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Disruption of actin filaments in *Zea mays* by bisphenol A depends on their crosstalk with microtubules

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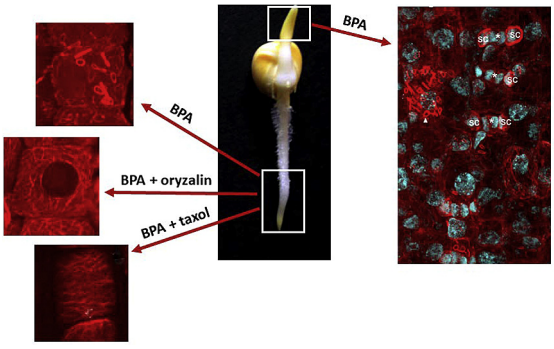
HIGHLIGHTS

- Actin filaments constitute a subcellular target of bisphenol A in plant cells.
- Microtubule-actin "cross-talk" mediates the observed effects on actin.
- Ontogenesis of stomatal complexes is highly disturbed by bisphenol A.

GRAPHICAL ABSTRACT



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Dedication: This paper is dedicated to the memory of Stavropoulou Konstantina, student of Biology at the Aristotle University of Thessaloniki, Greece

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ABSTRACT

Bisphenol A (BPA) is a widespread environmental pollutant, reportedly harmful to living organisms. In plant cells, BPA was shown to disrupt microtubule (MT) arrays and perturb mitosis, but its effects on filamentous actin (F-actin) have not been explored. Here we studied the effects of BPA on actin filaments (AFs) in meristematic root tip and leaf cells of *Zea mays*, by fluorescent labeling and confocal microscopy. Considering the typical dynamic interaction between MTs and AFs, the effects on these two essential components of the plant cytoskeleton were correlated. It was found that BPA disorganized rapidly AFs in a concentration- and time-dependent manner. The fine filaments were first to be affected, followed by the subcortical bundles, resulting in rod- and ring-like conformations. The observed differences in sensitivity between protodermal and cortex cells were attributed to the deeper location of the latter. Depolymerization or stabilization of MTs by relevant drugs (oryzalin, taxol) revealed that AF susceptibility to BPA depends on MT integrity. Developing leaves required harder and longer treatment to be affected by BPA. Ontogenesis of stomatal complexes was highly disturbed, arrangement of AFs and MT arrays was disordered and accuracy of cell division sequence was deranged or completely arrested. The effect of BPA confirmed that subsidiary cell mother cell polarization is not mediated by F-actin patch neither of preprophase band organization. On the overall, it is concluded that AFs in plant cells constitute a subcellular target of BPA and their disruption depends on their crosstalk with MTs.

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1. Introduction

Bisphenol A [BPA, 2,2-bis-(4-hydroxyphenyl)propane) is a synthetic organic chemical widely produced for manufacturing polycarbonate plastics and epoxy resins, which are finally applied in the production of many daily necessities, including food packaging, protective coatings, building materials, water-supply pipes, electric and electronic equipment, thermal paper, infant and baby bottles, medical facilities and dental sealants (Staples et al., 1998). BPA can be solubilized from these products and escape to the environment (Halden, 2010), hence it is considered as an emerging organic pollutant (Clarke and Smith, 2011; Zhang et al., 2016). The primary route of BPA pollution is effluents from wastewater treatment facilities and landfill sites with reported concentrations, in some cases, up to 17.2 mg/L (Yamamoto et al., 2001; Asakura et al., 2004). Percolation of BPA from BPA-based products is closely related to contamination of domestic sewage by BPA (Kang et al., 2007), while BPA may leach into the environment from plastic wastes as well (Yamamoto and Yasuhara, 1999).

The environmental toxicology of BPA is extensively being studied and reviewed in animals and human cell lines. Shown to be an estrogen-like chemical mimicking endogenous hormones, BPA is assigned as an endocrine disrupting chemical (Asakura et al., 2004), correlated to several maladies, including infertility (Maffini et al., 2006; Wang et al., 2016), dysfunction of thyroid hormones (Moriyama et al., 2002), complex immuno-modulating effects (Ahmed, 2000) and maybe carcinogenesis (Keri et al., 2007).

In plants, much fewer studies have been conducted and the effects of BPA have not been well established. Most existing studies are mainly focused on vegetative growth (Ferrara et al., 2006), seed germination (Dogan et al., 2010) and reproductive development (Speranza et al., 2011). Plants can rapidly absorb BPA from water and metabolize it to several glycosidic compounds (Nakajima et al., 2004; Noureddin et al., 2004). More recently, BPA was shown to affect physiological mechanisms of plants, such as nitrogen assimilation (Sun et al., 2013), photosynthesis (Qiu et al., 2013) and mineral nutrition (Nie et al., 2015). Moreover, it was shown to act as a plant hormone disrupter (Frejd et al., 2016).

Even fewer studies have linked the ultrastructural and mitotic malformations induced by BPA treatment with the observed growth impediment. For example, in pea (*Pisum sativum*) BPA deranged interphase and mitotic microtubule (MT) arrays, inducing the formation of macrotubules, preventing cell division and ultimately hampering plant growth (Adamakis et al., 2013). In the Greek endemic gymnosperm *Abies cephalonica* BPA also disrupted interphase and mitotic MT arrays, with prometaphase, metaphase and anaphase spindles appearing sharply pointed, sigmoid or in multipolar conformations (Adamakis et al., 2016). Both studies concluded that the mitotic MT arrays of plants are very sensitive to BPA, as it has been reported in animal cells (Metzler and Pfeiffer, 1995), but also that the effect of BPA is plant-specific. In pollen tubes of *Picea meyeri*, BPA disturbed Ca^{2+} efflux and disrupted actin filament (AF) organization, resulting in abnormal actin-dependent vesicle trafficking and further deranging the deposition of cell wall components, ultimately affecting pollen tube growth (Chang et al., 2015). Judging from this study, it is hypothesized that filamentous actin (F-actin) could be also a target of BPA toxicity.

To further elucidate the effects of BPA on plant AFs, in the present study the effects of BPA on the AF cytoskeleton were studied in root and leaf cells of *Zea mays*, a representative monocotyledon and major crop. Moreover, given that rearrangements of MTs and AFs are often interdependent (Collings et al., 2006; Collings, 2008) and cytoskeletal cross-talk might be enhanced during stress conditions (e.g. Sampathkumar et al., 2011), combined BPA treatments with anti-microtubule drugs (oryzalin, taxol) were also conducted to examine whether the above hypothesis could be valid under BPA stress.

2. Materials and methods

2.1. Preliminary experiments

Seeds of maize (*Zea mays* L. cv. Aris, kindly provided by the National Cereal Institute of Thessaloniki, Greece) were germinated for 3 days on filter paper soaked with distilled water. Then, three groups of 30 selected seedlings of about equal length were treated with 20, 50 and 100 mg/L BPA aqueous solution, as previously described (Adamakis et al., 2013). One more group of 30 seedlings grown in BPA-free aqueous solution was considered as the control. Special care was taken that the growing roots remained continuously immersed in the respective solution. Root length of each individual seedling was measured after 12, 24 and 48 h of exposure and their average was calculated.

To assess the effects of BPA on germination parameters, the response of maize seeds soaked in water and in 100 mg/L BPA solution was studied under controlled conditions. Three groups of 30 seeds from each of the treatments were placed on 90 mm-diameter Petri dishes on Whatman filter paper. Seeds were kept in a germinator at 25 °C in darkness for 96 h. Then, the germination percentage (GP, %), the germination index (GI) and seedling vigor index (SVI) were calculated. The GI was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by following the formula:

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \ldots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

The SVI was calculated according to Dezfuli et al. (2008) following the formula:

SVI = [Seedling length (cm) x Germination percentage]

Data were presented as mean ± standard error (SE) of three independent experiments. Statistical analyses (ANOVA with Dunnett's multiple comparison test) were performed using Graph Pad software (San Diego, CA, USA), with significance at $P < 0.05$.

2.2. Actin filament staining and tubulin immunofluorescence

For root specimens, 3-day-old maize seedlings were treated for 1, 2 and 3 h with 20, 50 and 100 mg/L BPA. In parallel, treatments with the anti-tubulin drugs oryzalin and taxol, which depolymerize and stabilize MTs, respectively (Morejohn and Fosket, 1991), were applied on the root tips as such: seedlings pretreated with either 5 μM oryzalin for 24 h or 20 μM taxol for 3 h were then subjected to combinations of either 100 mg/L BPA + 5 μM oryzalin for 3 h or 100 mg/L BPA + 20 μM taxol for 3 h. For leaf specimens, maize seeds were directly germinated on filter paper soaked with 100 mg/L BPA solution, with only the roots being immersed on the BPA solution. The chemicals and reagents used in this study were purchased from Applichem (Darmstadt, Germany), Merck (Darmstadt, Germany) and Sigma (Taufkirchen, Germany), unless stated otherwise.

For MT visualization, tubulin immunostaining was applied according to Adamakis et al. (2013). All the steps were performed at room temperature, unless stated otherwise. In short, root tips were excised and fixed for 45 min in 8% (w/v) paraformaldehyde (PFA) in PEM buffer (50 mM PIPES, 5 mM EGTA, 5 mM $MgSO_4 \cdot 7H_2O$), pH 6.8, and then washed in PEM. Following a 40 min cell wall digestion in