Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Sazada Siddqiui, Saad Abdurahamn Muhammad Al Amri, Huda Ahmed Al Ghamdy, Wadha Saad Saeed Alqahtani, Sarah Mohammed Alquyr, Habab Merghani Yassin (2021) Impact of Bisphenol A on seed germination, radicle length and cytogenetic alterations in *Pisum sativum* L.. *Caryologia* 74(2): 103-109. doi: 10.36253/caryologia-1230

Received: March 02, 2021

Accepted: June 11, 2021

Published: October 08, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

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Impact of Bisphenol A on seed germination, radicle length and cytogenetic alterations in *Pisum sativum* L.

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Abstract. Bisphenol A (BPA) is a global transpiring pollutant and an endocrine disruptor present in the environment which has a substantial harmful effect on plants. In the present study, its effects on seed germination, radicle length and cytogenetic alterations were investigated in *P. sativum* root tip cells. *P. sativum* seeds were germinated after treating with various concentrations of BPA (2 mg/L, 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L) at 24±1°C for 72 hours and the cytogenetic variations were assessed. The investigation showed that BPA reduced the percentage of seed germination, mitotic index, radicle length (at higher concentrations) and instigated a rise in chromosomal anomalies in a dose-related manner. In total, there is an enhanced occurrence of c-mitosis, stickiness, bridges, fragments and laggards in the BPA treated root tip cells of *P. sativum* seeds.

Keywords: BPA, Seed germination, Mitotic index, Chromosomal anomalies, *Pisum sativum* L.

INTRODUCTION

Bisphenol A (BPA, 2,2-bis-(4- hydroxyphenyl) propane) is an important transpiring pollutant (Clarke and Smith 2011). BPA is an abundantly mass-produced industrial chemical widely used in the manufacture of various domestic and daily use items like baby feeding plastic bottles, protecting coverings, packing of drinks, food items and in the linings of metal cans used for storing beverages and food products. Globally every year BPA is manufactured industrially in huge quantities approximately 0.0037 billion metric tonnes (Mihaich et al. 2009). It is constantly released in marine environment by municipal, agriculture and industrial effluents (Gatidou et al. 2007; Pothitou and Voutsa 2008; Fu and Kawamura 2010). With leaching of BPA by plastics and containers used for keeping food, drinks and beverages, human beings are exposed to it by consuming food and drinks stored in

these containers (Huang et al. 2012) and it poses a risk for the health of all human beings (Le et al. 2008; Wagner and Oehlmann 2009; Cooper et al. 2011). Human beings are also at risk by eating fish found in aquatic waters polluted by BPA (Mita et al. 2011). Agrarian soils usually get polluted by biosolids containing BPA found in sewage sludge (Gatidou et al. 2007; Stasinakis et al. 2008). Through extensive research work on BPA, it has been found that it is an endocrine disruptor (Staples et al. 1998; Le et al. 2008; Clarke and Smith 2011). Small organisms living in soils and plants could come in contact with soils polluted by BPA (Yamamoto et al. 2001; Staples et al. 2010). Moreover, not many studies have analyzed the toxicologic effects of BPA in plants which absorbs and accumulates it (Ferrara et al. 2006). Though, it has been established that plants can form BPA-glycosides by metabolizing BPA (Noureddin et al. 2004), clastogenic as well as phytotoxic influence of BPA were defined (Ferrara et al. 2006). Due to the impact of BPA on the pollen of kiwifruit in a dose-related manner, there is a substantial inhibition of tube development and its elongation (Speranza et al. 2011). Lately, the mitotic and chromosomal anomalies were found in cells of root meristem of *Allium cepa* L treated with 50, 100, 150 and 200 mg/L BPA concentration for five days (Jadhav et al. 2012). BPA treatment with 0.044-0.44 mM concentration inhibited the segregation of chromosomes, obstructed the cytokinesis completion, disrupted mitotic MT arrays and interphase and stimulated microtubules creation in *P. sativum* (Adamakis et al. 2013). Moreover, BPA treatment influences leaf blade differentiation in *Arabidopsis thaliana* significantly (Pan et al. 2013) and in BPA treated seedlings of soybean, it reduced the photosynthetic constraints and growth indexes (Qiu et al. 2013).

In animals, Bisphenol A has revealed to put forth xenoestrogenic action (Wang et al. 2021). However, the influence of BPA on plants are not clearly understood. Though BPA is consumed regularly and disposed, it may persist in the soil and can potentially cause detrimental effects on the plants. Further, there is not sufficient studies available in the literature about its genotoxic effects on plants (Palani and Panneerselvam 2007). In the present study, we have evaluated the adverse effects of BPA on seed germination, radicle length, mitotic index and chromosomal anomalies in cells of *P. sativum* root tips.

MATERIAL AND METHODS

Purchase of BPA and seeds

From a seed shop in Saudi Arabia, pea seeds (*P. sativum* variety ARKIL, $2n = 14$) were bought. Through Sigma–Aldrich Merck (Darmstadt, Germany) Bisphenol A (BPA) (BPA, 2,2-bis-(4- hydroxyphenyl) propane is procured from Bayouni Trading Co. Ltd., Jeddah, Saudi Arabia. Bisphenol A, CAS number is 80-05-7. Its melting point is 158-159 °C and its solubility in water at 25°C is 123–300 mg/L. The molecular weight of BPA is 228.29 and its chemical formula is $C_{16}H_{18}O_2$.

Seed treatment with BPA

For 5 minutes, seeds were sterilized in 0.1% $HgCl_2$ solution and they were washed in distilled water 2-3 times. Thirty seeds were soaked in BPA solutions of each concentrations (2 mg/L, 5 mg/ L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L) for 3 hours. For control group, a group of thirty seeds was soaked in distilled water. Seeds were repeatedly shaken for sufficient air supply. Thirty sterilized seeds were then spread over three Whatman filter papers, grade one and then kept in Petri-dishes (150 mm x 15 mm diameter). For more readings, these Petri dishes were kept in a Biological Oxygen Demand incubator (BOD) at a temperature of 24 ± 2^0C. As per the procedure defined by Rank (2003), root elongation toxicity and seed germination tests were performed. Radicle length were measured and germination of seeds were recorded, each day on an interval of 24 hours for 3 consecutive days. In similar settings, this test was done thrice. Toxicity was stated as compared with control, the difference of germination of seeds and root elongation.

Cytotoxicity and genotoxicity evaluations

To assess the cytotoxicity and genotoxicity evaluations caused by BPA in *P. sativum* plant, the root tips of germinated seeds were used as a source of mitotic cells. The root tips were washed in water. In a blend of ethanol and acetic acid (3:1–v/v, Merck), roots in length 2 cm were fixed (approximately 2 days). Staining of fixed roots were done with Schiff's reagent, as defined by Feulgen and Rossenbeck (Mello and Vidal 1978) and the slides were made by applying the meristematic region as per the protocol stated by Siddiqui et al. (2007). By documenting the variations in the meristematic cells mitotic index (MI), cytotoxicity was evaluated. By means of scoring various kinds of chromosomal anomalies (CAs), genotoxicity was assessed.

Each slide was observed and coded blind. By using light microscope under oil immersion, chromosomal anomalies and mitotic index in metaphase and anaphase plates were examined. At least 250 cells were scored from every single slide and mitotic index was computed. Chromosomal anomalies such as sticky chromosome,

c-mitosis, laggards, bridges and fragments were examined in at least 150 metaphase and anaphase plates for each slide and stated in percentage.

Statistical analysis

By using Graph Pad software (San Diego, CA, USA), statistical analysis (ANOVA with Dunnett's multiple-comparison test) having significance at P<0.05 was carried out. Data were exhibited in the form of mean ± standard error (SE).

RESULTS

Effect of BPA treatment on seed germination

At 24 h interval, in control group 77.33% of seeds germinated which increased to 85% and 99% at 48 h and 72 h respectively (Table 1). In seeds treated with lower concentration of BPA (2 and 5 mg/L), percentage of seed germination decreased (p<0.001 and p<0.05) at 24 h. Similarly, a significant decrease was observed at 48 h (p>0.05) and 72 h (p<0.01 and p<0.001) compared to control. In all time periods, on and above a concentration of 10 mg/L treatment with BPA caused a very significant decrease in germination percentage of seed in a dose-related manner, as compared to control. Lowest percentage of seed germination was reported at 20 mg/L (65% at 72 h) and at 25 mg/L (50.22% at 24 h, 60% at 48 h) in BPA treated seeds.

Effect of BPA treatment on radicle length

In control group, the radicle length increased with increase in time which was 4.0 ± 0.05 at 72 h (Fig. 1).

Table 1. Germination rates of *P. sativum* treated with different concentrations of BPA.

Concentrations of BPA	Seed germination (%)		
	24 h	48 h	72 h
00.00	77.33 ± 0.33	85.0 ± 0.88	99.0 ± 3.11
2 mg/L	72.77 ± 0.88[a]	84.0 ± 0.68	88.0 ± 2.09[b]
5 mg/L	74.33 ± 0.77[c]	78.0 ± 3.20	82.2 ± 0.33[a]
10 mg/L	66.66 ± 0.15[a]	70.0 ± 1.15[a]	75.0 ± 0.88[a]
15 mg/L	61.22 ± 0.03[a]	68.0 ± 1.15[a]	70.0 ± 1.33[a]
20 mg/L	55.33 ± 0.66[a]	61.0 ± 0.88[a]	65.0 ± 0.77[a]
25 mg/L	50.22 ± 0.42[a]	60.0 ± 0.55[a]	65.6 ± 0.66[a]

[a]p<0.001 compared to control; [b]p<0.01 compared to control; [c]p<0.05 compared to control.
Data are mean of three replicates ± SEM; 00.00 = Control group.

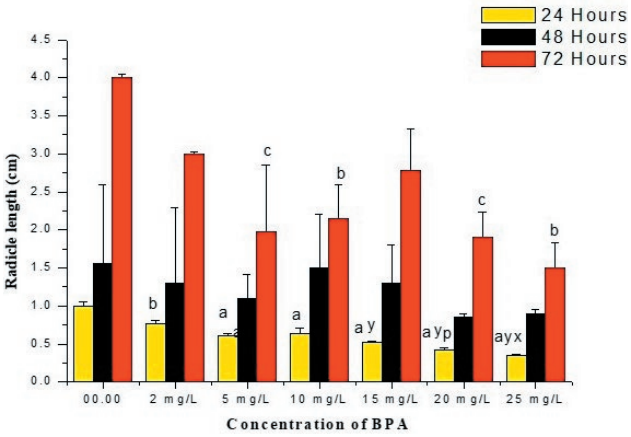

Figure 1. Effect of different concentrations of BPA on the radicle length of *P. sativum*. [a]p<0.001 compared to control; [b]p<0.01 compared to control; [c]p<0.05 compared to control. [Y]p≤0.001v/s 15, 20, 25 mg/L; [X]p≤0.01 v/s 25 mg /L; [p]p≤0.05 v/s 20 mg/L. Data are mean of three replicates ± SEM; 0.0 = Control group.

At 24 h interval, significant decrease in radicle length was observed in seeds exposed to BPA in a dose-related manner. Furthermore, there was no statistically significant difference reported from 2 mg/L to 25 mg/L BPA treatment at 48 h as compared to control. In 2 mg/L, 10 mg/L and 15 mg/L BPA treated seeds no statistically significant difference was noticed but in 5 mg/L and 20 mg/L significant decrease in radicle length was reported and in 25 mg/L very significant decrease was observed at 72 h. In BPA treated seeds lowest root length was recorded in 20 mg/L at 48 h (0.85 ± 0.04) and in 25 mg/L at 24 h (0.35 ± 0.02) and at 72 h (1.5 ± 0.33). Maximum root length was recorded in 2 mg/L (0.77 ± 0.04) at 24 h, 10 mg/L (1.5 ± 0.7) at 48 h and at 2 mg/L (3.0 ± 0.03) at 72 h in BPA treated seeds.

Effect of BPA treatment on mitotic index

The control presented a mitotic index of 17.78 ± 5.66 (Fig. 2). However, further increase in BPA concentration caused a decline in the mitotic index in a dose-related manner. As compared to control, at a lesser concentration of BPA (2 and 5 mg/L), the mitotic index was non-significantly lower. When compared with control, in seeds treated with 10 mg/L BPA, the mitotic index was significantly less (p<0.05), in 15 mg/L the mitotic index was found to be very significantly lower (p <0.01) and in seeds treated with 20 and 25 mg/L BPA, the mitotic index was highly significantly lower (p<0.001). In seeds treated with 25 mg/L BPA, the lowest mitotic index (5.45 ± 2.05) was determined.

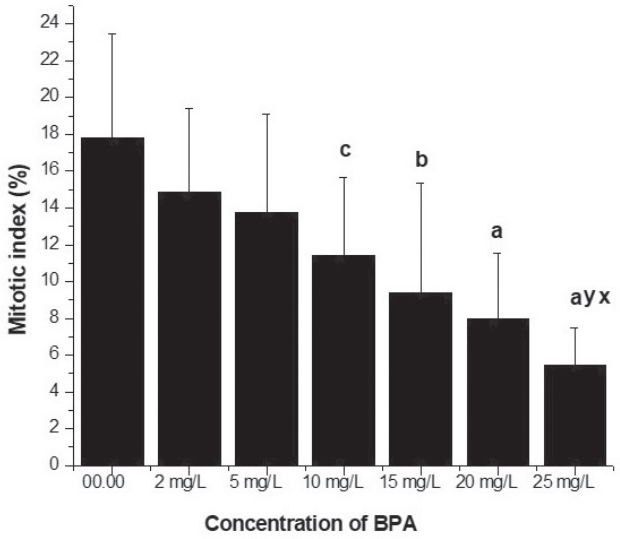

Figure 2. Effect of different concentrations of BPA on the mitotic index in root tip cells of *P. sativum*. [a]p<0.001 compared to control; [b]p< 0.01 compared to control; [c]p< 0.05 compared to control. [Y]p≤0.001v/s 15, 20, 25 mg/L; [X]p≤0.01 v/s 25 mg /L; Data are mean of three replicates ± SEM; 0.0 = Control group.

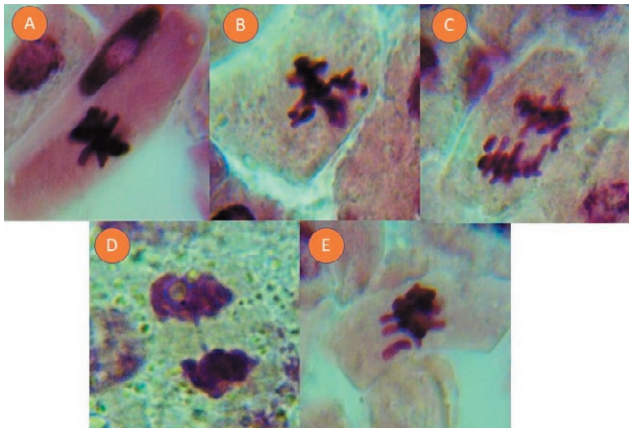

Figure 3. Chromosomal anomalies induced by BPA in *P. sativum* root tip cells. (A) Sticky chromosome, (B) C-mitosis, (C) Laggards, (D) Bridge at anaphase (E) Fragment.

Effect of BPA treatment on chromosomal anomalies.

As shown in Table 2 and Fig. 3 treatment with BPA caused numerous mitotic anomalies in *P. sativum*. In control, the occurrence of abnormal metaphase-anaphase plates was 00 ± 00. In the present study, in case of root tips of *P. sativum* enhanced occurrence of chromosomal anomalies such as sticky chromosomes, c-mitosis, laggards, bridges and fragments were observed in various doses of BPA treatment (Table 2, Fig. 3). Treatment with BPA resulted in a dose-related increase in the percentage of root tip cells with abnormal metaphase-anaphase plates.

In lower concentration (2 mg/L of BPA treatment), minimum chromosomal anomalies such as fragments (0.42 ± 0.01), c-mitosis (0.52 ± 0.01), sticky chromosomes (0.61 ± 0.01), laggards (0.83 ± 0.06) and bridges (0.91 ± 0.02) were found which were non-significant (p>0.05) when compared with control. Highest percentage of bridges (10.72 ± 2.2), c-mitosis (8.1 ± 2.15), fragments (6.78 ± 0.56), sticky chromosomes (6.1 ± 0.77) and laggards (6.01 ± 2.56) were found in 25 mg/L BPA treated root tip cells.

Sticky chromosomes were highly significant (p<0.001) in 5 to 25 mg/L, c–mitosis was found to be significant (p<0.05) at 25 mg/L, laggards were found to be significant (p<0.05) at 20 mg/L, bridges were found to be very significant (p<0.01) at 10 mg/L and highly significant (p<0.001) at 15 to 25 mg/L (p<0.01) and fragments were found to be very significant (p<0.01) at 15 mg/L and highly significant (p<0.001) at 20 to 25 mg/L when compared with control.

DISCUSSION

The outcome of the present study revealed that BPA inhibits and delays the germination of seeds, mitotic index, radicle length and chromosomal anomalies in

Table 2. Chromosomal anomalies in metaphase–anaphase plates in root tip cells of *P. sativum* treated with different concentrations of BPA.

Anomalies in 150 plates	Concentrations of BPA						
	00.00	2 mg/L	5 mg/L	10 mg/L	15 mg/L	20 mg/L	25 mg/L
Sticky chromosome (%)	00 ± 00	0.61 ± 0.01	2.78± 0.09[a]	4.99 ± 0.90[a]	4.25± 0.04[a]	7.80 ± 0.44[a]	6.10 ± 0.77[a]
C-mitosis (%)	00 ± 00	0.52 ± 0.01	3.15 ± 1.12	2.25 ± 1.20	5.70 ± 1.13	6.70 ± 3.20	8.10 ± 2.15[c]
Laggards (%)	00 ± 00	0.83 ± 0.06	1.32 ± 0.91	2.45 ± 1.01	6.75 ± 2.05	8.15 ±3.25[c]	6.01 ± 2.56
Bridges (%)	00 ± 00	0.91 ± 0.02	2.25 ± 1.00	4.23 ± 1.20[b]	5.78 ± 0.09[a]	7.62 ± 1.50[a]	10.72 ± 2.2[a]
Fragments (%)	00 ± 00	0.42 ± 0.01	0.71 ± 0.45	1.75 ± 0.76	2.91 ± 0.66[b]	4.62 ± 0.78[a]	6.78 ± 0.56[a]

[a]p<0.001 compared to control; [b]p<0.01 compared to control; [c]p<0.05 compared to control. Data are mean of three replicates ± SEM; 0.0 = Control group.

seeds of *P. sativum* in a dose-related manner. It was shown in our experimental outcome that there is a substantial concentration-effect of BPA on the germination of seeds, mitotic index, radicle length and chromosomal anomalies in seedlings of *P. sativum* (Table 1, 2 and Fig. 1-3).

Seed germination is inhibited by BPA (Zhiyong et al. 2013; Pan et al. 2013; Dokyung *et al.* 2018)). Similar findings have been found by the present study that BPA delays and inhibits the germination of *P. sativum* seeds. Seed germination is affected by various causes for example light, temperature of incubation, humidity and oxygen level (Isabelle et al. 2000). Eunkyoo et al. (2004) proved that an essential helix-loop-helix transcription feature PIF3-like 5 (PIL5) protein was a significant adverse regulator of phytochrome-mediated germination of seeds. It is known that etiolated seedlings generally have higher quantities of phytochromes A (Hanumappa et al.1999), therefore, the possible functioning of BPA on phytochromes in seeds germination phase is interesting.

In the present test, it was revealed that BPA showed inhibitory effects on root length in *P. sativum* treated with different doses. This may be caused by the noxious influence of BPA in root tips mitotic cell division (Adamakis et al. 2013; 2016; Amer 2017; Dokyung et al. 2018. In *P. sativum* the root tip mitotic index is directly associated with decrease in root length. The same influence of BPA on mitotic index was recorded (Pan et al. 2013; Jadhav et al. 2012). Primary roots elongation is facilitated by relating hormonal signal paths and a variety of enzymes for example phospholipase D, auxin and phosphatidic acid (Ohashi et al. 2003; Li et al. 2006; Saini et al. 2013). Though, the paths comprised in the molecular process related to the elongation of roots altered by BPA is not known.

The decrease in the quantity of mitotic cells in root tips treated with BPA may be because of its mode of action on the progress of cell cycle. Synthesis of DNA may be inhibited by BPA (Adamakis et al. 2019; Ozge et al. 2019) or in G2 stage of cell cycle, BPA could also obstruct the cells and thus blocking them to enter into mitosis. Moreover, BPA might affect enzymes for DNA-repair, by altering the structure of proteins present in the enzymes or in mitotic cells, by decreasing the formation of enzymes at transcription phase that could induce chromosomal anomalies (Ozge et al. 2019; Nasir et al. 2018).

P. sativum seeds treated with BPA showed numerous chromosomal anomalies in root tips mitotic cells for example c-mitosis, bridges, laggards, fragments and sticky chromosomes. The occurrence of chromosomal anomalies increases with increase in BPA concentration. In cell division, spindle fiber arrangement and its movement are a mechanism reliant on ATP (Can et al. 2005; Nasir et al. 2018; Adamakis et al. 2019). Because of decreased synthesis and obtainability of ATP, arrangement of spindle fiber in root tips treated with BPA, cells might get influenced, and it could disturb the chromosomal organization at metaphase plate and chromosomal migration to opposite poles in anaphase. The irregularity in spindles formation and segregation of chromosomes in mitosis, will cause chromosomal anomalies like laggards, bridges and sticky chromosomes.

In BPA treated root tips, C-mitosis is generally linked to spindle defects (Shahin and El-Amoodi, 1991). Since earlier studies have shown that BPA is a strong inhibitor of spindle microtubule organization (George et al. 2008; Adamakis et al. 2013; Xin et al. 2014; Adamakis et al. 2016) which may explain high incidence of C-mitosis in BPA group.

The bridges found in the cells of BPA treated root tips are possibly produced by breaking and merging of chromosome bridges which got enhanced with treatment by BPA. Chromosome bridges may be formed because of stickiness of chromosomes and consequent collapse of freed anaphase separation or because of an uneven translocation or chromosome segment inversion (Gomurgen 2000; Siddiqui 2012; Siddiqui and Al-Rumman 2020 a and b). Moreover, chromosome fragments may get formed because reactive oxygen species can induce double strand breaks in DNA.

CONCLUSION

Conclusively, the outcome of this investigation revealed that BPA has substantial repressing effects on seed germination and enhances chromosomal anomalies in *P. sativum* root tip cells. As found in the present study and on the basis of the occurrence of numerous types of chromosomal abnormalities, it is rational to presume that BPA might reveal genotoxic effect on plants at elevated concentrations. Moreover, a greater insight into the manner of BPA noxiousness in crops for example *P. sativum* is vital.

ACKNOWLEDGMENTS

We are grateful to Deanship of Scientific Research at King Khalid University, Abha, Kingdom of Saudi Arabia for providing technical and administrative support.

FUNDING

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through general research program under grant number GRP-33-41.

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