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Phytotoxicity of chiral herbicide bromacil: Enantioselectivity of photosynthesis in *Arabidopsis thaliana*

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HIGHLIGHTS

- It is necessary to assess the direct effects of PSII herbicides on photosynthesis.
- Phytotoxicity of bromacil is investigated in an enantiomeric level.
- Bromacil disturbed enantioselectively the photosystem II of *Arabidopsis thaliana*.
- S-bromacil caused severer damage to photosynthesis of *Arabidopsis* than R-bromacil.
- Photosynthesis should be considered for phytotoxicity assessment of herbicides.

GRAPHICAL ABSTRACT

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ABSTRACT

With the wide application of chiral herbicides and the frequent detection of photosystem II (PSII) herbicides, it is of great importance to assess the direct effects of PSII herbicides on photosynthesis in an enantiomeric level. In the present study, the enantioselective phytotoxicity of bromacil (BRO), typical photosynthesis inhibition herbicide, on *Arabidopsis thaliana* was investigated. The results showed that S-BRO exhibited a greater inhibition of electron transmission in photosystem I (PSI) of *A. thaliana* than R-BRO by inhibiting the transcription of *fnr* 1. S-BRO also changed the chlorophyll fluorescence parameters Y (II), Y (NO), and Y (NPQ) to a greater extent than R-Bro. Transcription of genes *psb*O2, *Lhcb*3 and *Lhcb*6 was down-regulated in an enantioselective rhythm and S-BRO caused more serious influence, indicating that S-BRO did worse damage to the photosystem II (PSII) of *A. thaliana* than R-BRO. This study suggested that S-BRO disturbed the photosynthesis of plants to a larger extent than R-BRO and provided a new sight to evaluate the phytotoxicity of chiral herbicides.

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Abbreviations: ANOVA, analysis of variance; BRO, bromacil, 5-bromo-3-*sec*-butyl-6-methyluracil; CD, circular dichroism; Chla/Chlb, chlorophyll a/chlorophyll b; ETR, electron transport rate; HPLC, high performance liquid chromatography; LHC, light-harvesting complex; MS Medium, Murashige & Skoog Medium; NADPH, nicotinamide adenine dinucleotide phosphate; PCR, polymerase chain reaction; PSI, photosystem I; PSII, photosystem II.

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1. Introduction

The past four decades have witnessed the rapid increase of herbicides consumption in China and the proportion of all pesticides has climbed from 20% in 1960 to 48% in 2005 (Zhang et al., 2011; Ye et al., 2009a). This rapid increase is a growing threat to the environment due to their toxic effects on plants of nearby area, despite the primary goal is to enhance the crop productivity. As we know, plants are intimately connected with the natural environment, and their growth is strongly dependent on the availability of light. It has been reported that the photosystem II (PSII) herbicides are the most frequently detected at the highest concentrations and the approximately 50% of commercially available herbicides act by inhibiting the chloroplast electron transport chain (Flores et al., 2013; Jones and Kerswell, 2003). Their mode of action is associated with the perturbation of photosynthetic electron flow which affects carbon fixation. Photosynthesis is important for plants and the disturbance to photosystem may occur before obvious inhibition to growth (Chen et al., 2015). The effects of PSII herbicides on photosynthesis reduce primary production, leading to ecosystem deterioration. Therefore, providing regulators and managers for herbicides is necessary to assess the direct effects of PSII herbicides on photosynthesis.

Chiral herbicide family represents about quarter of the pesticide active ingredients and this ratio has been increased by introducing compounds with more complex structures (Garrison, 2006; Wen et al., 2009; Ye et al., 2009b). Previous studies have reported that chiral herbicides displayed enantioselective disturbance to plants though they share the identical physical and chemical properties due to their different interactions with enzymes and biological receptors in organism (Wang et al., 2007; Qian et al., 2015; Wen et al., 2011; Wen et al., 2010). A few studies have demonstrated that chiral herbicides may disturb the photosynthesis of plants in an enantioselective manner (Qian et al., 2013; Zhang et al., 2013), however, the studies of the enantioselective effect of PSII herbicides on photosynthesis are poorly limited (Omokawa and Takahashi, 1994; Huppatz, 1996).

Bromacil (5-bromo-3-*sec*-butyl-6-methyluracil, BRO) is a broad spectrum herbicide used for nonselective weed and bush control on non-cropland areas, as well as for selective weed control on a limited number of crops, such as citrus fruit and pineapple. It has been reported to interfere with photosynthesis in the PSII of plants (Retzinger and Mallory-Smith, 1997). It was reported that between 55,000 and 117,000 lb of BRO was used on California citrus crops from 1992 to 2001 (Turner and Branch, 2003). In fact, due to the high mobility in soils, BRO has been detected in groundwater wells and aquatic habitats at levels up to 5 μg/L (Alavi et al., 2008; Mena et al., 2014; Spurlock et al., 2000; Wilson and Boman, 2011), which lead both animals and plants exposed to BRO. Considering the carcinogenic potential shown in the inhibition to growth and nutrient uptake of human cell lines (Hurley, 1998; Venkat et al., 1995; Zilkah et al., 1981), BRO is classified as a possible carcinogen by the US Environmental Protection Agency (Pfeuffer and Matson, 2001).

It should be noticed that BRO is also a chiral compound with a pair of enantiomers for the carbon chiral center (Fig. 1A). The toxicity of BRO to plants has been investigated recently (El-Nahhal and Hamdona, 2015; Liu et al., 2013). But, unfortunately, BRO was studied as race mate and no information is available on the enantioselective toxicity of BRO to plants. In this study, we selected *Arabidopsis thaliana* as model plants, to learn about the potential enantioselective effect of BRO on plants in terms of photosynthesis and photosynthetic pigment levels, chlorophyll fluorescence parameters and some genes expression of proteins in PSI and PSII were measured. The aim of this study was to detect the effect of BRO on photosystem at enantiomer level and to provide a new sight to evaluate the phytotoxicity of chiral herbicides.

2. Materials and methods

2.1. Chiral separation of BRO

Racemate BRO (97% purity) was provided by the Kangbaotai Fine Chemical Company (Wuhan, China). Chiral separation of BRO enantiomers was performed on Waters-2535 series HPLC systems with chiral column (Lux 5u Cellulose-1, 250 × 4.60 mm) purchased from Phenomenex Company (Torrance, CA, USA). The composition of mobile phase was 9:1 of hexane/isopropanol with a flow rate of 0.5 mL/min. 5 μL sample was injected every time for analysis and the temperature of column was 30 °C. Other solvents used in this study were analytical or HPLC grade and all glassware were sterilized in an autoclave.

2.2. Plant growth inhibition

Wild type *A. thaliana* was used as test plants. Following surface sterilization with 4% (*v*/*v*) sodium hypochlorite for 1 min, seeds of *A. thaliana* were rinsed three times with 75% (*v*/*v*) ethanol and repeated with sterile water. Then test plants were cultivated in 24-well culture cluster containing Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) and BRO for 3 weeks at 23 ± 2 °C with the photoperiod of 16 h light and 8 h dark. BRO enantiomers (S-BRO, R-BRO) and race mate BRO (Rac-BRO) solutions were dissolved in isopropanol with a final solvent concentration of 0.1% (*v*/*v*) isopropanol for each treatment and the exposure concentrations were set at 1 μM and 2 μM, which were represented as BRO-1 and BRO-2 respectively. Growth inhibition experiments at each concentration of BRO enantiomers and Rac-BRO were performed in triplicate.

2.3. Determination of chlorophyll and carotenoid content in plant tissue

Chlorophyll and carotenoid contents were extracted from a known fresh (weight 0.1–0.5 g) leaves with 100% (*v*/*v*) acetone solution for 24 h in the dark. After centrifugation (3000 rpm for 10 min at 4 °C), the supernatant was collected and quantified photo-metrically by the UV-2401PC spectrophotometer (Shimadzu corporation, Japan) at 470, 645 and 662 nm. The experiment was performed in triplicate. The concentrations of chlorophyll and carotenoids were calculated according to the previous methods (Türlerinde et al., 1998).

2.4. Chlorophyll fluorescence analysis

The determination of chlorophyll fluorescence parameters was performed by LI-6400 chlorophyll fluorometer (Maxwell and Johnson, 2000). The value of the ratio of variable fluorescence to maximal fluorescence (F_v/F_m), electron transport rate (ETR) were measured and analyzed with the gradually strengthened actinic light, as well as some photochemical and non-photochemical quenching coefficients including the yield of photochemical quantum (Y (II)), the non-photochemical quenching that are not photo-protective (Y(NO)) and non-photochemical quenching coefficient (Y(NPQ)) were calculated according to previous study (Lazar, 2015). All the parameters were measured in triplicates.

2.5. RNA extraction, reverse transcription and real-time PCR analysis

3-week-old *A. thaliana* leaves were collected to isolate the total RNA using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer. The cDNAs were synthesized from total RNA using a reverse transcriptase kit (Toyobo, Tokyo, Japan). Real-time PCR was carried out using an SYBR Green Realtime PCR Master Mix (Toyobo CO., LTD). The gene primers were listed in Table 1. The relative gene expression among the treatment groups was quantified using the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001). *Actin*2 was used as a housekeeping gene to normalize the expression changes. Values were presented as

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