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Effects of Polyamines (Putrescine, Spermidine and Spermine) on Root Tip Mitosis and Chromosomes in *Allium cepa* L.

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Summary In the present study effects of the polyamines; putrescine (Put), spermidine (Spd) and spermine (Spm) have been studied on root tip mitosis of *Allium cepa* L. Root tips of *A. cepa* were treated with a series of polyamine (PA) concentrations, ranging from 0.01, 1 and 2 mM for 3, 6, 12, 24 and 48 h. The roots were examined in permanent root tip squash preparations stained by the Feulgen technique. This research has confirmed that PAs have various effects on chromosomes. PA concentrations used, generally had a marked mitodepressive effect on mitotic index (MI) but at 6 h treatment of Spm, Spd and Put at all concentrations had increased MI. Polyamines affected the relative duration of each mitotic stage as compared to the control. They also increased frequency of abnormal mitosis.

The types of abnormalities induced are: chromosome stickiness, c-metaphase, anaphase and telophase bridges, disturbed chromosomes of anaphase and telophase stages, lagging and forward chromosomes at anaphase and telophase. Binucleate cells and micronuclei formation at interphase cells were also observed.

Key words Allium test, Polyamines, Putrescine, Spermidine, Spermine, Aberrant chromosomes, Mitotic index.

Polyamines are essential components of living organisms and their implications in the physiological and biochemical processes have long been studied (Slocum *et al.* 1984, Smith 1985). In plant cells, the diamine putrescine (Put), triamine spermidine (Spd) and tetraamine spermine (Spm) constitute the major PAs. They are small polycations found in most organisms or cells and are essential for cellular proliferation and normal cellular functions (Tabor and Tabor 1984, Pegg 1988). Recently, the relationship between PA level and plant cell division/cell proliferation has received considerable attention (Torrigiani *et al.* 1987, Voigt and Bohley 2000).

The first attempt to evaluate these, higher plants bioassay was undertaken within the US Environmental Protection Agency's Gene-Tox Program and reported in 1982 by Constantin and Owens. In 1991 the International Programme on Chemical Safety (IPCS) published the results from any initial phase-I study on plant assay. Rank and Nielsen (1997) showed that Allium test was a simple method and can be used in IPCS studies. Also Allium test has been used a number of laboratories all over the world (Grant 1982).

The aim of this study is to investigate the effects of PA's on mitotic cell division and chromosomes in the root tip cells of *Allium cepa*.

Materials and methods

Root meristems raised from common onion (*Allium cepa* L. $2n=16$) were used as assay sys-

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tem. The bulbs of onions used had a size of 17–20 mm and a weight of 4–5.5 g. The yellow shallows and the dry parts inside the root primordia were removed before the test was carried out. Bulbs of onions were placed in small jars with their basal ends dipping in distilled water and germinated at room temperature (20±2°C). After 72 h, when the roots were 1.5 cm in length, transferred to jars containing Put, Spd, Spm solutions at various concentrations and distilled water (control). Nine onions were set up at each concentration. Roots of *A. cepa* were treated with a series of concentrations, ranging from 0.01 mM, 1 mM and 2 mM for 3, 6, 12, 24 and 48 h. Root tips were fixed in acetic alcohol (1 : 3). Samples of root tips were stained according to standard Feulgen procedure as previously described (Gömürgen, 2000). Three replicates were performed for each treatment and scoring was made from the 3 roots of each replicate. The MI was calculated for each treatment as a number of dividing cells/100 cells. The cytological abnormalities were scored in the mitotic cells and the results were shown in the tables and figures. The most common abnormalities were presented with micrographs.

Data obtained from the different treatments were statistically analyzed. The results of the treated groups were compared to the controls. The significance between the mean results was determined according to the t-test at the 5% level.

Results and discussion

The effects on MI and the frequency of mitotic phases are given in Table 1 for the treatment of Spm, in Table 2 for the treatment of Spd and in Table 3 for the treatment of Put.

As could be seen from Tables 1–3, three levels of Spm, Spd and Put at 6 h increased the MI but generally decreased at 48 h depending on concentrations compared to the control ($p<0.05$). Additionally, 0.01 and 1 mM Put at 3 h, 0.01 mM Put at 12 and 24 h, also significantly increased the MI.

These findings were partially similar to those of previous studies. Basu *et al.* (1988) observed that cell division in suspension cultures derived from coconut leaves was increased by Spd (optimum 10^{-6} M). Ünal *et al.* (2002) stated that Put decreased MI in diploid and tetraploid wheats while increased MI in hexaploid wheats. Agazo *et al.* (1995) concluded that in the maize meristematic zone a strong reduction of MI, and in the elongation zone an inhibition of the cell elongation, occurred simultaneously after Spd treatment. Majewsaka-Sawka *et al.* (1997) were analyzed the influence of exogenous polyamines on the frequency of protoplast division and showed that the enhancement in division index was caused mainly by spermine. In our study, with the presence of PAs especially at 6 h treatment, the increase of MI supports that polyamines like Spm, Spd and Put have been involved in cell growth and differentiation, and are considered to be crucial components of the living cell.

On the other hand MI was reduced especially at the highest concentration (2 mM) at 48 h treatment of Spm, Spd and Put. Such a drop in the mitotic index indicates that PAs at the highest concentration and the longest period interferes with the normal sequence of mitosis thus preventing a number of cells entering the prophase state at interphase. The reduction in the mitotic activity at high concentration of PAs could be due to the inhibition of DNA synthesis.

In the present study the PAs caused a change in the frequencies of the different stages (Tables 1–3). The frequency of pro-metaphase increased after treating with low concentration (0.01 mM) of PAs at 3 and 6 h when compared to the control. With higher concentrations (1 and 2 mM) and almost all treatment periods the frequency of pro-metaphase decreased. The frequency of anaphase and telophase stages was decreased in general after treating with PAs at all concentrations and all periods. These changes in the stages of mitosis indicate that PAs effects the relative duration of each stage as compared with the control. Similar results were obtained after treating *A. cepa* root cells with herbicides (Gömürgen 2000, El Khodary *et al.* 1989).

It could be seen from Table 1, 2 and 3 that PAs exerted a marked mitodepressive action on mi-

Table 1. Effect of **Spm**, at different concentrations, on mitotic activities of *A. cepa* root tip cells for different period of time

				Prophase-Metaphase (%)		Anaphase (%)		Telophase (%)		
Time of treatment (h)	Concentration (mM)	Total cells examined	Total mitosis	Total	Abnormalities	Total	Abnormalities	Total	Abnormalities	MI mean±SE
3h	Control	9592	321	42.99	0	28.04	0.31	28.66	0	3.30±0.33
	0.01	12495	451	61.86	20.62	4.66	2.22	8.20	2.44	3.74±0.59
	1	7999	330	41.82	30.61	0.61	1.82	23.33	1.82	4.19±0.52
	2	9154	246	29.67	45.12	1.63	0	17.07	6.50	2.68±0.11
6h	Control	10941	168	50	0	23.21	0	26.79	0	1.51±0.11
	0.01	9718	345	59.71	28.70	2.90	1.16	7.54	0	3.53±0.13*
	1	11429	363	37.74	52.62	0.83	1.38	5.23	2.20	3.18±0.06*
	2	6367	189	60.85	25.40	1.59	0.53	8.99	2.65	2.98±0.30*
12h	Control	9954	264	71.97	0.76	8.71	0	18.18	0.38	2.63±0.24
	0.01	7219	226	55.31	26.99	3.54	1.33	12.39	0.44	3.13±0.06
	1	11629	328	27.13	42.38	0	0	24.39	6.10	2.90±0.26
	2	10188	258	46.12	36.82	1.94	0.39	9.30	5.43	2.51±0.21
24h	Control	9105	218	54.13	0	16.51	0	29.36	0	2.37±0.55
	0.01	9644	303	24.09	53.47	2.31	3.96	9.57	6.60	3.10±0.17
	1	7839	203	13.79	61.08	0.49	0	16.26	8.37	2.59±0.09
	2	8803	178	19.10	68.54	1.12	0	1.12	10.11	1.93±0.33
48h	Control	9012	351	41.02	0	23.93	0	35.04	0	3.91±0.42
	0.01	9151	241	45.64	33.20	7.05	5.39	8.30	0.41	2.63±0.12*
	1	7162	191	15.18	65.97	0.52	0	4.71	13.61	3.02±0.56
	2	8282	160	30	53.13	0	0.63	11.25	5	1.90±0.19*

* : Significant (P<0.05) when compared with treated groups and control

Table 2. Effect of **Spd**, at different concentrations, on mitotic activities of *A. cepa* root tip cells for different period of time

				Prophase-Metaphase (%)		Anaphase (%)		Telophase (%)		
Time of treatment (h)	Concentration (mM)	Total cells examined	Total mitosis	Total	Abnormalities	Total	Abnormalities	Total	Abnormalities	MI mean±SE
3h	Control	9592	321	42.99	0	28.04	0.31	28.66	0	3.30±0.33
	0.01	12124	491	66.19	13.85	3.26	2.04	13.03	1.63	4.14±0.30
	1	14099	558	49.46	36.74	0	1.08	6.45	6.27	4.20±0.66
	2	9248	307	59.61	28.34	0	0	6.51	5.54	2.94±0.51
6h	Control	10941	168	50	0	23.21	0	26.79	0	1.51±0.11
	0.01	10787	368	51.36	34.24	1.63	0.82	11.41	0.54	3.41±0.28*
	1	11354	400	34.25	47.50	0	0	11.75	6.50	3.50±0.26*
	2	8844	309	44.66	39.16	0	0.32	8.09	7.77	3.00±1.21*
12h	Control	9954	264	71.97	0.76	8.71	0	18.18	0.38	2.63±0.24
	0.01	11794	324	42.28	42.59	5.25	0.93	6.48	2.47	2.73±0.27
	1	13589	332	28.92	40.96	0.30	0.90	19.58	9.34	2.71±0.74
	2	9751	165	37.58	43.64	1.21	1.82	13.33	2.42	1.96±0.55
24h	Control	9105	218	54.13	0	16.51	0	29.36	0	2.37±0.55
	0.01	9184	238	34.87	51.26	2.52	2.10	4.20	5.04	2.60±0.16
	1	12537	332	27.11	50.90	0.30	0.60	4.52	16.57	2.66±0.19
	2	10590	276	39.86	36.23	0	0.36	11.23	12.32	2.58±0.34
48h	Control	9012	351	41.02	0	23.93	0	35.04	0	3.91±0.42
	0.01	10826	208	57.21	26.44	2.88	2.40	8.65	2.40	2.06±0.88*
	1	10337	131	20.61	55.73	0	0	6.11	17.56	1.29±0.27*
	2	10512	111	17.12	66.67	0	0	1.80	14.41	1.05±0.16*

* : Significant (P<0.05) when compared with treated groups and control

Table 3. Effect of **Put**, at different concentrations, on mitotic activities of *A. cepa* root tip cells for different period of time

				Prophase-Metaphase (%)		Anaphase (%)		Telophase (%)		
Time of treatment (h)	Concentration (mM)	Total cells examined	Total mitosis	Total	Abnormalities	Total	Abnormalities	Total	Abnormalities	MI mean±SE
3h	Control	9592	321	42.99	0	28.04	0.31	28.66	0	3.30±0.33
	0.01	8830	490	71.22	11.02	4.08	3.88	9.80	0	5.55±0.89*
	1	10089	472	63.77	2.12	2.75	5.08	24.36	1.91	4.77±0.23*
	2	11042	447	55.70	33.56	0.67	1.34	5.37	3.36	3.98±0.23
6h	Control	10941	168	50	0	23.21	0	26.79	0	1.51±0.11
	0.01	10525	542	66.79	11.08	2.03	2.58	14.58	2.95	5.10±0.46*
	1	8277	368	39.13	21.74	0.54	1.09	27.72	9.78	4.42±0.11*
	2	8847	259	34.36	45.17	0	0.39	13.51	6.56	3.01±0.13*
12h	Control	9954	264	71.97	0.76	8.71	0	18.18	0.38	2.63±0.24
	0.01	8752	434	61.98	12.21	2.30	4.38	17.97	1.15	4.96±0.14*
	1	9542	314	36.31	42.36	0	0	14.97	6.37	3.28±0.46
	2	10542	312	60.26	32.69	3.85	0	3.21	0	2.96±0.10
24h	Control	9105	218	54.13	0	16.51	0	29.36	0	2.37±0.55
	0.01	10065	485	45.21	36.88	4.17	3.75	8.54	1.46	4.77±0.30*
	1	8548	259	33.59	38.61	0	0	5.02	22.78	3.02±0.10
	2	9544	243	25.10	52.67	0	0.41	4.53	17.28	2.57±0.09
48h	Control	9012	351	41.02	0	23.93	0	35.04	0	3.91±0.42
	0.01	8326	368	59.78	9.24	8.15	6.52	13.04	3.26	4.42±0.07
	1	8659	257	17.12	57.59	0	0.78	8.95	15.56	2.98±0.36
	2	10392	173	24.86	27.75	2.31	0.58	9.25	35.26	1.71±0.25*

* : Significant (P<0.05) when compared with treated groups and control

Table 4. Frequencies of different types of pro-metaphase, anaphase and telophase abnormalities after **Spm** treatment

		% of pro-metaphase abnormalities				% of anaphase abnormalities						% of telophase abnormalities						
Time of treatment (h)	Concentrations (mM)	sticky metaphase	c-metaphase	disturbed metaphase	forward metaphase	disturbed anaphase	anaphase laggard	anaphase bridge	anaphase forward	anaphase laggard bridge	tripolar anaphase	telophase bridge	telophase laggard	telophase forward	tripolar telophase	polipioid	binuclei cell	micronucleus
3	Control	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0
	0.01	0	81.72*	12.90	5.38	70	10	10	10	0	0	0	0	0	0	61.54*	23.08	15.38
	1	97.03*	0	1.98	0.99	0	0	100	0	0	0	50	0	50	0	0	0	0
	2	86.49*	0	10.81	2.70	0	0	0	0	0	0	81.25*	0	0	0	0	0	18.75
6	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.01	57.58	30.30	12.12	0	100	0	0	0	0	0	0	0	0	0	0	0	0
	1	84.29*	1.57	13.61	0.52	0	0	100	0	0	0	100	0	0	0	0	0	0
	2	87.50	2.08	6.25	4.17	0	100	0	0	0	0	60	0	20	0	0	0	20
12	Control	50	0	0	50	0	0	0	0	0	0	100	0	0	0	0	0	0
	0.01	0	93.44*	6.56	0	100	0	0	0	0	0	0	0	0	0	100	0	0
	1	98.56*	0	1.44	0	0	0	0	0	0	0	90*	0	0	0	0	0	10
	2	88.42*	0	6.32	5.26	100	0	0	0	0	0	64.29	0	0	0	0	0	35.71
24	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.01	18.52	38.89*	33.33*	9.26*	0	0	100*	0	0	0	40	0	0	0	45*	0	15
	1	100*	0	0	0	0	0	0	0	0	0	88.24*	0	0	0	0	0	11.76
	2	86.89*	0	8.20	4.92	0	0	0	0	0	0	66.67*	0	0	0	0	11.11	22.22
48	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.01	53.75	16.25	18.75	11.25	76.92*	0	23.08	0	0	0	0	0	0	0	0	0	100
	1	94.44*	0	0	5.56	0	0	0	0	0	0	88.46*	0	0	0	0	0	11.54
	2	64.71	22.35	9.41	3.53	0	100	0	0	0	0	50	0	0	0	37.5	0	12.50

* : Significant (P<0.05) when compared with treated groups and control

Table 5. Frequencies of different types of pro-metaphase, anaphase and telophase abnormalities after **Spd** treatment

		% of pro-metaphase abnormalities				% of anaphase abnormalities						% of telophase abnormalities					
Time of treatment (h)	Concentrations (mM)	sticky metaphase	c-metaphase	disturbed metaphase	forward metaphase	disturbed anaphase	anaphase laggard	anaphase bridge	anaphase forward	anaphase laggard bridge	tripolar anaphase	telophase bridge	telophase laggard	tripolar telophase	poliploid	binuclei cell	micronucleus
3	Control	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0
	0.01	1.47	95.59*	1.47	1.47	90*	0	0	10	0	0	37.50	0	0	50	0	12.5
	1	58.54*	8.29	28.78*	4.39	33.33	16.67	33.33	0	16.67*	0	25.71	5.71	2.86	0	0	65.71*
	2	44.83	24.14	19.54	11.49	0	0	0	0	0	0	17.65	0	0	5.88	11.76	64.71
6	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.01	0.79	92.86*	6.35	0	33.33	0	33.33	33.33	0	0	0	0	0	0	0	100
	1	76.84*	2.63	17.37*	3.16	0	0	0	0	0	0	26.92	3.85	0	0	0	69.23*
	2	77.69*	0	18.18	4.13	0	100	0	0	0	0	12.5	0	0	0	0	87.50*
12	Control	50	0	0	50	0	0	0	0	0	0	100	0	0	0	0	0
	0.01	29.71	50*	19.57	0.72	33.33	33.33	33.33	0	0	0	12.50	0	0	25	0	62.50
	1	94.85*	0	0.74	4.41	0	33.33	0	0	66.67*	0	35.48	0	0	0	12.90	51.61
	2	94.44	0	0	5.56	0	33.33	33.33	0	0	33.33*	25	0	25	0	0	50
24	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.01	81.97*	13.93	2.46	1.64	0	40*	40	20	0	0	50	0	0	41.67	0	8.33
	1	96.45*	0.59	0.59	2.37	0	0	100	0	0	0	20	0	0	0	0	80*
	2	98*	1	0	1	0	0	100	0	0	0	50*	0	0	0	0	50
48	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.01	36.36	47.27	3.64	12.73	60	0	40	0	0	0	0	0	0	20	60	20
	1	100*	0	0	0	0	0	0	0	0	0	30.43	0	0	0	0	69.57
	2	67.57	0	32.43	0	0	0	0	0	0	0	0	0	0	0	0	100

* : Significant (P<0.05) when compared with treated groups and control

Table 6. Frequencies of different types of pro-metaphase, anaphase and telophase abnormalities after Put treatment.

		% of pro-metaphase abnormalities				% of anaphase abnormalities						% of telophase abnormalities						
Time of treatment (h)	Concentrations (mM)	sticky metaphase	c-metaphase	disturbed metaphase	forward metaphase	disturbed anaphase	anaphase laggard	anaphase bridge	anaphase forward	anaphase laggard bridge	tripolar anaphase	telophase bridge	telophase laggard	telophase forward	tripolar telophase	poliploid	binuclei cell	micronucleus
3	Control	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0
	0.01	0	92.59*	7.41	0	100*	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	100	0	0	0	0	100*	0	0	0	100	0	0	0	0	0	0
	2	86*	2	8	4	0	0	100	0	0	0	80*	0	0	0	0	20	0
6	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.01	18.33	81.67*	0	0	35.71	14.29*	28.57	21.43*	0	0	0	0	0	0	62.5*	0	37.5
	1	75	20	2.50	2.50	0	0	100	0	0	0	44.44*	0	0	0	11.11	0	44.44
	2	93.16*	1.71	5.13	0	0	0	100	0	0	0	76.47*	0	0	0	0	11.76	11.76
12	Control	50	0	0	50	0	0	0	0	0	0	100	0	0	0	0	0	0
	0.01	0	83.02	16.98	0	73.68*	0	26.32	0	0	0	0	0	0	0	100	0	0
	1	63.91*	25.56	10.53	0	0	0	0	0	0	0	60	20*	0	0	0	0	20
	2	0	100*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.01	5.65	85.88*	2.26	6.21*	77.78*	0	22.22	0	0	0	0	0	0	0	0	0	100
	1	57	10	10	23*	0	0	0	0	0	0	22.03*	10.17*	0	0	0	0	67.79*
	2	49.22	1.56	42.97*	6.25	0	100	0	0	0	0	9.52	0	0	0	0	16.67*	73.81*
48	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.01	29.41	58.82	11.76	0	41.67*	8.33*	41.67*	0	0	8.33*	16.67	0	0	33.33	0	0	50
	1	71.62*	4.05	16.22	8.11*	100	0	0	0	0	0	52.5*	0	0	0	0	32.5*	15
	2	68.75	16.67	10.42	4.17	100	0	0	0	0	0	22.95*	9.84*	0	6.56*	1.64	9.84*	49.18*

* : Significant (P<0.05) when compared with treated groups and control

tosis (except 6 h PA treatments) and induced a number of chromosomal aberrations. The high percentage of abnormalities was observed at prophase-metaphase stages in Table 1, 2 and 3. The percentage of mitotic abnormalities (prophase-metaphase) ranged from 2.12% after 3 h treatment with 1 mM Put to 68.54% after 24 h treatment with 2 mM Spm. However percentages of anaphase and telophase abnormalities were lower than pro-metaphase abnormalities.

Different kinds of chromosome aberrations and frequencies were presented in Table 4,5 and 6 for Spm, Spd and Put respectively. These PAs caused chromosome stickiness, c-mitosis, bridges, laggards, forwards, disturbed phases, multipolar anaphases, binucleate cells, poliploid cells, multipolar cells and micronucleus.

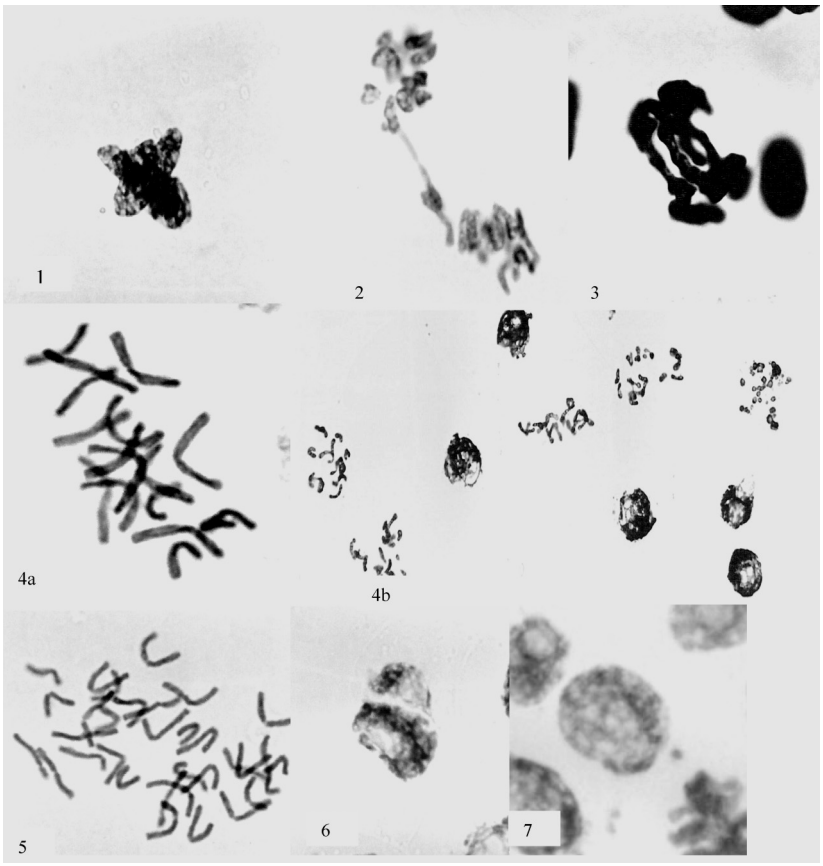
The most frequent type of abnormalities was stickiness (Fig. 1) for all PAs at almost all periods and concentrations. It was observed that all PAs significantly increased chromosome stickiness at almost all concentrations and periods. Stickiness has been attributed to the improper folding of chromosomal fibers, which makes the chromatids connected by means of subchromatid bridges (Badr and İbrahim 1987). Klasterska *et al.* (1976) and Mc Gill *et al.* (1974) suggested that chromosome stickiness arises from improper folding of the chromosome fiber into single chromatids and the chromosomes become attached to each other by subchromatid bridges. Chromosome stickiness reflects highly toxic effects, usually of an irreversible type, probably leading to cell death. Chromosomal stickiness has been attributed to an action on the proteins of the chromosomes.

Anaphase (Fig. 2) and telophase bridges (Fig. 3) involving one or more chromosome also the frequent type of anaphase and telophase abnormalities for all PA treatments. The ratio of cells with telophase bridges was significant in comparision to control. In the present study a correlation exists between stickiness and bridges produced in the cells of *A. cepa* affected with PAs. Chromosome bridges may be due to the chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to unequal translocation or inversion of chromosome segments. Perhaps a major interaction of the 3 polyamines in the cells may be with the proteins of the spindle apparatus and proteins which form an integral part of the chromosomes. This type of anomaly was also observed in the mitosis of *Vicia faba* cells after treatment with insecticide (Amer and Farah 1985).

The frequency of bridge formation without accompanying fragment(s) could only indicate that there was stickiness of the chromosomes perhaps most likely the result of cross-linkage of chromoproteins that are not really chromosome aberrations in a strict sense.

Another most common type of abnormality observed with all the concentrations and periods of Put treatments was c-metaphase (Fig. 4a, 4b, Table 6). C-metaphase was observed especially with 0.01 mM concentration for all periods of Spm and Spd treatments (Table 4 and 5). They were statistically significant as compared to the controls. This type of abnormality was the result of inhibition of spindle fiber formation. In this case it causes an arrest mainly at metaphase. C-mitosis was first described by Levan (1938) in root tips of *A. cepa* L. as an inactivated of the spindle followed by the random scattering of the condensed chromosomes. The most pronounced effect was the colchicine type of action resulting in the formation of C-metaphase figures which indicate inhibition of spindle fibers (Deysson 1968).

A number of other abnormalities are the result of this action in the spindle fiber. These are chromosome lagging, multipolar anaphases and micronucleated interphase cells. During the scoring of the chromosomes, poliploid cells (Fig. 5), binucleate cells (Fig. 6) and micronucleus (Fig. 7) were present in PA treated groups in addition to telophase aberrations. They are statistically significant at the micronucleated interphase and binucleated cells treated with high concentration of Put and for a long period of application. Metaphase with forward (Fig. 8), anaphase with laggard (Fig. 9), anaphase with forward (Fig. 10), telophase with laggard (Fig. 11) and tripolar anaphase (Fig. 12) were also rarely observed in *A. cepa* root cells after the some types and concentrations of PA treatments (Tables 4–6). At the root tip cells treated with Put at some of high concentrations and long periods, telophase with lagging chromosomes were significant as compared to the control. The in-

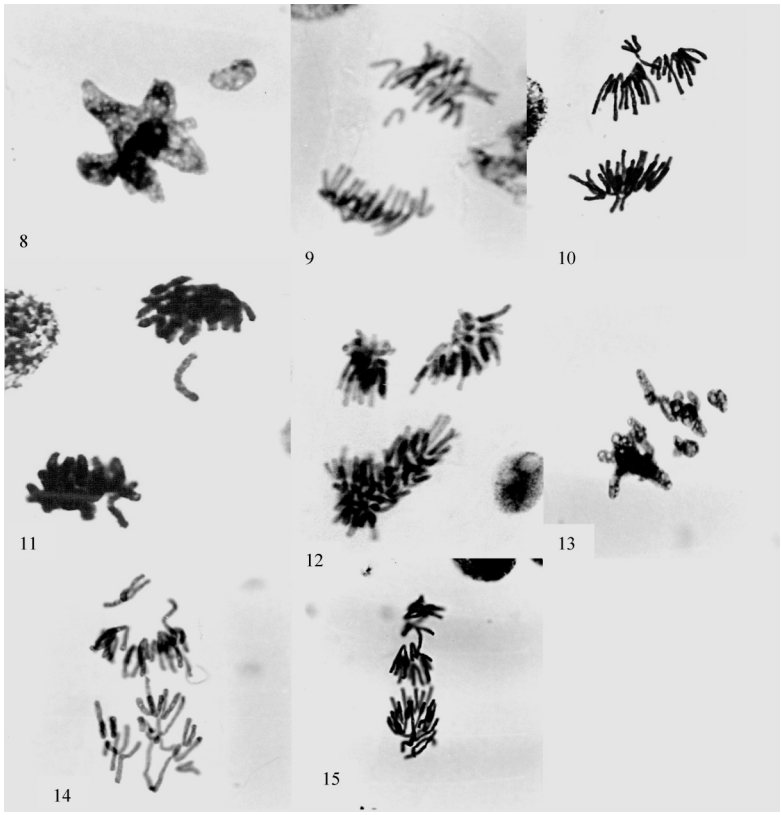


Figs. 1–7. Cytological abnormalities in root tip meristems of *A. cepa* treated with PAs; 1) chromosome stickiness, 2) anaphase bridge, 3) telophase bridge, 4a) c-metaphase, 4b) c-mitosis, 5) polyploid cell, 6) binucleate cell, 7) micronucleus.

duction of laggards could be attributed to the failure of spindle apparatus to organize and function in a normal way (Patil and Bhat 1992). As a rule, micronucleus formation is the result of acentric fragments or laggards being excluded from the nucleus during mitosis (Ma *et al.* 1995). The induction of micronucleus and multipolarity indicate the mutagenic potential of the applied polyamines.

Different treatments with PAs induced considerable percentage of disturbed metaphase (Fig. 13), disturbed anaphases (Fig. 14) and disturbed telophases (Fig. 15, Tables 4–6). In the mitotic cells treated especially with low concentration of Put, disturbed anaphase almost at all periods were significantly higher than the controls ($p<0.05$). In the cells treated with 0.01 mM Spm for 24 h (Table 4) and 1 mM Spd for 3 and 6 h (Table 5) and with 2 mM Put for 24 h (Table 6), the disturbed metaphases were significantly higher than the controls. Disturbed stages were noticed frequently after all treatments with three polyamine titers. It could be attributed to the interference of the polyamines with tubilin and/or polymerization of the microtubule subunits forming the spindle apparatus (Pickett-Heaps and Spurck 1982).

Various kinds of chromosome aberrations were observed in this study. These chromosomal aberrations have been considered as reliable indicators of mutagenic activity (Mohandas and Grant 1972). Ünal *et al.* (2000), similar to our observations, have stated that Put increased mitotic abnormalities by affecting both chromosomes and spindle fibers. Immunochemical studies of spermidine and spermine have showed that these polyamines are associated with highly compacted mitotic



Figs. 8–15. Cytological abnormalities in root tip meristems of *A. cepa* treated with PAs; 8) metaphase with forward chromosome, 9) anaphase with laggard, 10) anaphase with forward chromosome, 11) telophase with laggard, 12) tripolar anaphase, 13) disturbed metaphase, 14) disturbed anaphase, 15) disturbed telophase.

chromosomes (Hougaard *et al.* 1987) although they have more stabilizing than regulating effect on the chromatin structure during the cell cycle.

This research has confirmed that PAs have various effects on chromosomes. PA concentrations used, generally had a marked mitodepressive effect on MI but at 6 h treatment of Spm, Spd and Put at all concentrations had increased MI significantly. At the same time PAs caused various chromosomal aberrations. According to these results, it can be concluded that most probably PAs at high level has a genetoxic effect.

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