated in the one-half strength Hoagland's solution (pH 7.0) within a greenhouse under 70/80% (day/night) relative humidity (RH), 25/20 °C (day/night) temperature, and a 14:10 (light:dark) photoperiod with a light intensity of 300 μmol/m^2/s measured at the top of the plants.

When the third true leaves were completely expanded (approximately 30 d after germination), 50 pots of healthy plants (six plants per pot) of similar size were selected to be treated with the prepared BPA solutions (1.5, 17.2 and 50.0 mg/L, pH 7.0). There were three randomly selected pots of soybean seedlings used for exposure to each dose of BPA solution. The control plants were cultured in half-strength Hoagland's solution without BPA. The solution was renewed every 3 d to ensure adequate nutrients for plant growth and maintaining stable pH values. After 7 d of BPA exposure, the new leaves at the same nodes that had completely expanded in plants at different growth stages were selected for determination of each index in the control and BPA treatment groups. The remaining plants were transferred to half-strength

Please cite this article in press as: Jiao, L., et al., Bisphenol A effects on the chlorophyll contents in soybean at different growth stages, Environmental Pollution (2017), http://dx.doi.org/10.1016/j.envpol.2017.01.042