Asian Journal of Water, Environment and Pollution, Vol. 13, No. 2 (2016), pp. 107–110.
DOI 10.3233/AJW-160021

Short Note

Effect of Bisphenol-A on Nucleic Acids during Germination in Mung Bean Seedlings

K. Jayasri*, K. Padmaja and P. Eswara Prasad

Department of Veterinary Biochemistry, College of Veterinary Science
Sri Venkateswara Veterinary University, Tirupati – 517502, Andhra Pradesh, India
✉ jayasrikanteti@yahoo.co.in

Received February 21, 2015; revised and accepted December 21, 2015

Abstract: The extensive use of bisphenol-A (BPA) based polymers leads to widespread environmental contamination. Since agricultural soils are often enriched with activated sewage sludge, biosolids that may contain BPA, organisms dwelling in the soil and of course the rooted plants could encounter BPA from that source. However, few studies have examined the toxicological effects of BPA in plants which are able to take up and accumulate BPA. Since germination stage is more sensitive, the present study was carried out on the toxic effect of BPA on nucleic acids in mung bean seedlings. Controls were treated with distilled water and other lots of seeds were treated separately with 0.3mM, 0.4mM, 0.7mM concentration of BPA in petridishes. The plant growth was retarded in a dose dependent manner with BPA treatment. The nucleic acids were isolated and estimated on 4th day of germination. Both DNA and RNA contents were found to be decreased with BPA exposure. The purified samples subjected to electrophoresis further revealed the DNA degradation in a dose dependent manner.

Key words: Bisphenol-A, nucleic acids, DNA, RNA, germination.

Introduction

Bisphenol-A is an industrially important compound widely used as a monomer for the production of polycarbonates, plastics and also in epoxy resins used in internal protective lining for food and beverage cans (Ben-Jonathan and Streinmetz, 1998). To meet high demands, BPA is synthesized in large quantities i.e. 3.7 million MT per year worldwide. As a result, a significant quantity of BPA may escape or be discharged into the environment either from plastic manufacturer bodies or due to the extensive use of polymers/plastics (Ye et al., 2009). Leaking of BPA from plastic and metal waste in landfills is the potential source of environmental contamination (Vomsaal and Huges, 2005). BPA presence was repeatedly confirmed in atmosphere, waste water effluents, raw sewage and sewage sludge (Fu and Kawamura, 2010). Agricultural soils are often enriched with activated sewage sludge and biosolids that may contain BPA.

Plants can readily absorb BPA through their roots from agricultural soils and water. Significant reduction in DNA and RNA contents in BPA-treated animals was observed by Sangai and Verma (2011). Under optimal conditions, seed imbibitions and metabolic reactivation lead to synthesis of total RNA and proteins. It is reported earlier that alteration of total RNA or some species of mRNA indicates the exposure of toxicity or stress (Reuzeau and Cavalie, 1997). The adverse effects of BPA on cell division and growth of *Pisum sativum* was reported to be due to its action as anti-micro tubule agent. MT arrangement of BPA-treated cells were disrupted in all mitotic stages and cytokinesis arresting all cell cycle phases (Ioannis-Dimosthenis et

*Corresponding Author

al., 2013). Even though the rate of synthesis of nucleic acids is high during early growth of seedlings, there are few studies on the toxic effects of BPA on nucleic acids during germination in plants. Hence, BPA toxicity on nucleic acids is studied during germination in Mung bean seedlings.

Materials and Methods

Mung bean (*Phaseolus vulgaris* L) seeds were washed with tap water, allowed to imbibe in water for five hours and spread over on the moist filter paper in petri dishes. Controls were maintained with distilled water and other lots of seeds were treated separately with 0.3mM, 0.4mM, 0.7mM concentration of BPA dissolved in distilled water. Seedlings were maintained under natural day light for 4 d at day and night temperatures of 30±2°C. Experiments were carried out on 4th day of germination.

DNA Isolation

DNA is isolated from plant tissue using Hipura plant DNA isolation kit by CTAB (Cetyl trimethyl ammonium bromide) method. Lyophilized plant tissue (400 mg) was ground using 9 ml of pre-warmed CTAB extraction buffer and incubated at 64°C with occasional inversion for 60-90 minutes. The samples were cooled and 5 ml of chloroform:octanol (24:1) is added. The samples were centrifuged at 3000 rpm for two minutes at room temperature. The top aqueous layer is treated with 25 µl of RNase A (20 mg/ml) and incubated at room temperature for 30 minutes. To it 6 ml of isopropanol is added and mixed gently by inversion until a white fluffy DNA precipitate was observed. The tubes are centrifuged at 3000 rpm for 20 minutes. To the pellet, 8 ml of 70% ice cold ethanol is added in cold condition and centrifuged again at 3000 rpm for five minutes. The pellet is dissolved in 1 ml of elution buffer. The purified DNA is stored at –80°C for further analysis.

RNA Extraction

RNA content was estimated according to the protocol using Hipura multipurpose RNA purification kit. Plant tissue (100 mg) is ground properly using mortar and pestle in liquid nitrogen to get fine powder and allowed liquid N to evaporate. Homogenized tissue is mixed with 1 ml of RNA x press reagent and transferred to 2 ml collection tube which is incubated at 15-25°C for five min. To 1 ml of RNA x press reagent 200 µl of chloroform is added. After proper mixing, it is kept at 20°C for 10 minutes, and then the tubes were centrifuged at 12,000 g for 10 minutes at 4°C. The upper aqueous layer containing RNA is transferred to another collection tube and 1 ml of P B R (binding solution) is added. After thorough mixing 775 µl of ethanol is added and mixed by gentle pipetting. The above solution, 700 µl is taken in a tube with spin column and centrifuged at 10,000 rpm for one minute. The flow through is discarded. The same is repeated with remaining solution. Pre-wash solution (500 µl) is added to the tube with spin column and centrifuged at 10,000 rpm for one minute. The flow through is discarded. The centrifugation is repeated twice with 500 µl of wash solution. The column is transferred to a new 2 ml collection tube and centrifuged twice at 8000 g for one minute using 50 µl of ethanol. Then finally spinned using 100 µl of RNase-free water. The column is discarded and RNA collected and stored at –80°C for further analysis.

The amount of DNA and RNA was estimated using nanodrop. The purified DNA is subjected to gel electrophoresis.

Results

The seedling growth was found to be retarded with BPA exposure in a dose dependent manner compared to the control. Similar observations were noticed by Ioannis-Dimosthenis et al. (2013) in seedlings of *Pisum sativum.*

The DNA content decreased significantly in a dose dependent manner with increase in BPA concentration. The percentage of reduction in DNA content was found to be 36%, 42% and 54% with 0.3mM, 0.4mM and 0.7mM BPA treatment respectively (Table 1). It was reported that decrease in DNA concentration in BPA-treated mice was probably a result of impaired DNA synthesis (Sangai and Verma, 2011). The decreased DNA content may be due to result of block in cell division. Similar results were observed by Prasad and Strzalka (2002) in plants where there is a reduced efficiency of DNA synthesis due to heavy metal toxicity. The electrophoretic pattern further confirmed the degradation of DNA with increase in concentration of BPA (Figure 1).

The decrease in total RNA levels was more with increase in concentration of BPA. The RNA content was decreased by 5.5%, 26% and 58% with 0.3mM, 0.4mM and 0.7mM concentration of BPA respectively (Table 2). It was reported that high germination ability of seeds was characterized by larger amount of total RNA (Reuzeau and Cavalie, 1997). Similarly, our results

Table 1: Effect of bisphenol-A on DNA content in Mung bean seedlings

Treatment	*DNA (µg/g)*	*% reduction*
Control	132.52 ± 52.1	
0.3mM BPA	84.39 ± 2.88	36%
0.4mM BPA	75.93 ± 3.21	42%
0.7mM BPA	60.19 ± 2.28	54%

Values are Mean ± SD, $P < 0.001$

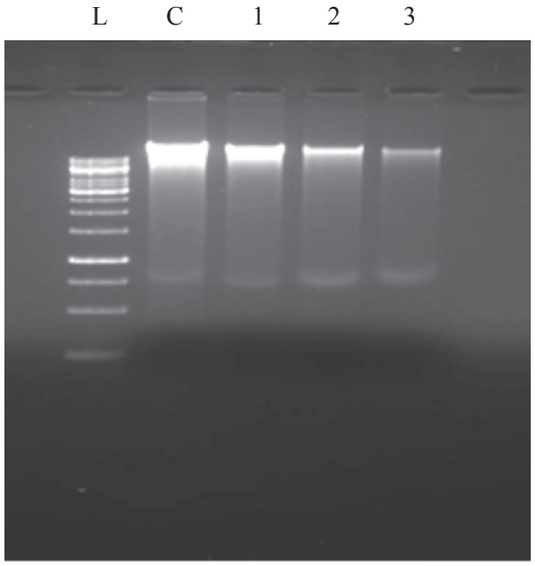

Figure 1: Electrophoretic pattern of DNA samples. L – ladder, C – control, 1 – 0.3mM BPA 2 – 0.4mM BPA, 3 – 0.5mM BPA

showed more seed germination in controls along with the increase in RNA content. Further Dhanakar and Solanki (2005) reported that RNA content was decreased with increase in copper and zinc concentration in *vigna mungo* (L). The metals such as Cr, Ni, Cd and Pb have been reported to decrease RNA synthesis and in turn decrease in RNA content (Schmidt, 1996).

A significant decrease in nucleic acid levels due to BPA toxicity is indicating disturbed nucleic acid metabolism. Atkinson and Roy (1995) found that BPA is converted to BPA-O-quinone. The semiquinone or quinone derivatives of BPA may be the ultimate DNA binding metabolites. This interaction might prevent RNA polymerase transcribing the DNA and can inhibit the formation of mRNA. This may be due to binding of BPA metabolites to DNA forming adducts and impairment of the process of transcription (Edmonds et al., 2004) Our results are in agreement with Jana and

Table 2: Effect of bisphenol-A on RNA content in Mung bean seedlings

Treatment	*RNA (µg/g)*	*% reduction*
Control	519.93 ± 4.67	
0.3mM BPA	91.18 ± 8.54	5.5%
0.4mM BPA	383.6 ± 5.47	26%
0.7mM BPA	217.33 ± 9.29	58%

Values are Mean ± SD, $P < 0.001$

Choudhuri (1984) who found a decrease in DNA and RNA content with heavy metal stress. Retarded growth of seedlings could be correlated with the degradation of DNA and RNA (Osborne et al., 1980).

References

Atkinson, A. and D. Roy (1995). *In vitro* conversion of environmental estrogenic chemical bisphenol-A to DNA binding metabolite(s). *Biochem. Biophys. Res. Commun.*, **210:** 424-433.

Ben-Jonathan, N. and R. Steinmetz (1998). Xenostrogens: The emerging story of bisphenol-A. *Trends Endocrinol. Metab.*, **8:** 124-128.

Dhankhar, R. and R. Solanki (2011). Effect of copper and zinc toxicity on physiological and biochemical parameters in *Vigna mungo* (L.) Hepper. *International Journal of Pharma and Bio Sciences.*, **2(2):** 553-565.

Edmonds, J.S., Nomachi, M., Terasaki, M., Morita, M., Skelton, B.W. and A.H. White (2004). The reaction of bisphenol-A-3, 4-quinone with DNA. *Biochem. Biophys. Res. Commun.*, **319:** 556-561.

Fu, P. and K. Kawamura (2010). Ubiquity of Bisphenol-A in the atmosphere. *Environ. Pollu.*, **158:** 3138-3143.

Ioannis-Dimosthenis, S., Adamakis, Emmanuel Panteris, Anna Cherianidou and Eleftherios, P. Eletheriou (2013). Effects of bisphenol-A on microtubule arrays in root meristematic cells of *Pisum sativum* L. *Journal of Mutation Research*, **750:** 111-120.

Jana, S. and M.A. Choudhuri (1984). Synergistic effects of heavy metal pollutants on senescence in submerged aquatic plant. *Water, Air & Soil Pollution*, **21(1-4):** 351-357.

Osborne, D.J., Sharon, R. and R. Ben-Ishai (1980). Studies on DNA integrity and DNA repair in germinating embryos of rye (Secale cereale). *Israeli Journal of Botany*, **29:** 259-275.

Prasad, M.N.V. and K. Strzalka (2002). Physiology and Biochemistry of heavy metal toxicity and tolerance in plants. Kluwer Academic Publishers, Dordrecht.

Sangai, N.P. and R.J. Verma (2011). Quercetin alleviates Bisphenol-A induced changes in nucleic acids and protein content in mice. *Acta Polonia Pharmaceutica-Drug Research*, **68(6):** 867-873.

Schmidt, W. (1996). Influence of chromium(III) on root associated Fe(III) reductase in *Plantago lsnceolata* L. *J. Exp. Bot.*, **47:** 805-810.

Vom saal, F.S. and C. Hughes (2005). An extensive new literature concerning low dose effects of Bisphenol-A show the need for a new risk assessment. *Environmental Health Perspectives*, **113(8):** 926-933.

Ye, X., Pierik, F.H., Angerer, J., Meltzer, H.M., Jaddoe, V.W., Tiemeier, H., Hoppin, J.A. and M.P. Longneeker (2009). Levels of metabolies of organophosphate pesticides, phthalates and bisphenol-A in pooled urine specimens from pregnant women in Norway mother and child cohent study. *Int J. Hyg. Env-Health*, **212:** 481-491.

Yong-Sheng Tian, Xiao-Fen Jin, Xiao-Yan Fu, Wei Zhao, Hong-juan Han, Bo Zhu Man-Liu and Quan-Hong Yao (2014). Microarray analysis of differentially expressed gene responses to bisphenol-A in Arabidopsis. *The Journal of Toxicological Sciences*, **39(4):** 671-679.