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Mitotic Effect of Water Extract of Different *Ipomoea* Species on *Allium cepa* L.

[1]A. Mondal, [2]G. Kabir, [3]N. Yasmin, [3]A.M.S. Alam and [3]H.A. Khatun
[1]Deratment of Botany, Manda MS College, Manda, Naogoan, Bangladesh
[2]Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh
[3]Professor Sultanul Alam Cytogenetics Laboratory, Department of Botany,
University of Rajshahi, Rajshahi 6205, Bangladesh

Abstract: The present investigation on ten species of *Ipomoea* was made from mutative points of view. For testing the mutagenic effectiveness of ten *Ipomoea* species their fresh leaves were used and the test was made on root tip cells of onion. Five different concentrations of the leaf extracts and two different treatments were considered. Cytological observations revealed that all the doses of leaf extracts caused mitotic anomalies. The anomalies were observed mostly at metaphase, anaphase and telophase. Bridge and ring chromosomes were common at anaphase. Few anomalies like c-metaphase, fragments and laggards were also observed. The frequency and type of abnormalities caused due to mutagenic effect of the leaf extracts of ten *Ipomoea* species were recorded under different treatment duration and doses. DMRT indicated the highest mutagenicity due to aqueous extract of *I. carnea* and lowest due to *I. aquatica*. In the present investigation, it was observed that mitotic anomalies increased with an increase of the doses and treatment duration. The overall doses of *I. aquatica* leaf extracts showed negligible anti-mitotic effects in *Allium cepa* roots compared to that of other *Ipomoea* species.

Key words: Mitotic effect, water extracts, *Ipomoea* sp., *Allium cepa*

INTRODUCTION

The effect of plant extracts on seed germination, seedling growth and on chromosome behaviour has attached considerable interest. Le Tourneau *et al.* (1956) found the aqueous extracts of some plants inhibited seed germination and seedling growth of wheat and peas. Mitodepressive and radiomimetic effects of plant extracts have been found by several researchers (Pandya, 1975; Shehab, 1979; Shehab *et al*., 1978). But no such information regarding its mutagenic effects is available. The seeds of *I. hederacea* contain a resin glycoside pharbitisin and alkaloids such as lysergol, chanoleavine, penniclavine, isopenniclavine and elymoclavine. The young shoots of *I. quamoclit* contain small amounts of alkaloids, resin glycosides and quamoclins I-IV (Ghani, 1998).

The toxic effects of leaf extracts of *Ipomoea carnea* in the onion root tip cells were also observed by Alam *et al.* (1987). They observed that the leaf extracts affected the mitotic frequency and caused some anomalies in root tip cells of onion. The edible species *I. aquatica* is naturally grown in different marshy lands throughout the Bangladesh and the poor people in Bangladesh are accustomed to consume the leaves as the major dietary constituents. However, information about the constituents of aqueous extracts of the related ten species of *Ipomoea* and their cytological effects are not adequate. Therefore an attempt was made to observed the mitotic effect of the leaf extracts of ten *Ipomoea* species in vegetative chromosome of *Allium cepa*.

MATERIALS AND METHODS

Fresh leaves of the ten species of *Ipomoea* and locally available bulbs of onion, *Allium cepa* L. were used as plant materials in this experiment in the year of 2003 in Prof. S. Alam Cytogenetics laboratory of the Botany Department of Rajshahi University, Bangladesh

Five grams of the fresh and mature leaves of each species of *Ipomoea* were ground in a mortar by the pestle with 50 mL distilled water and that was used as the stock solution. Five different concentrations viz., D_1 (10%), D_2 (15%), D_3 (25%), D_4 (50%) and D_5 (75%) were prepared by mixing 10, 15, 25, 50 and 75 mL of the extract with 90, 85, 75, 50 and 25 mL of distilled water, respectively. The control (distilled water) was designated as D_0.

Allium cepa bulbs were placed in small beakers with their basal ends dipping in distilled water at room temperature. About after 60 h, when the roots grew up to

Corresponding Author: Dr. A. Mondal, Department of Botany, MS College, Manda, Naogaon, Bangladesh

1.0-2.0 cm in length, water was replaced by different concentrations of the water-soluble extract of ten *Ipomoea* species and were treated for 6 and 12 h. The control (D_0) was kept undisturbed and untreated. After each time of treatment, the roots were washed in distilled water and fixed in Carnoy's fixative (1:3 aceto-alcohol). After 48 h of fixation root tips were transferred to 70% alcohol and stored in the refrigerator (9°C).

In order to collect the cytological data from the root tip cells of *Allium cepa*, temporary slides were prepared by hydrolysing the root tips in 50% HCl for 8 min, mordanting in 2% iron alum for 6 min and staining in 0.5% haematoxylin for 7 min. For each treatment slides were prepared from 5 different root tips and screening was made under microscope. Photomicrographs were taken from the desired preparations.

Analysis of data: Analysis of variance and Duncan's multiple range tests were made following Zaman *et al.* (1982). In case of ANOVA, the replication was omitted, because the data were recorded as percentage values and the materials were used in laboratory condition. Before making ANOVA the percentage values were transferred into angular values. When the values were found to range from of 0 to 100%, then they were substituted to 1/4n and 100 -1/4n for 0 and 100%, respectively. Before transformation all zero values were replaced by 1/4n (= 0.0042) and 100 by 100 -1/4n (= 99.9958). For example, table 0 values were replaced by 0.37, 0.11 values by 1.90 and 0.12 by 1.99 according to archin $\sqrt{\text{Percentage}}$ transformation appendix.

RESULTS AND DISCUSSION

Mutagenic effectiveness of ten *Ipomoea* species was determined by making the test on root tip cells of onion. Root tip cells treated with leaf extracts of *Ipomoea* species were observed for finding out any sort of cellular or chromosomal anomalies. Type and frequency of abnormalities caused of due to mutagenic effect of leaf extracts of *Ipomoea* species were recorded under different treatment duration and doses.

Cytological observations revealed that all the doses (10, 15, 25, 50 and 75%) of leaf extracts caused mitotic abnormalities. The abnormalities were observed mostly at metaphase, anaphase and telophase. Bridge and ring of the chromosomes were common at anaphase and telophase. Moreover, few other anomalies like c-metaphase, fragments, laggards, micronuclei, etc. were also observed at these stages of mitotic cell division (Fig. 1-7).

In control (D_0) and at lower concentration (10%) of extracts onion bulbs were found to emerge their roots but at highest concentration (75%) no root emergence or their growth were observed. The percentage of anomalies at the least concentration (D_1) was found to be somewhat high (2.09%) compared to 1.01% in the control D_0.

Despite of no treatment with the leaf extracts the apical meristem of *Allium cepa* showed cytological abnormalities with a very low frequency and that might be due to automutagenic substances (Sharma and Sen, 1954; Rieger and Michaelis, 1958, 1959; Dubinin and Scerbako, 1962).

In the present study percentage of chromosomal abnormalities in dividing cells were found to vary due to different leaf extracts under several concentrations (Table 1).

Lowest percentage (0.10) of mitotic abnormalities were observed due to mutagenic effect of the extracts of *I. maxima* and *I. turpethum* at low doses for short duration. The highest concentration (D_5) for long treatment duration (12 h) caused highest abnormalities (2.21%) with the extracts of *I. carnea*. On the other hand, 25% (D_3) concentration of the extracts of *I. carnea* for 12 h treatment duration caused 1.13% anomalies which was close to that frequency (1.15%) of abnormalities caused at highest concentration of the leaf extracts of *I. aquatica*. On the other hand 50% (D_4) concentration of the extracts of *I. paniculata* for 12 h. treatment duration caused 1.32% abnormalities and it was also close to 1.34% anomalies caused by 75% (D_5) concentration of the leaf extracts of *I. pulchella* for 12 h treatment duration.

The percentage of chromosomal anomalies was very low at the lower concentration of extracts but it was found to increase with an increase of the concentration. With the increase of treatment duration chromosomal anomalies were also found to increase. Similar results were found with the extracts of *Pulicaria crispa* on the mitotic index. Percentage of anomalies in onion, where increase of anomalies were found with an increase of the concentration as well as treatment duration (Shehab, 1979; Shehab and Adam, 1983). Such a result was also observed by Sudharsan and Reddy (1971) after treatment of *Vicia faba* root tips with leaf extracts of *Lathyrus sativus*. Similar results were also reported by Kabarity and Malallah (1980) in the root tip cells of onion due to the effect of *Cata edulis* leaf extracts.

Analysis of Variance (ANOVA) was made on mutagenic effect of the leaf extracts at different doses and treatment duration and they were found to be highly significant statistically (Table 2). Duncan's Multiple Range Test was also made to find out the mutagenic all the *Ipomoea* species individually (Table 3).

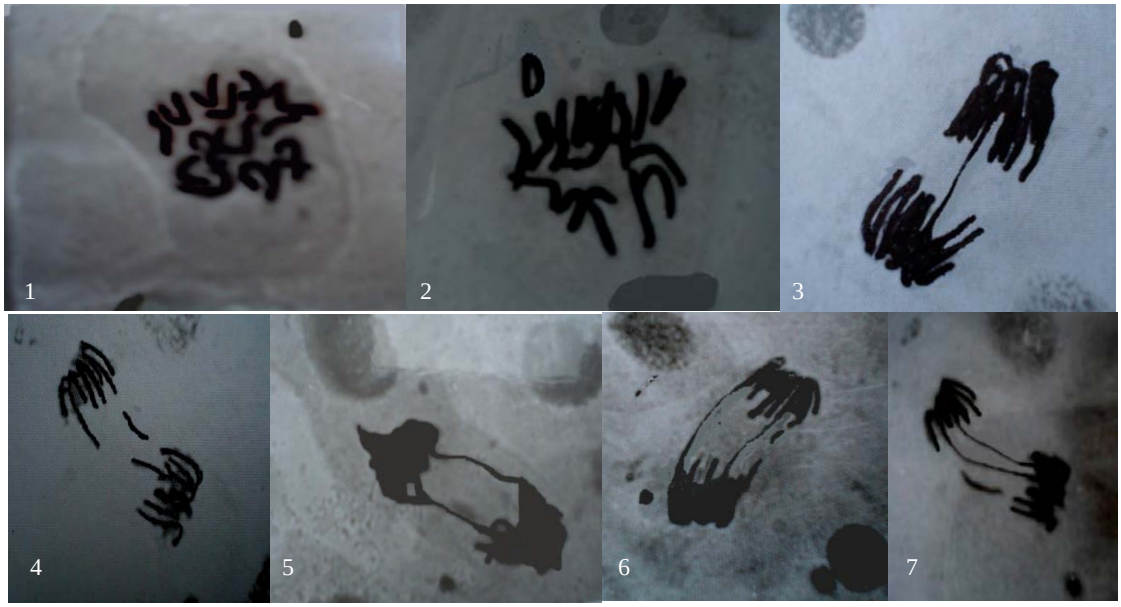

Fig. 1-7: Photomicrographs showing different chromosomal anomalies (ca 10X×1000) in root tips cells of *Allium cepa* due to mutagenic effect of the leaf extracts, 1: C-metaphase, 2: Metaphase with ring chromosome, 3: Anaphase with bridge chromosome, 4: Late anaphase with laggard, 5 and 6: Telophase with bridge, micronuclei and fragment, 7: Telophase with bridge and laggard

Table 1: Mitotic abnormalities in root tip cells of *Allium cepa* treated with different concentrations of the leaf extracts of ten species of *Ipomoea*

		Dose and duration												
		D_0 (Control)		D_1 (10%)		D_2 (15%)		D_3 (25%)		D_4 (50%)		D_5 (75%)		Total abnormalities (%)
Leaf extracts of	Parameters	6 h	12 h	6 h	12 h	6 h	12 h	6 h	12 h	6 h	12 h	6 h	12 h	
I. aquatica	Total cells studied	951	1025	908	865	920	810	1047	898	760	877	1155	960	4.88
	Total abnormal cells	0	0	1	1	2	2	3	4	5	7	10	11	
	% of abnormal cells	0	0	0.11	0.12	0.22	0.24	0.29	0.44	0.65	0.79	0.87	1.15	
I. carnea	Total cells studied	1125	1000	922	843	713	884	872	808	914	892	730	768	10.77
	Total abnormal cells	1	1	2	3	3	5	7	9	12	14	14	17	
	% of abnormal cells	0.08	0.10	0.22	0.36	0.42	0.57	0.84	1.13	1.32	1.60	1.92	2.21	
I. hederacea	Total cells studied	910	1220	976	937	740	808	747	890	592	727	856	713	5.83
	Total abnormal cells	0	1	1	1	1	3	4	6	4	7	8	9	
	% of abnormal cells	0	0.08	0.10	0.11	0.14	0.37	0.52	0.66	0.68	0.98	0.93	1.26	
I. maxima	Total cells studied	929	1075	840	994	915	980	619	752	1017	913	669	755	6.07
	Total abnormal cells	0	1	1	1	1	2	3	5	8	9	8	10	
	% of abnormal cells	0	0.09	0.12	0.11	0.11	0.20	0.48	0.66	0.79	0.99	1.20	1.32	
I. paniculata	Total cells studied	872	840	1025	853	986	877	793	808	1127	909	895	847	6.30
	Total abnormal cells	0	0	1	1	2	3	3	4	9	9	12	13	
	% of abnormal cells	0	0	0.10	0.12	0.20	0.34	0.39	0.48	0.81	0.99	1.34	1.53	
I. pes-tigridis	Total cells studied	1130	1080	726	611	818	891	952	881	732	828	704	834	8.19
	Total abnormal cells	1	0	1	1	2	3	6	7	8	11	11	15	
	% of abnormal cells	0.09	0	0.14	0.16	0.24	0.34	0.65	0.80	1.09	1.32	1.56	1.80	
I. pulchella	Total cells studied	915	842	908	865	920	810	1047	898	760	877	1155	960	6.38
	Total abnormal cells	0	0	1	1	2	2	3	4	5	7	10	11	
	% of abnormal cells	0	0	0.12	0.12	0.23	0.32	0.49	0.69	0.82	1.03	1.27	1.34	
I. quamoclit	Total cells studied	998	850	926	830	716	700	772	726	835	878	772	673	7.67
	Total abnormal cells	1	0	1	1	2	4	5	6	8	10	10	11	
	% of abnormal cells	0.10	0	0.11	0.12	0.28	0.56	0.65	0.83	0.96	1.13	1.30	1.63	
I. turbinata	Total cells studied	9.87	1180	715	665	782	730	662	835	890	957	956	970	8.75
	Total abnormal cells	0	1	1	1	2	4	6	9	11	13	14	15	
	% of abnormal cells	0	0.08	0.14	0.15	0.26	0.55	0.90	1.08	1.23	1.35	1.46	1.55	
I. turpethum	Total cells studied	730	991	1008	922	826	991	1005	1049	896	833	841	940	6.92
	Total abnormal cells	0	1	1	1	2	4	5	8	8	9	11	14	
	% of abnormal cells	0	0.10	0.10	0.11	0.24	0.40	0.50	0.70	0.89	1.08	1.31	1.49	

Table 2 : Analysis of variance (ANOVA) for mutagenic effectiveness of the leaf extracts of ten species *Ipomoea*

Source of variation	df	Sum of square	Mean square	F-values
Extract	9	25.3551	2.8172	21.3748***
Dose (D)	5	487.8454	97.5691	740.2815***
Hour (H)	1	5.8388	5.8388	44.3005***
DXH	5	3.0829	0.6166	4.6783**
Error	99	13.0461	0.1318	
Total	119			

CV% = 9.90 ** = Highly significant at 0.01%, *** = Very highly significant at 0.001%

Table 3: Duncans Multiple Range Test (DMRT) for mutagenic effectiveness of the leaf extracts of ten *Ipomoea* species

Leaf extracts of	Percentage (%) mutagenic effect	Ranks
I. aquatica	3.16g	10
I. carnea	4.85a	1
I. hederacea	3.55f	9
I. maxima	3.58f	8
I. paniculata	3.60f	7
I. pes-tigridis	4.18c	3
I. pulchella	3.70f	6
I. quamoclit	3.99d	4
I. turbinate	4.37b	2
I. turpethum	3.81e	5

The highest mitotic abnormalities (4.85%) was observed due to the aqueous extract of *I. carnea* and the lowest (3.16%) was observed due to that of *I. aquatica*. The extracts of *I. hederacea*, *I. maxima*, *I. paniculata* and *I. pulchella* showed almost similar significant effect to cause mitotic abnormalities.

Response of different concentration of the extracts were also manifested (Table 4). Mitotic anomalies increased with an increase of the concentration and the highest (6.79%) value for chromosomal anomalies were found with D_5 (75%) and the lowest (2.09%) one was found with the least dilution D_1 (10%). Depression of cell division was marked obviously in the lowest concentration D_1 (10%) when the mitotic anomalies reached up to 2.09%. Obviously it was high when compared to 1.01% in the control (D_0).

The highest concentration (D_5) was found to have more deleterious effect. The percentage of the mitotic abnormalities increased also with the increase of treatment duration. The frequency of anomalies was found always low at lower concentrations and as well as lower treatment duration (Table 5). The over all means values at higher treatment duration (12 h) was always high (4.10%) and low (3.66%) always at lower treatment duration.

Conforti *et al.* (1992) studied the mutagenic action of the anthocyanin pigments of *I. batatas* with meristem cells of onion and they found that at different concentrations its mutagenic action had a little but not significant effect. In the present investigation, the overall doses of *I. aquatica* showed no such strong anti-mitotic effect in *Allium cepa* roots compared to that of other species of

Table 4: Mean values for chromosomal anomalies against each concentration of the extracts and their ranking

Percentage of doses	Mean values (over extracts)	Ranks
D_5 (75%)	6.79a	1
D_4 (50%)	5.74b	2
D_3 (25%)	4.56c	3
D_2 (15%)	3.09d	4
D_1 (10%)	2.09e	5
D_0 (0%) (control)	1.01f	6

Table 5: Over all mean values for chromosomal anomalies and ranks of two different treatment durations

Hours	Mean values (over all)	Ranks
12	4.10 a	1
6	3.66 b	2

Ipomoea. This species may have some inhibitory effect due to the presence of rich number of pigments. Ghani (1998) reported the presence of a small amount of alkaloid in the young shoot of *Ipomoea quamoclit* and *I. hederacea* and rich number of pigments in *I. aquatica*. Yoshimoto *et al.* (1999) stated that the anthocyanin pigment may decrease the mutagenic activity of this sort of mutagens as heterocyclic amines. They purified two anthocyanin pigments from purple-coloured sweet potato. It may be stated here that the edible species *I. aquatica* exhibited such mutagenic efficiency, as *I. carnea* caused, although that by *I. aquatica* was of low frequency.

Tewary *et al.* (1964) isolated β-sitosterol, tricantian and saponin from the leaves of *I. carnea* and suggested that the saponin is probably triterpenoid in nature and it may have some inhibitory effect. Thus, the presence of this alkaloid in *I. carnea* is not unlikely.

REFERENCES

Alam, S., G. Kabir, M.N. Amin and M. Islam, 1987. Mitotic effect of leaf extract of *Ipomoea carnea* on *Allium cepa*. Cytologia, 52: 721-724.

Conforti, F.N., G.M. Varella and A.A. da-Silva, 1992. Use of beetroot pigment as a food colourant. Alimentos-e- Nutricao, 4: 33-34.

Dubinin, N.P. and V.K. Scerbako, 1962. Control of the natural mutation process by means of cysteamine and streptomycin. Proc. Acad. Sci. USSR., 145: 427-429.

Ghani, A., 1998. Medicinal Plants of Bangladesh (Chemical Constituents and Uses). Dhaka: Asiatic Society of Bangladesh, pp: 201-204.

Kabarity, A. and G. Malallah, 1980. Mitodepressive effect of khat extract in the meristematic region of *Allium cepa* root tips. Cytologia, 45: 733-738.

Le Tourneau, D., G.D. Failes and H.G. Heggeness, 1956. The effect of aqueous extracts of plant tissue on germination of seeds and growth of seedlings. Weeds, 4: 363-368.

Pandya, S.M., 1975. Effect of *Celosia argentea* extracts on root and shoot growth of bajra seedlings. Geobios, 2: 175-178.

Rieger, R. and Michaelis, 1958. Investigations of the cytology and metabolic physiology of the active mesistem of the root-tip of *Vicia faba* L. I. The influence of under water soaking of seeds on the rate of chromosome aberrations. Chromosome, 9: 238-257.

Rieger, R. and Michaelis, 1959. Investigations on cytology and metabolic physiology of the active meristem of the root *V. fava*. III. Further findings on the production and effect of automutagenic metabolic products. Biol. Zbl., 78: 291-307.

Sharma, A.K. and S. Sen. 1954. Study of the effect of water on the nuclear constituents. Genet. Iberica, 6: 151-195.

Shehab, A.S., 1979. Cytological effects of Medicinal plants in Qutar I. Mitotic effect of water extract of *Pulicaria crispa* and *Allium cepa*. Cytologia, 44: 607-613.

Shehab, A.S. and Z.M. Adam. 1983. Cytological effects of Medicinal plants in Qatar III. Meiotic effect of water extract of *Anastatia hierochuntica* L. on *Allium cepa*. Cytologia, 48: 343-348.

Shehab, A.S., H.A. Hakeem and Abu-El-Kheir, 1978. Cytological effects *of Achillea fragrantissima* extract on *Allium cepa* and *Vicia faba*. Cytologia, 43: 623-629.

Sudharsan, R. and S.S. Reddy, 1971. Cytological studies in *Vicia faba* L. treated with leaf extracts of two varieties of *Lathyrus sativus* L. Cytologia, 36: 702-715.

Tewary, J.P., Datta, K.C. and S.S. Mishra, 1964. Phytochemical and pharmacological investigations of *Ipomoea carnea* leaves. Sci. Technol., 2: 4.

Yoshimoto, M., S. Okuno, M. Yoshinaga, O. Yamakawa, M. Yamaguchi and J. Tamada, 1999. Antimutagenicity of sweet potato (*Ipomoea batatas*) roots. Biosci. Biotechnol. Biochem., 63: 537-541.

Zaman, S.M.H., K. Rahim and H. Manotosh, 1982. Simple lessons from Biometry. Bang. Rice Research Institute, Dacca, Bangladesh.