



Effect of BPA on the germination, root development, seedling growth and leaf differentiation under different light conditions in *Arabidopsis thaliana*



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HIGHLIGHTS

- We model six light environments in which effect of BPA on seedling was elucidated.
- Low-dose BPA caused an increase in the growth indices and lateral root formation.
- High-dose BPA shows an inhibition effect in a dose-dependent manner.
- BPA has no notable role in light response in germination and early seedling growth.
- BPA influences the leaf blade differentiation in a light-dependent manner.

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ABSTRACT

Bisphenol A (BPA) is a well-known environmental toxic substance, which exerts unfavorable effects through endocrine disruptor (ER)-dependent and ER-independent mechanisms to threaten ecological systems seriously. BPA may also interact with other environmental factors, such as light and heavy metals, to have a synergetic effect in plants. However, there is little data concerning the toxic effect of BPA on the primary producers-plants and its possible interaction with light-dependent response. Here, the effects of BPA on germination, fresh weight, tap root length, and leaf differentiation were studied in *Arabidopsis thaliana* under different parts of light spectrum (dark, red, yellow, green, blue, and white light). Our results showed that low-dose BPA (1.0, 5.0 μM) caused an increase in the fresh weight, the tap root length and the lateral root formation of *A. thaliana* seedlings, while high-dose BPA (10.0, 25.0 μM) show an inhibition effect in a dose-dependent manner. Unlike karrikins, the effects of BPA on germination fresh weight and tap roots length under various light conditions are similar, which imply that BPA has no notable role in priming light response in germination and early seedling growth in *A. thaliana*. Meanwhile, BPA exposure influences the differentiation of *A. thaliana* leaf blade significantly in a light-dependent manner with little to no effect in dark and clear effect under red illumination.

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1. Introduction

Environmental contaminant bisphenol A (BPA; 2, 2-bis-(4-hydroxyphenyl) propane), which is utilized to make polycarbonate plastics, is found in everything from baby bottles and reusable water bottles to the linings of food cans. BPA is continuously brought into the aquatic environment through means of industrial,

agricultural, municipal effluents and so on. With BPA leaching from the plastics, humans are constantly exposed to this chemical from drinking or taking food from these containers (Huang et al., 2012). Detection of BPA in fetal tissues and human fluid, such as urine, blood and amniotic fluid (Calafat et al., 2005; Vom Saal and Hughes, 2005), confirms the significant exposure of humans through their diet (Tomohiro and Tatsuyuki, 2012), which indicates that BPA has penetrated into all aspects of human life. Furthermore, Honkanen et al. (2004) reported that the morphology, histological structure, and habitual behavior of salmon yolk-sac fry which exposed to BPA at the concentrations of 10, 100, and 1000 mg L⁻¹, have been obviously changed.

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Data from our group and others indicate that the amount of BPA to which humans are exposed may cause adverse health effects (Vandenberg et al., 2010; Mei et al., 2013). At the same time, agricultural soils are often enriched with activated sewage sludge biosolids which may contain BPA (Gatidou et al., 2007; Stasinakis et al., 2008), and therefore plants could encounter BPA from organisms dwelling in the soil and hazardous landfill leachates (Yamamoto et al., 2001). But few studies have examined the toxicological impacts of BPA in plants that are able to take up and accumulate BPA phytotoxic, clastogenic and bioaccumulation effects of the environmental endocrine disruptor bisphenol A in various crops grown hydroponically (Ferrara et al., 2006; Adamakis et al., 2013). Although Nakajima et al. (2002) have proved plants can metabolize BPA to BPA-glycosides, phytotoxic and clastogenic impacts of BPA on plants were determined (Ferrara et al., 2006). More recently, Jadhav et al. (2012) found that the genotoxicity of BPA on root meristematic cells of *Allium cepa* L, which was exposed to 50, 100, 150 and 200 mg L⁻¹ BPA, was reported in terms of numbers of observed chromosomal aberrations. Nakajima et al. (2002) demonstrated that tobacco seedlings can absorb and metabolize BPA to the β -glucoside of BPA. Although it remains unknown about the metabolic mechanism of BPA in plants, the metabolic products of BPA can be considered as detoxified forms (Schmidt and Schuphan, 2002). Plants, the primary producers, synthesize organic substances and provide energy for the whole ecosystem, on which the mechanism of BPA phytotoxicity remains obscure.

Environmental compounds may reshape the light response of plant and therefore influence the germination and seedling development. Such as karrikins, a class of seed germination stimulants identified in smoke from wildfires can enhance light responses during germination and seedling development in *Arabidopsis thaliana* (Nelson et al., 2010). Nelson et al. (2009) demonstrated that karrikins can trigger *Arabidopsis* seed germination in a light-dependent manner through a mechanism requiring gibberellic acid (GA) synthesis, which enhances the expression of the GA biosynthetic genes GA3ox1 and GA3ox2 and has little effect on the sensitivity to exogenous GA. Prior to radicle emergence, abscisic acid and GA levels in seed are hardly affected by karrikins treatment. Thus, it is intriguing to know whether environmental pollutants, such as BPA, can influence seed germination and seedling development in a light-dependent way similar to karrikins, and if so, the toxic effects of environmental pollutants need further investigation in great detail related to plant biochemical and photobiological processes.

Plants contain three well-characterized photoreceptor kinds such as the phytochromes (more sensitive to red than to blue), cryptochromes (specifically blue light sensitive) and phototropins (especially sensitive to blue light), as well as an uncertain green light receptor, which are involved in regulating many aspects of plant growth and development (Sullivan and Deng, 2003; Whitelam and Halliday, 2007; Goggin et al., 2008; Zhang et al., 2011). Most studies have assessed light quality effects on the germination, leaf, hypocotyls, root or other plant vital activities such as stomatal opening via means of isolated guard cells combined with recordings on single guard cells (López-Figueroa, 1991; Roelfsema and Hedrich, 2005; Galen et al., 2007; Bae and Choi, 2008; Millar et al., 2010; Nelson et al., 2010; Barrero et al., 2012). However, the molecular mechanism underlying the response to different growth spectra on plant development are not known in detail, although the involvement of photoreceptors has been examined using mutants and by an evaluation of changes in gene expression (Hogewoning et al., 2010; Zhang et al., 2011).

Light responses, the first and most important stage of photosynthesis, is a complex process, and it is reflected by many indices together, such as chlorophyll content, chlorophyll fluorescence parameters, the activity of Mg²⁺-ATPase and so on (Govindjee and van Rensen, 1978). Sun et al. (2012) have completed an

interesting study about interactive effects of cadmium and acid rain on photosynthetic light responses in soybean seedlings, and have proved that single treatment with Cd²⁺ or acid rain decreased the absorption of light energy, electron transport, conversion of light energy and photophosphorylation. The decrease in these photosynthetic light responses processes must lead to the inhibition of the photosynthesis in plants; therefore it was necessary to know how environmental contaminants affect the light responses of plants and eventually affect the production.

All of the above results suggest that plants hold additional means to adapt to a changing light environment reminding us that plants are sensitive to a broad series of inputs to shape plant form and function. It is well known that germination can be triggered by different environmental factors, e.g., heat shock, light, absorption of water and certain chemicals. BPA toxicity has attracted attention in recent years due to its widespread release into the environment from polycarbonate bottles at high temperature and so on. Thus, it is important to investigate how plants adapt to BPA and whether light environment and biochemistry environment are correlated in germination and seedling development.

The experiments presented in this report provide some guidelines for elucidating the effect mechanism of BPA on the growth of plants in six different light environments: white, red (obtained by red filtering), yellow (obtained by yellow filtering), green (obtained by green filtering), blue (obtained by blue filtering), and dark (obtained by aluminum foil). The results also provide experimental foundation for foreseeing the different light environmental hazards of the accumulation of BPA in soil.

2. Materials and methods

2.1. Seed material

Freshly matured *A. thaliana* wild-type Columbia seeds were obtained from University of Science and Technology of China (Hefei, China). Mature seeds collected from the *A. thaliana* plants were placed into PE pipe, in which there were some anhydrous cupric sulfate particles used as drier. The mature seed were tested for viability prior to the germination tests, and the rate of germination is more than 98%.

2.2. Filters

The filters (XL-H8000), made of Polyvinyl Chloride (PVC), were purchased from Hangzhou Haipu Technology Co., Ltd. (Hangzhou, China). They were made into rectangular bags and sealed by parafilm after the Petri dishes put into it. The light intensity was measured by an optical power test instrument (SGN-II), which was manufactured by Tianjin Gangdong Sci. & Tech. Development Co., Ltd. (Tianjin, China). Under each filter, the light intensity was 47.1 μ W (white), 45.1 μ W (red filtering), 46.3 μ W (yellow filtering), 44.9 μ W green (green filtering), 45.5 μ W blue (blue filtering), and 0 μ W (aluminum foil).

2.3. Reagents

BPA was purchased from Sigma. The compositions of MS medium with 0.7% agar were all obtained from Sinopharm Chemical Reagent Co., Ltd. (Hefei, china). The deionized water was produced by the deionized water machine in our laboratory in Anhui province, China.

2.4. Preparation of BPA and control solution

Based on the preliminary experiments in our laboratory (data not shown), a BPA solution at four concentrations of 1, 5, 10, and

25 μM were selected to cultivate *A. thaliana* seedlings. Different concentrations of BPA in MS medium were prepared by adding 0.1 M mother liquor of BPA dissolving with anhydrous ethanol at the corresponding amount of 5 μL , 25 μL , 50 μL and 125 μL into 500 mL MS medium. Simultaneously, we add the amount of 5 μL , 25 μL , 50 μL and 125 μL of anhydrous ethanol into 500 mL MS medium as control.

2.5. Seed germination and growth conditions

The seeds of *A. thaliana* were sterilized in NaClO (5%) solution for 5 min followed by six rinsing with distilled water. After that seeds were placed in 60-mm-diameter MS medium petri dishes (20 seeds per plate, five replicates for each treatment), in which supplemented with 30 g L⁻¹ sucrose, vitamins, 0.7% (w/v) agar and different concentrations of BPA. Then put these petri dishes containing *A. thaliana* seeds into refrigerator for vernalization last 3 d. Following vernalization, Petri dishes wrapped different color filtering were transferred to an artificial climate (providing light by a bank of 12 fluorescent tubes) with the germination conditions of 16 h photoperiod: 8 h dark and constant temperatures of 22 °C. Soon afterwards, seed germination was scored every 12 h for 72 h.

2.6. Determination of growth indices

The *A. thaliana* seedling growth indices include the germination rate, average fresh weight, average tap root length, the number of blades distribution. Tap root length was measured with a ruler. To measure average fresh weight, the plants were removed from the petri dishes and immediately weighed to prevent dehydration. We used the deviation between the group of BPA and the control to present the number of blades distribution.

2.7. Statistical analysis

Differences between the treatments were analyzed by the analysis of variance (ANOVA) in Excel and Origin 8.5. *T* test was applied to determine the significance between different treatments ($p < 0.05$).

3. Results and discussion

3.1. Effect of BPA and light spectrum on seed germination

The response of vernalized (nondormant) seeds to subsequent light stratification varied depending upon the light spectrum and BPA. Fig. 1(A and B) and Fig. S1 revealed that seeds exposed to different light spectrum in 16 h light: 8 h dark cycles or 24 h dark (equivalent to standard germination conditions) germinated immediately, reaching 90% germination after 36 h and continuing onto a maximum of 97% by the end of the subsequent 36 h. However, seed germination in *A. thaliana* was a little inhibited by 1 μM BPA in white, dark, yellow, blue, green or red light, with the rate of germination being lower than that of control, but all the inhibited effect was not significant. In red and green, the rate of germination was much higher than that of control at first, while a trend of low followed by high rate was observed at last. Dark and red light induced the lower rate of germination than others light qualities, no matter whether it was the control group or the BPA group, while the control of yellow light exhibited much higher rate of germination than other 8 groups.

Fig. 1(A and B) and Fig. S2 show the results of the germination indices in *A. thaliana* seeds, which were treated with 5 μM BPA and exposed to different light spectrum in 16 h light: 8 h dark or 24 h dark cycles. 5 μM BPA positively affected the rate of germination

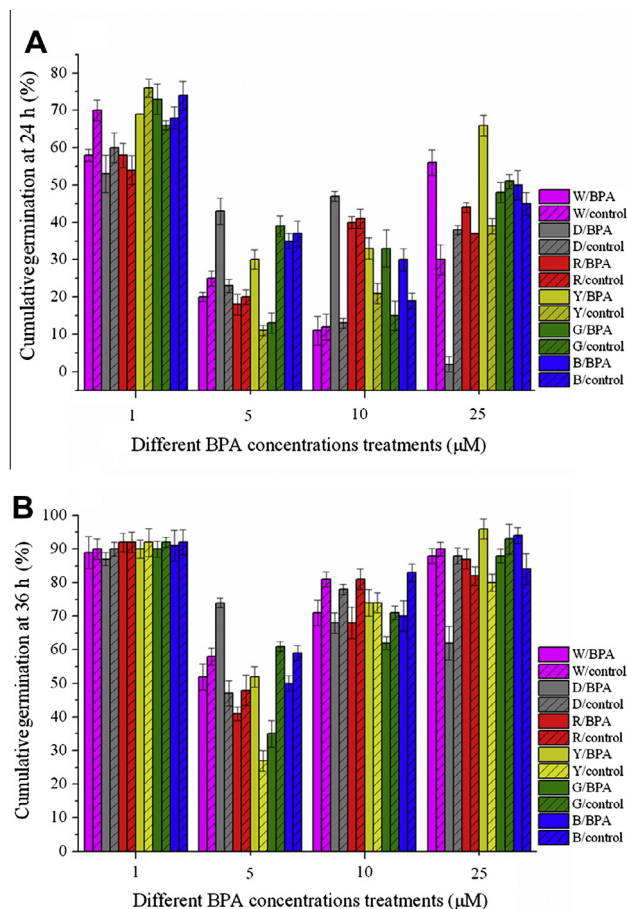


Fig. 1. The seed germination ratio of *Arabidopsis thaliana* at 24 h (A) and 36 h (B) in response to different parts of light spectrum (dark as “D”, red as “R”, yellow as “Y”, green as “G”, blue as “B”, and white light as “W”), treated with 1.0 μM BPA, 5.0 μM BPA, 10.0 μM BPA and 25.0 μM BPA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in dark and yellow, however, seed germination in *A. thaliana* seeds was inhibited by BPA in white, blue, green or red light, with the rate of germination being lower than that of control, while all the germination was lower than 90% by the end of the 60 h.

After 6 d (3 d vernalisation plus 3 d germination) of stress with 10 μM BPA, dark and all light spectrum band except for yellow light significantly inhibit the germination indices of *A. thaliana* seeds by comparing with the control in Fig. 1(A and B) and Fig. S3, although the rate of germination was a little higher than that of control at 12–24 h in white, green and blue.

Fig. 1(A and B) and Fig. S4 revealed that 25 μM BPA promoted seeds to germinate immediately in white, yellow, red and blue, reaching 90% germination after the 36 h and continuing onto a maximum of 97% by the end of the subsequent time. BPA exhibited a little but not significant inhibition effect on germination in dark and green.

During measurements of germination, we noticed that BPA-mediated inhibition of the germination process was not light-dependent, because BPA also significantly affect the germination in dark. In white and blue, BPA had little effect on germination while there was the same promotion and inhibition trend in red and yellow, however, BPA had significantly effect in green and dark with low concentrations of BPA promoting seed germination and high concentrations inhibiting seed germination. An angiosperm seed decided to germinate or not based on integration of various environmental signals such as water, temperature and light.

Eunkyoo et al. (2004) confirmed that a basic helix-loop-helix transcription factor PIF3-like 5 (PIF5) protein was a key negative regulator of phytochrome-mediated seed germination. It is known that etiolated seedling usually contains high levels of phytochromes A (Hanumappa et al., 1999), so the possible role of BPA on phytochromes during seeds germination stage is intriguing.

3.2. Effects of BPA and light spectrum on the fresh weight of *A. thaliana* seedlings

Fig. 2 shows the results of the growth indices on the fresh weight of *A. thaliana* seedlings treated with BPA at the different concentrations and exposed to different light spectrum. According to the *t*-test, Fig. 2(A) reveals that BPA positively affect the fresh weight of *A. thaliana* seedlings, which were treated with 1.0 μ M BPA and exposed to different spectral bands in 16 h light: 8 h dark cycles for 14 d. There was highly significant and positive influence of the fresh weight under yellow and blue light, with the *p*-value being 0.008786 and 0.004569, respectively, compared with the control. There was significant affection on the fresh weight under white, red and green light, compared with the control, with the *p*-value being 0.04536, 0.02184 and 0.04973, respectively. Under dark conditions, the positive effect of BPA on fresh weight is not observed.

Fig. 2(B) illustrated that 5.0 μ M BPA had high significant and positive effects on the fresh weight under white, dark, red and yellow light, with the *p*-value being 0.009513, 0.003628, 0.008542 and 0.007139, respectively, compared with the control. There was significant and positive effect on the fresh weight under blue light, with the *p*-value being 0.03379, compared with the control.

Under green light, BPA induced a slight increase in fresh weight, but the increase was insignificant.

When *A. thaliana* seedlings were treated with 10.0 μ M BPA in Fig. 2(C), the indices mentioned above was high significantly affected by BPA under white and green light, with the *p*-value being 0.008819 and 0.003592, respectively, compared with the control. There was significant and positive effect on the fresh weight under red light, with the *p*-value being 0.04713, compared with the control. With the *p*-value being 0.02918 and 0.03965, respectively, 10.0 μ M BPA significantly suppressed the increase of fresh weight under yellow and blue light, compared with the control. Under dark, BPA induced a slight increase in fresh weight, compared with the control, but the increase was insignificant.

After 14 d of stress with BPA, all light spectrum band and dark significantly inhibit the fresh weight of *A. thaliana* seedlings treated with 25.0 μ M BPA in comparison with the control in Fig. 2(D). These results clearly illustrated that low-dose BPA plays a positive role in stimulating the fresh weight of *A. thaliana* seedlings increase. Conversely, high-dose BPA show an inhibition effect.

How did BPA affect the accumulation of the fresh weight in *A. thaliana* seedlings? As we all know, the growth and development of plants are based on the synthesis and accumulation of organic substances which depends on photosynthesis. Thus, the main reason why environmental contaminants affect the growth of plants might be the change in the photosynthesis of plants (Kummerova et al., 2006). Qiu et al. (2013) also proved that high concentration of BPA showed a significant inhibition effect on the growth of soy-bean seedlings, which was related to the decrease in the photosynthesis because of the decrease in the content of Chl and the change in chlorophyll fluorescence. The result of BPA affecting the accumulation of the fresh weight in *A. thaliana* seedlings confirms that

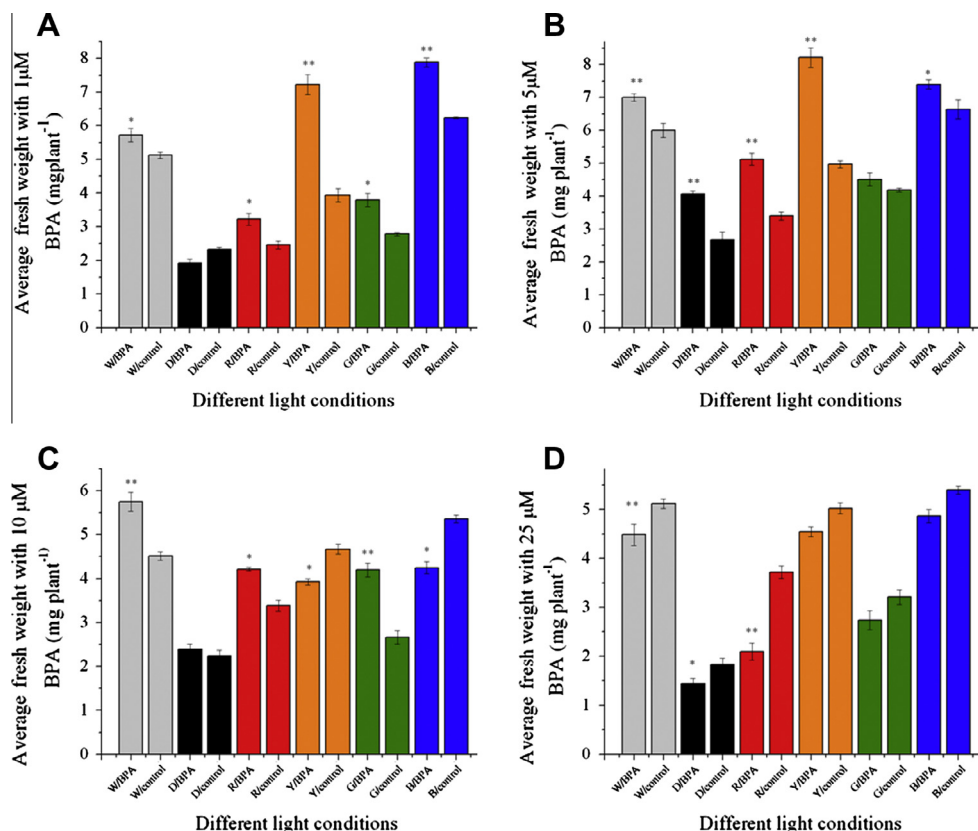


Fig. 2. The growth indices on the fresh weight of *Arabidopsis thaliana* seedling in response to different parts of light spectrum (dark as "D", red as "R", yellow as "Y", green as "G", blue as "B", and white light as "W"), treated with 1.0 μ M BPA (A), 5.0 μ M BPA (B), 10.0 μ M BPA (C), and 25.0 μ M BPA (D). Data represents mean \pm SEM of $n = 60$ *Arabidopsis thaliana* seedling. *0.01 < P < 0.05, ** P < 0.01. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the *A. thaliana* is more sensitive to toxic effects than soybean. Because the effect on soybean photosynthesis observed in Qiu et al. (2013) was only significant at 17.2 mg L⁻¹ (75 μ M) and 50 mg L⁻¹ (219 μ M) BPA, which is 3–9 times higher than the highest concentration used in our study. The 7 mg L⁻¹ (30.7 μ M) treatment in Qiu et al. (2013) is closest to the highest concentration of 25 μ M used on *A. thaliana* in the current study, and this concentration had no effect in soybean. Thus, it was concluded that the decrease of fresh weight in *A. thaliana* seedlings treated with high concentration of BPA (Fig. 2C and D) maybe also resulted from the decrease in the photosynthesis; however, a further investigation is necessary to clarify the biochemical mechanism underlining the effect of BAP on the plant photosynthesis.

3.3. Effects of BPA and light spectrum on the tap roots length of *A. thaliana* seedlings

To test the effect of BPA and light spectrum on the tap roots growth, *Arabidopsis Col-0* seedlings grown in continuous white, dark, red, yellow, green and blue light for 14 d (14-day-old seedlings) in MS medium which were treated with different concentrations of BPA. As shown in Fig. 3(A), 1 μ M BPA can promote the tap root elongation in the dark and all light spectrum. Under white, red, yellow and blue light, the promoting effect was very significant, with the *p*-value being 0.01832, 0.04338, 0.03585, and 0.04366, respectively, compared with the control. There were a slight increase in tap root elongation under dark and green light, but the increase was insignificant.

Fig. 3(B) clearly suggests that 5 μ M BPA can partly stimulate the tap root elongation. For example, the tap root length was significant longer than that of control in dark, red and green, with the *p*-value being 0.001916, 0.01965 and 0.01166, respectively.

Compared with the control, there were a slight elongation in tap root length in white and blue, but the elongation was insignificant. However, 5 μ M BPA exhibited a slight repression effect on tap root growth under yellow light.

Fig. 3(C) describes the effect of 10 μ M BPA on the tap roots length of *A. thaliana* seedlings in different light spectrum. The results indicated that the tap root length was significant shorter than that of control in white and red, with the *p*-value being 0.0005415 and 0.04224, respectively. Compared with the control, 10 μ M BPA exhibited a slight repression effect on tap root growth under other light spectrum and dark.

The effect of 25 μ M BPA on the tap roots length of *A. thaliana* seedlings in different light spectrum are shown in Fig. 3(D). 25 μ M BPA can significantly restrain the tap root elongation than that of control in the white, dark, red, yellow, green, and blue, while the *p*-value were 0.02453, 0.005424, 0.000031, 0.04713, 0.005989 and 0.01816, respectively.

These results indicated that low-dose BPA stimulated the tap root of *A. thaliana* seedlings elongation. Conversely, high-dose BPA exhibited a repression effect. The elongation of primary roots is mediated by interacting hormonal signaling pathways and a range of enzymes such as auxin, phospholipase D (PLD) and its product phosphatidic acid (PA) (Ohashi et al., 2003; Li et al., 2006; Saini et al., 2013). However, the pathways which are involved in the molecular mechanism of the tap root elongation affected by BPA are still unknown.

3.4. Effects of BPA and light spectrum on the distribution of *A. thaliana* blades

The influence of light spectrum and 1 μ M BPA on the leaf development of *A. thaliana* seedling grown was shown in Fig. 4(A). Under

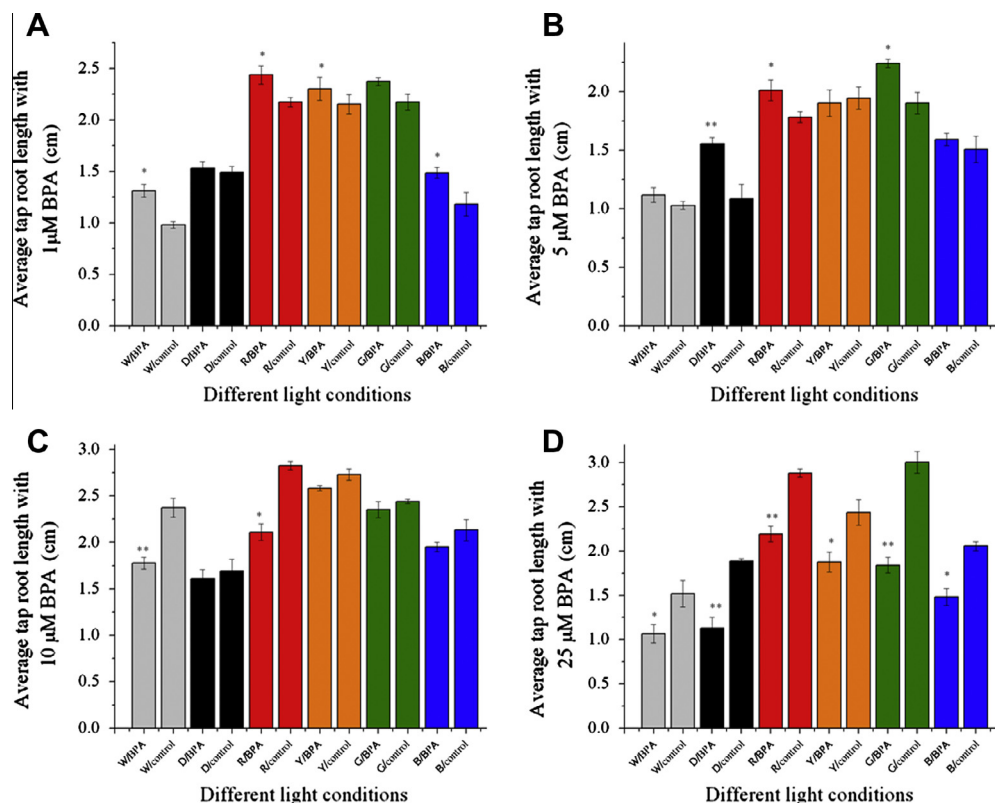


Fig. 3. The growth indices on the tap roots length of *Arabidopsis thaliana* seedling in response to different parts of light spectrum (dark as “D”, red as “R”, yellow as “Y”, green as “G”, blue as “B”, and white light as “W”), treated with 1.0 μ M BPA (A), 5.0 μ M BPA (B), 10.0 μ M BPA (C) and 25.0 μ M BPA (D). Data represents mean \pm SEM of *n* = 60 *Arabidopsis thaliana* seedling. *0.01 < *P* < 0.05, ***P* < 0.01. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

any light, independently of quality, 1 μM BPA seemed to positively affect leaf number/plant, while there was no impact on the distribution of *A. thaliana* blades in dark. BPA significantly promoted the blades differentiating into more blades under all light, and the degree of promotion corresponds with the relative effectiveness of each light as a differentiation stimulant: Yellow > Red > Green > Blue > White.

Fig. 4(B) shows the interaction between BPA and light spectrum on the differentiation of *A. thaliana* blades, while the concentration of BPA was fixed at 5 μM . The results indicated that BPA positively promoted the blades differentiating into more blades, no matter under the light or dark conditions. The degree of promotion corresponds with the relative effectiveness of all light qualities as a differentiation stimulant: Yellow > White > Red > Green > Blue > Dark.

The effect of 10 μM BPA and light spectrum on the distribution of *A. thaliana* blades showed in Fig. 4(C) reveals that BPA can positively promoted the blades differentiating into more blades under all light except for in dark, and the degree of promotion corresponds with the relative effectiveness of all light qualities as a differentiation stimulant: White > Green > Blue > Red > Yellow, while there was little effect on blades differentiation in dark.

Fig. 4(D) revealed that 25 μM BPA inhibit *A. thaliana* blades differentiation in a light-dependent manner, and the degree of inhibition corresponds with the relative effectiveness of all light qualities as a differentiation inhibitor: Red > Blue > Green > White > Yellow, while there was little effect on blades differentiation in dark.

The results illustrated that BPA affect the distribution of *A. thaliana* seedling in a dose-dependent and light-dependent manner. It was known that dark-grown seedlings exhibit etiolated growth, characterized by long hypocotyls, small and closed cotyledons with undifferentiated chloroplasts, and the repression of light-regulated genes, however, light inhibits hypocotyl growth and promotes cotyledon opening and expansion, chloroplast differentiation and the activation of light-regulated genes during photomorphogenesis

(De Lucas et al., 2008). Although, the mechanism underlying this antagonistic interaction remains unclear, we can conjecture that BPA may induce a change of chloroplast differentiation and the activation of light-regulated genes to control the distribution of blades. Qiu et al. (2013) found that when soybean seedlings were treated with BPA at low concentration, the growth indices were increased obviously compared with those of the control, which was not related with the photosynthesis, the content of chloroplast and the chlorophyll fluorescence, while soybean seedlings were treated with BPA at high concentrations, the growth indices, net photosynthetic rate, content of chlorophyll, Fv/Fm, UPSII, and ETR were much lower than control. The results indicated that the inhibition in the *A. thaliana* blades differentiation treated with 25 μM BPA might be related to the decrease in photosynthesis because of the decrease in the content of chlorophyll and the change in chlorophyll fluorescence.

3.5. Effects of BPA and light spectrum on the lateral root of *A. thaliana* seedlings

The effect of light spectrum and BPA on lateral root formation of *A. thaliana* seedling was shown in Figs. 5 and 6. BPA stimulated lateral root formation in a dose-dependent manner under white, red, yellow, green and blue light, while the first three low-dose BPA (the data of 5 μM and 10 μM BPA treatments are not shown) promoted the lateral root formation, yet 25 μM BPA curbed the lateral root formation, however, in darkness, all concentrations of BPA destroyed the lateral root formed. Lateral root formation is an important model with which to study cell patterning and differentiation in *A. thaliana* seedling.

As we all know, elevated levels of free auxin inhibit root growth and induce formation of lateral roots. Is Auxin pathway involved in the molecular mechanism of the lateral root formation affected by BPA? Burkhard et al. (2001) investigated that the *A. thaliana*

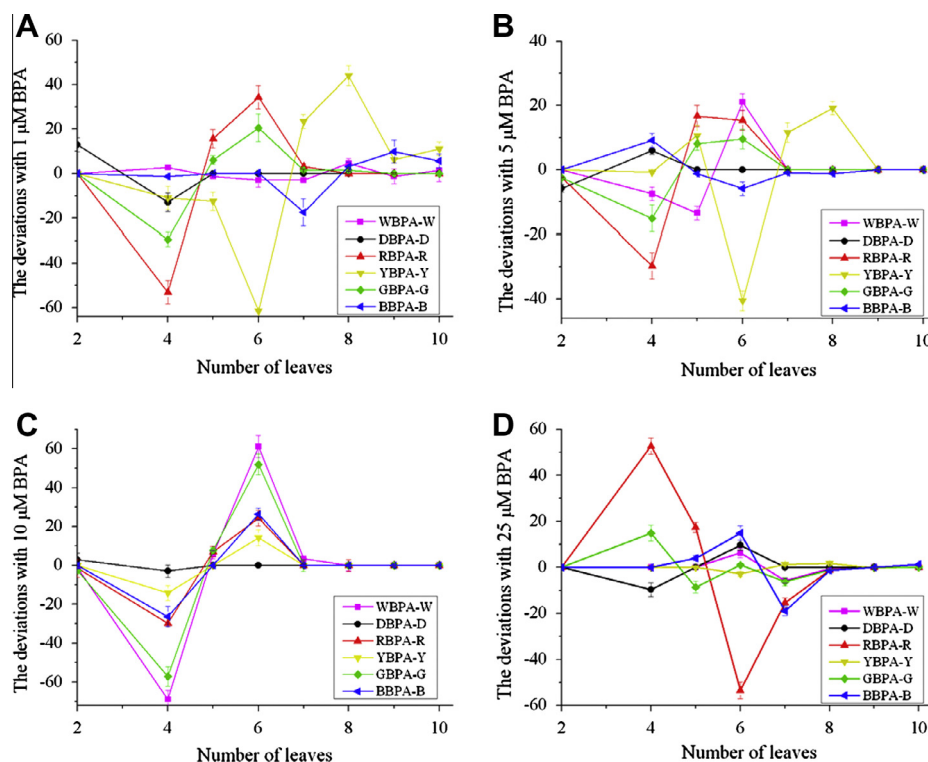


Fig. 4. The growth indices on the leaf distribution of *Arabidopsis thaliana* seedling in response to different parts of light spectrum (dark as "D", red as "R", yellow as "Y", green as "G", blue as "B", and white light as "W"), treated with 1.0 μM BPA (A), 5.0 μM BPA (B), 10.0 μM BPA (C) and 25.0 μM BPA (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Fig. 5. The growth indices on lateral root formation for single representative plants harvested from the different light treatments (dark as “D”, red as “R”, yellow as “Y”, green as “G”, blue as “B”, and white light as “W”), treated with 1.0 μ M BPA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

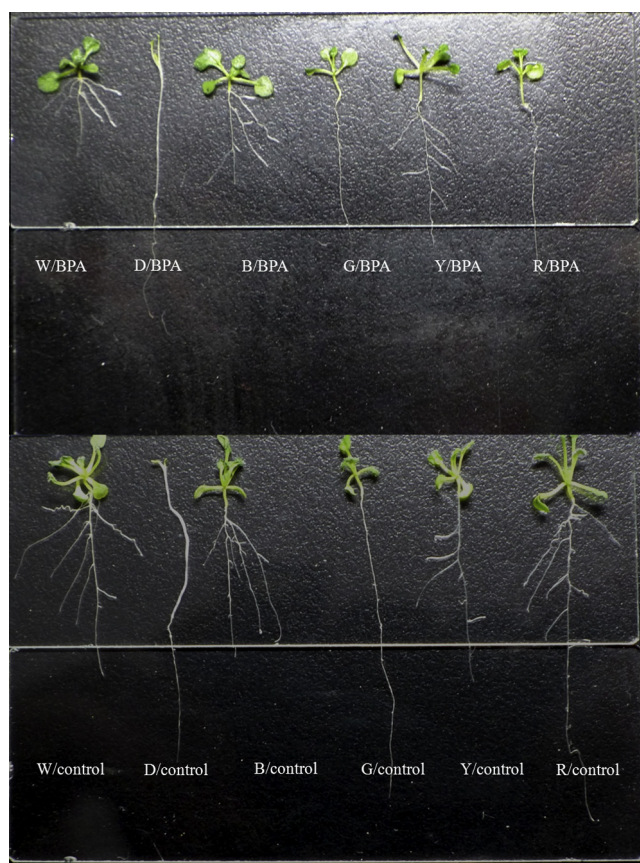


Fig. 6. The growth indices on lateral root formation for single representative plants harvested from the different light treatments (dark as “D”, red as “R”, yellow as “Y”, green as “G”, blue as “B”, and white light as “W”), treated with 25.0 μ M BPA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

seedlings exhibited decreased root growth and increased lateral root formation at last with the increase of auxin levels through impaired auxin transport. Maybe the interaction of BPA with auxin signaling is a possibility which needs to be tested.

It is also worthwhile noting that our present work was performed in solid medium conditions excluding real and natural soil–plant systems. To better evaluate the toxic effect of BPA in natural conditions, the degradation and adsorption of BPA in soil need to be considered. Under anaerobic conditions in the soil, [Ying and Kookana \(2005\)](#) showed little degradation of BPA during the 70-d study, so BPA should be able to affect soil and groundwater quality in a relatively long time scale. [Qiang et al. \(2013\)](#) show that the studied BPA present in the activated sludge cannot be degraded during sludge ozonation deficiency. The effect of BPA on plants in natural soil–plant systems will be studied in the future.

4. Conclusions

Our work demonstrated that BPA can influence the germination and early seedling growth in a dose-dependent manner. The BPA-mediated inhibition or promotion process in germination, may be not light-dependent, as BPA also significantly affects the germination in dark. Meanwhile, effects of BPA on fresh weight and tap roots length under different light conditions are similar, which imply that BPA has no notable role in priming light response in early seedling growth. Furthermore, BPA exposure influences the differentiation of *A. thaliana* leaf blade significantly in a light-dependent manner with little to no effect in dark and clear effect under red illumination.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2013.09.081>.

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