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DETERMINATION OF GENOTOXIC EFFECT OF BORON ON *ALLIUM CEPA* ROOT MERISTEMATIC CELLS

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Abstract

The effect of boron on the mitotic index of *Allium cepa* root meristematic cells were investigated. By using the growth inhibition test LD_{50} value was determined first and then different doses of varied concentrations of boron were introduced to onion tuber roots. Distilled water was used as control. Since *Allium cepa* cell cycle is 24 hours, application process was carried out at 12, 24 and 48 hours. Mitotic index and mitotic phase frequences were calculated separately for each dose introduced. The most observed abnormalities were c-metaphase, prometaphase and disturbed anaphase-telophases. In addition to these, anaphase bridge, poliploidy and late chromosome particules were also observed. In the interphase disturbed nuclei, micronucleus and binuclear cells were determined. The data were analysed by use of chi-square and student t-test. According to the results obtained the genotoxic effect of boron on the test organism was observed.

Introduction

The first study on mitosis was carried out by Levan (1938) by using colchicin on *Allium cepa* root meristem cells. In addition to this *Vicia faba, Tradescantia paludosa, Pisum sativum, Hordeum vulgare,* and *Crepis capillaris* were also used for the same purpose (Amer & Ali, 1983; Dryanosvka, 1987, Kluge & Podlesak, 1985; Amer *et al*., 1999; Dimitrov, 1994). Allium test is the most common one in order to determine the toxicity in the labs because of the storage and easy growing pecularities of Allium. (Fiskesjö, 1985; Rank *et al*., 2002; Yüzbaşıoğlu *et al*., 2003; Smaka-Kincl *et al*., 1996; Evseeva *et al*., 2003; Kara *et al*., 1994; Grant, 1992**)**. Moreover this system is well correlated with the data obtained from procaryotic systems (Fiskesjö, 1985).

Boron and its derivatives or boron containing materials are used in a lot aspects of our daily life (Moore *et al*., 1997; Beyer *et al*., 1983). In this background, any possiblity of damage of boron to the the living cells should be considered. A number of reports indicate that its harmful affects had varied from one organism to another (Nobel, 1981; Guhl, 1996; Martinez *et al*., 1986; Bringmann & Kuhn, 1980; Kluge & Podlesak, 1985; Sage *et al*., 1989). The objective of this study was to determine any possible affects of boron by using Allium root meristematic cell test which is one of the reliable genotoxic test used in laboratories.

Material and Methods

Allium cepa (2n=16) was used as test organism. Boric acid was purchased from Sigma, Karmen and HCl from Fluka, absolute alcohol from Kimetsan, glycerine and acetic acid from Carlo erba.. Distilled water was employed as control. The data obtained from the study were analysed by use of chi-square and student t-test by mean of SPSS 10.0 software.

Growth inhibition test was carried out to determine the doses which had cytogenetic effect on the cells. For this purpose LD_{50} was determined first. In order to determine this concentration, 5 tubers of onions for each experiment were germinated in boric acid solutions of 1000, 750, 500, 400, 300, 200, 100, 50, 25, and 10 ppm concentrations, respectively. They were left at room temperature (~21°C ± 4°C) for 96 hours. The same process was carried out for the controls. After germinating the roots, randomly 10 root tips were taken out and measured metrically. LD_{50} value was considered as the concentration which retarded the growth of root by 50% when compared to the control**.** Administration doses used in the experiments were $2xLD_{50}$, $3/2xLD_{50}$**,** LD_{50}, $1/2xLD_{50}$ and $1/4xLD_{50}$

For the determination of cytogenetic parameters, the experiments were carried out by the method of Yüzbaşıoğlu *et al*., (2003). For this, 400, 300, 200, 100 and 50 ppm boron concentrations were employed. Distilled water was used for control group. Since cellular cycle of *Allium cepa* is 24 hours, examining periods were fixed as 12, 24, and 48 hours. The root tip were fixed in 3:1 mixture of ethanol and glacial acetic acid. Microscopic slides were prepared by squashing the tips in acetocarmine 1% (w/v). Cells division and cytogenetical abnormalities were observed and photographed under a BX50 Olympus research microscope

Results and Discussion

Root growth increased up to 50 ppm concentration of boron (Table 1). A decrease in root length was noticed at doses of 100 ppm and above. Growth inhibition was found at 200 ppm of boric acid. Over 100 ppm concentration, roots became dark coloured, more thick and gel like. These results supports that boron is necessary for growth but the line between useful and harmful concentration is very thin.

When same concentrations of boron were introduced at 12, 24 and 48 hours, results showed different effects on mitotic index (Table 2). The data obtained from this experiment was analysed by chi-square test and student t-test, and statistically meaningful value was considered as $p<0,05$.

Table 1. Results of the growth inhibition test.

Doses (ppm)	Average length (mm)	Growth (%)	Increase (+) or decrease (-) in growth (%)
Control	17,12	100,0	0,00
10	24,64	143,92	+ 43,92
25	19,86	116,00	+ 16,00
50	18,32	107,00	+ 7,00
100	14,50	84,69	- 15,31
200	9,70	56,65	- 43,35
300	5,34	31,19	- 68,81
400	5,10	29,78	- 70,22
500	3,68	21,49	- 78,51
750	2,18	12,73	- 87,27
1000	1,60	9,34	- 90,66

Table 2. The effects of boron on mitotic index of *Allium cepa* root meristem

Concentration (ppm)	Dividing cell number	Mitotic Index ± Stantard deviation (SD)	Mitotic Phases (%)			
			Prophase	Metaphase	Anaphase	Telophase
Control-12 hours	390	39 ± 0,021	47,57	22,33	14,56	15,53
50	368	36,8 ± 0,020	42,92	16,50	21,69	18,86
100	204	20,4 ± 0,020a	29,55	25,00	25,00	20,45
200*	360	36 ± 0,021	57,84	12,04	7,22	22,90
300*	340	34 ± 0,021a	86,17	6,38	1,07	6,38
400*	230	23 ± 0,019a	70,37	18,53	5,55	5,55
Control- 24 hours	386	38,6 ± 0,018	42,96	17,18	13,29	26,57
50	337	33,7 ± 0,021a	55,56	13,58	6,17	24,69
100*	394	39,4 ± 0,021	61,54	7,69	23,08	7,69
200*	311	31,1 ± 0,020a	75,25	4,12	5,15	15,46
300*	254	25,4 ± 0,020a	70,58	3,53	9,42	16,47
400*	123	12,3 ± 0,019a	73,08	11,54	7,69	7,69
Control- 48 hours	366	36,6 ± 0,021	35,95	13,49	11,79	38,77
50	511	51,1 ± 0,022a	42,34	22,52	9,02	26,12
100*	308	30,8 ± 0,021a	69,35	12,90	4,85	12,90
200	261	26,1 ± 0,020a	46,66	6,66	8,34	38,34
300*	330	33 ± 0,021a	61,45	13,25	8,43	16,87
400*	237	23,7 ± 0,020a	69,56	2,17	10,87	17,40

a $p < 0.05$ (student-t test)
* $p < 0.05$ (chi-square testi).

There has been a number of differences in mitotic index in all concentrations and all times. The highest values in difference were obtained after 48 hours examination in 50 ppm boron and the lowest one in 24 hours application of 400 ppm ($p<0,05$). When the mitotic phase frequencies was compared with the control group in varied time applications, there has also been statistically meaningful results (Table 2). Higher concentration of the boron caused an increase of prophase and decrease of telophase frequency, except for 12 hours application. Since metaphase and anaphase frequency formed as a result of cell division their numbers also varied as increase or decrease.

The results of anomaly types and their ratios produced by boron are given in Table 3. When the results were analysed by student t-test, there was statistically meaningful differences at the all values except for 50 ppm concentration application for 12 hours ($p<0,05$). The most observed anomaly types were c-mitosis, prometaphase and disturbed anaphase and telophase. C-mitosis was found to be the most common anomaly (45,54 %), followed by prometaphase (13,3 %), and disturbed anaphase and telophase (9,34 %), respectively. Apart from these, stickiness (8,97 %), late chromosome (3,96 %), anaphase bridge formation (2,69 %), and polyploidy (2,32 %) were also observed. In the interphase cells, disturbed nucleus (21,48 %), micronucleus formation (12,63 %), and binuclear cells (11,18 %) were observed. But these anomalies observed in the interphase showed no statistically meaningful differences (Fig. 1). When the growth inhibition on root was more than 45%, it indicated the presence of a substance which was either sublethal or toxic to the examined organism (Fiskesjö, 1985; Hidalgo *et al*., 1989; Wierzbicka, 1988; Antonsiewicz, 1990). From the point of toxicity, it would suggest that 300, 400, 500, 750 and 1000 ppm of boron is toxic to *Allium cepa* root meristematic cells. There are reports on the growth inhibition of borate on to some other organisms (Guhl, 1996; Martinez *et al*., 1986; Bringmann & Kuhn, 1980).

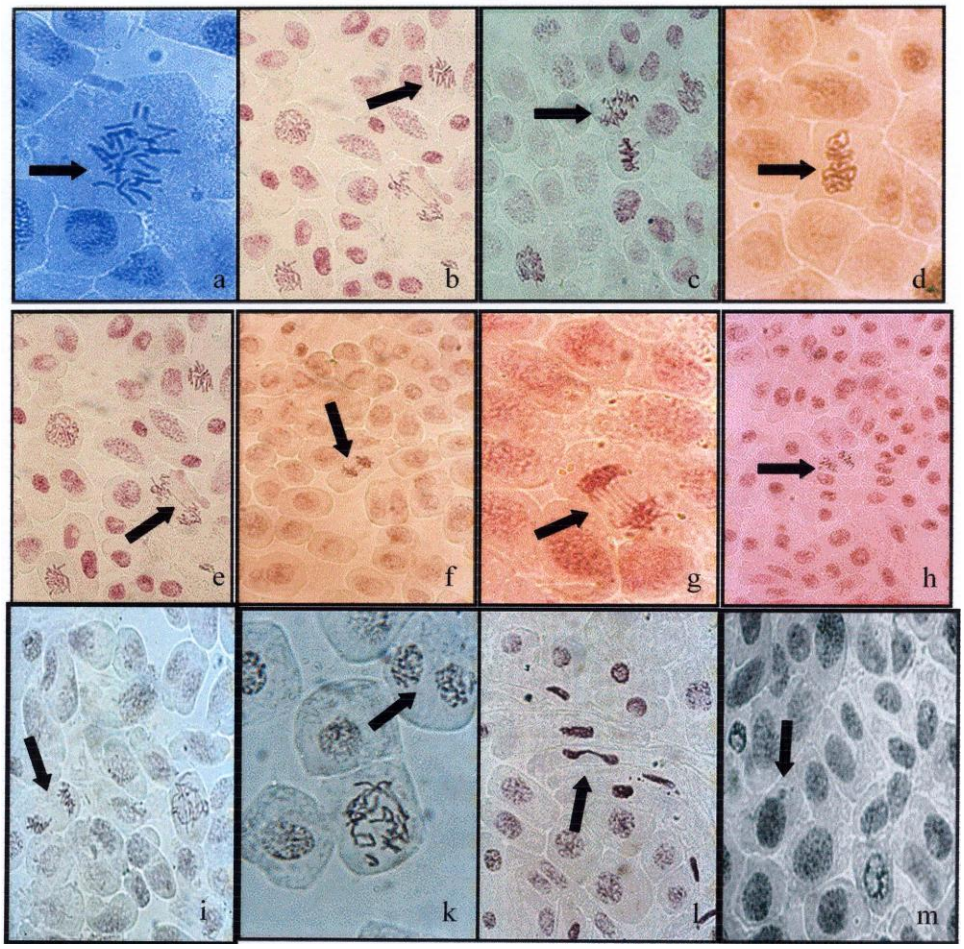

Fig. 1. Anomalies caused by boron on root meristematic cells of *Allium cepa*.
a- c-metaphase (x100), b- prometaphase (x40), c- polyploidy (x40), d- stickiness (x40), e- monobridge (x40), f- dibridges (x40), g- tribridges (x100), h- late chromosome (x40), i- disturbed anaphase-telophase (x40), k- binuclear cell (x100), l- nucleus anomaly(x40), m- micronucleus (x40)

According to Badr & Ibrahim (1987) decrease of mitotic index level shows that experimental material had mitodepressive effect resulting in the inhibition of cells acces to mitosis. It means that boron disturbs the normal cell cycle process by preventing biosynthesis of DNA and/or microtubule formation (Sadia & Vahidy, 1994). This is explained by antimitotic effect (Hidalgo *et al*., 1989; Yüzbaşıoğlu *et al*., 2003). Hence, this effect could be formed by decreased ATP level or suppression of the engine of energy production (Jain & Andsorbhoy, 1988).

C-mitosis, the most common anomaly observed, may occur due to disturbed microtubules by borate and this results c-mitosis aneuploidy (Fiskesjö, 1988). The other anomalies such as late chromosome, disturbed anaphase-telophase could be caused by the

effect of boron on microtubule formations (Amer & Ali, 1983). Stickiness could be because of sub-chromatid linkage between chromosomes (Mc-Gill *et al*., 1974) or chromosomes lose their movement abilities due to presence of boron and they get stuck in anywhere, and can not go to final destination (Ajay & Sarbhoy 1988). This is also explained as physical adhesion of the proteins of the chromosome (Patil & Bhat, 1992). Stickiness is accepted as an indicator of toxicity which results in cell death (Fiskesjö, 1985; El-Ghamery *et al*., 2000). Interphase nucleus anomalies could be because of the toxic effect of boron. Micronuclei break the microtubules and cause acentric chromosome fragments and it ends in aneuploidy and poliploidy (Chauhan *et al*., 1986; Chauhan & Sundararaman, 1990). Binuclear cells are accepted as the inhibition of cytokinesis in any control points of the cellular cycle (Ateeq *et al*., 2002). Anaphase bridges were observed mainly on monobridges, but di- and tribridges were also observed though in less number. This could happen during the translocation of the unequal chromatid exchange or due to dicentric chromosome presence. This bridges cause structural chromosome mutations (El-Ghamery *et al*., 2000).

The results of our study explain the genotoxic effect of boron, it would be better if it is examined by other eucaryotic test systems.

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