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Brassinosteroid alleviates polychlorinated biphenyls-induced oxidative stress by enhancing antioxidant enzymes activity in tomato

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HIGHLIGHTS

- PCBs decreased biomass accumulation and photosynthesis in tomato dose dependently.
- PCBs mist spray induced photoinhibition and damage to photosynthetic apparatus.
- EBR increased photosynthetic capacity and biomass accumulation under PCBs stress.
- Overproduction of H_2O_2 and $O_2^{\cdot-}$ by PCBs induced membrane lipid peroxidation.
- EBR alleviated PCBs-induced oxidative stress by stimulating antioxidant enzymes.

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ABSTRACT

Polychlorinated biphenyls (PCBs) are persistent organic pollutants often found in the atmosphere. Phytoremediation of airborne PCBs is an emerging new concept to minimize potential human exposure. However, effects of atmospheric PCBs on plant growth, photosynthesis and antioxidant defence system are poorly understood area. Brassinosteroids have been reported to alleviate different abiotic stresses including organic pollutants-induced stress. Hence, we studied the effects of PCBs and 24-epibrassinolide (EBR) on biomass accumulation, photosynthetic machinery and antioxidant system in tomato plants. PCBs (0.4, 2.0 and 10 μg/l) mist spray significantly decreased dry weight, photosynthesis, chlorophyll contents in a dose dependent manner. Both stomatal and non-stomatal factors were involved in PCBs-induced photosynthetic inhibition. Likewise, the maximal photochemical efficiency of PSII (Fv/Fm), the quantum efficiency of PSII photochemistry (Φ_{PSII}) and photochemical quenching coefficient were increasingly decreased by various levels of PCBs, suggesting an induction of photoinhibition. Increased accumulation of H_2O_2 and $O_2^{\cdot-}$ accompanied with high lipid peroxidation confirmed occurrence of oxidative stress upon PCBs exposure. Meanwhile, antioxidant enzymes activity was decreased following exposure to PCBs. Foliar application of EBR (100 nM) increased biomass, photosynthetic capacity, chlorophyll contents and alleviated photoinhibition by enhancing Fv/Fm, Φ_{PSII} and qP. EBR significantly decreased harmful ROS accumulation and lipid peroxidation through the induction of antioxidant enzymes activity. Our results suggest a protective role of EBR against PCBs stress which may strengthen phytoremediation approaches by enhancing plant tolerance.

1. Introduction

Polychlorinated biphenyls (PCBs) are one of the widely distributed persistent organic pollutants (POPs). They are toxic, bioaccumulative and able to reach most remote area by long-range atmospheric transport (Erickson and Kaley, 2011; Fitzgerald et al., 2011; Schwitzguébel et al., 2011; Tato et al., 2011). PCBs are of 209 congeners having good thermal and chemical stability as well as electric insulating property. These properties led PCBs to widespread industrial applications such as dielectric fluids (in transformer and capacitors), air and gas compressor lubricants, hydraulic fluids, coolants, plasticizers, fire retardants, paint,

Abbreviations: APX, ascorbate peroxidase; BRs, brassinosteroids; CAT, catalase; Chl a, chlorophyll a; Chl b, chlorophyll b; Ci, intercellular CO_2; DW, dry weight; EBR, 24-epibrassinolide; Fv/Fm, the maximal photochemical efficiency of PSII; FW, fresh weight; POD, guaiacol peroxidase; gs, stomatal conductance; GR, glutathione reductase; MDA, malondialdehyde; PAHs, polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls; Pn, net CO_2 assimilation rate; PPFD, photosynthetic photon flux density; qP, photochemical quenching coefficient; ROS, reactive oxygen species; RuBisCO, ribulose-1,5 *bis*phosphate carboxylase/oxygenase; SD, standard deviation; SOD, superoxide dismutase; Φ_{PSII}, the quantum efficiency of PSII photochemistry.

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pesticide extenders, textiles and wax extenders etc. (Erickson and Kaley, 2011). Although production of PCBs had been terminated since mid '70 s, many products remained in service even after decades (Erickson and Kaley, 2011; Wu et al., 2011). Moreover, some of these equipments were sealed and disposed without sufficient consideration and proper management which resulted in leakage, vaporization and subsequent release to environment (Wu et al., 2011). That is why, PCBs are now being detected in different environmental compartments such as air, water, sediment, soil and biota (Fitzgerald et al., 2011; Li et al., 2011; Tato et al., 2011; Wu et al., 2011).

The atmospheric concentration of 7 indicator PCBs ($\sum_7$PCBs) lies between 4.0 and 27.5 pg m^{-3} in 11 background sites geographically distributed throughout China (Wu et al., 2011). In addition, the concentrations of PCBs in indoor air are often much higher than those in ambient air. Long-term exposure to indoor air PCBs levels potentially increased the body burdens of the residents in older homes (Fitzgerald et al., 2011). In China, total PCB contents in the soil of Tianjin region have been reported 4.02 ng g^{-1} with greatest value (12.65 ng g^{-1}) in the coastal sites of that region (Li et al., 2011). Indeed, soils and sediments are the major reservoirs of PCBs, whilst air is the dominant medium of global transport (Wania and Daly, 2002). The enrichment of PCBs in above ground air layer is correlated with net emission fluxes from the soil (Tato et al., 2011).

PCBs are uptaken, translocated and subsequently metabolized in plants (Van Aken et al., 2009; Schwitzguébel et al., 2011). Air to plant transfer pathway plays important role in the uptake of organic semi-volatile chemicals like PCBs which is involved in the global cycling of POPs and the potential food chain contamination (Collins et al., 2011). PCBs concentration in cow milk is directly correlated with the PCBs concentration in vegetation of that locality, which is a serious threat to human health (Tato et al., 2011). It is to be noted that the contamination of plant leaves by PCBs-like compounds mainly occurs via air rather than via the soil-root system (Kömp and McLachlan, 2000). Semi-volatile chemicals enter the leaf either crossing the cuticle or directly via stomata (Collins et al., 2011). The ability to assimilate aerosols and chemical compounds from air and liquid solutions is an important industrial characteristic of higher plants (Korte et al., 2000). These properties provide the opportunity to use plants as air purifier or green air filter for semi-volatile organic pollutants. Meanwhile, hybrid poplar trees have been proposed as a feasible treatment candidate for scavenging airborne PCBs from nearby sources (Beebe, 2011). PCBs are effectively scavenged by poplar leaves which reduce airborne PCBs and thus prevent human exposure.

Phytoremediation is a promising cost effective technology that uses higher plants to rehabilitate soil, air and groundwater contaminated with toxic compounds like PCBs (Van Aken et al., 2009; Beebe, 2011; Schwitzguébel et al., 2011). Plants suffer from phytotoxicity under high level of toxin exposure. Organic pollutants upon entry induce generation of reactive oxygen species (ROS) and subsequent oxidative stress (Korte et al., 2000; Oguntimehin et al., 2010; Schwitzguébel et al., 2011). Plants use rate limiting antioxidant systems composed of both enzymatic and non-enzymatic antioxidants to minimize harmful concentration of ROS (Apel and Hirt, 2004). However, effects of PCBs on photosynthesis and antioxidant systems in higher plants are poorly understood area. Usually plants lack the catabolic enzymes necessary for complete metabolism of recalcitrant organic compounds (Van Aken et al., 2009). One of the strategies to overcome this inherent limitation is to introduce efficient foreign genes involved in the degradation of toxic chemicals. Another strategy could be hormonal supplementation which would increase antioxidant and detoxification capacity to tolerate stressful conditions. "Assisted phytoremediation by plant growth regulators" is similar kind of concept recently proposed by Barbafieri and Tassi (2011). Nowadays, plant defence inducers like naphthyl-acetic acid (a plant hormone analogue) and BION® (plant resistance inducer against diseases) are being used to enhance plant detoxification capacity (Schwitzguébel et al., 2011).

Brassinosteroids (BRs) are a group of steroidal phytohormones that coordinate different aspects of plant growth, development and defense (Bajguz and Hayat, 2009; Cui et al., 2011; Jiang et al., 2012). BRs have also been reported to alleviate abiotic stresses like high or low temperature, moisture stress, drought, salinity, pesticidal injury, organic pollutant and heavy metal stress (Bajguz and Hayat, 2009; Xia et al., 2009a,b; Cui et al., 2011; Sharma et al., 2011; Ahammed et al., 2012a). BRs increase photosynthesis, antioxidant and detoxification potential and thereby improving plant tolerance under different stresses (Bajguz and Hayat, 2009; Xia et al., 2009a; Sharma et al., 2011; Ahammed et al., 2012a). BRs could be considered as potential candidate phytohormones for "Assisted phytoremediation by plant growth regulators" (Barbafieri and Tassi, 2011). Hence, we hypothesized that BRs could enhance plant tolerance under high atmospheric PCBs level. We used tomato as a model plant system to study the effects of different levels of PCBs and BRs on growth, photosynthesis, chlorophyll fluorescence, accumulation of H_2O_2 and $O_2^{\cdot-}$, lipid peroxidation and antioxidant enzyme system. We expected an anti-stress role of BRs against PCBs which would give an opportunity to use BRs to strengthen phytoremediation approaches.

2. Materials and methods

2.1. Plant material and growing conditions

Seeds of tomato (*Solanum lycopersicum* L. cv. Hezuo 903) were treated in hot water (55 °C) for 30 min and imbibed in water (25 °C) for another 3 h. Later on, the seeds were sown in 10 cm diameter plastic pots (two seeds per pot) containing a mixture of peat, vermiculite and perlite (6:3:1, v:v:v) for germination and subsequent growth. The experiment was carried out in controlled growth chamber under following conditions: temperature 25/17 °C (day/night), mean relative humidity 80%, photosynthetic photon flux density (PPFD) 600 μmol m^{-2} s^{-1} and photoperiod of 14/10 h (day/night). Appropriate moisture was maintained by regular watering. After germination and early establishment, only one healthy seedling was kept per pot, which was watered daily and fertilized twice in a week with Hoagland's nutrient solution.

2.2. Treatment application, sample harvesting and dry weight analysis

A working solution of 100 nM 24-epibrassinolide (EBR, Sigma–Aldrich, St. Louis, USA) was prepared by dissolving solute in ethanol followed by dilution with MilliQ water (ethanol: water (v/v) = 1:10000). Thirty days after sowing, uniform sized tomato plants were selected and foliar parts were sprayed with or without 100 nM EBR. Treatments without EBR were simultaneously sprayed with MilliQ water containing same ratio of ethanol. The concentration of EBR was chosen from our preliminary experiment, whilst this concentration was found most effective to promote xenobiotics metabolism in our previous studies (Xia et al., 2009b; Ahammed et al., 2012b). After 24 h of EBR pretreatment, entire foliar regions of plants were sprayed with 0, 0.4, 2.0 and 10.0 0 μg/l PCBs (Aroclor 1242, Dr. Ehrenstofer GmbH, Augsburg, Germany). Aroclor 1242 is a commercial PCBs mixture containing 42% (w:w) chlorine (Erickson and Kaley, 2011). Aroclor 1242 was supplied in cyclohexane by manufacturer which was diluted with MilliQ water to get desired concentration (cyclohexane: water (v/v) = 1: 40000). Plants under 0 μg/l PCBs treatment were also sprayed with equal concentration of cyclohexane like others. PCBs-treated plants