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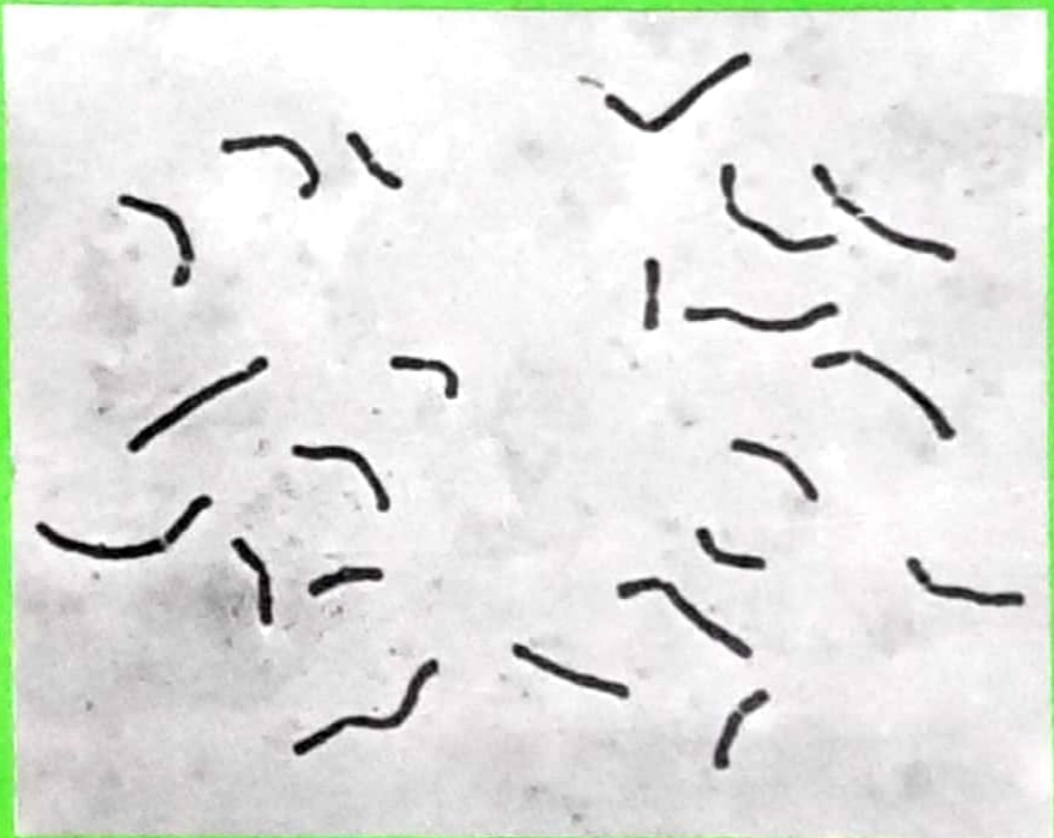

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ANTIMUTAGENIC BEHAVIOUR OF AN ANTIOXIDANT (ASCORBIC ACID) IN TWO MUTAGEN TEST SYSTEMS

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SUMMARY

The present study was aimed to unravel the effect of ascorbic acid on sodium azide induced mutagenicity in *Salmonella typhimurium* strains and chromosomal aberrations in *Allium cepa*. Sodium azide is a well known respiratory inhibitor and has been reported to induce base pair substitution mutations in microbes and chromosomal aberrations in eukaryotes. The investigation revealed that ascorbic acid reduced significantly the his^+ revertants induced by sodium azide in *S. typhimurium* and chromosomal aberrations in *A. cepa*. It was also observed that pre-treatment was more effective in reducing micronuclei and physiological chromosomal aberrations induced by sodium azide, whereas the reduction in clastogenic aberrations did not differ appreciably from one mode of treatment to another. It is postulated that probably ascorbic acid may be blocking the conversion of sodium azide to its ultimate mutagenic form.

Key Words : Antimutagenicity, *Salmonella*, *Allium*, ascorbic acid.

INTRODUCTION

Modulatory effects of chemicals/natural substances on environmental mutagens have a great significance due to their eventual application in evolving prophylactic measures against cancer. Ascorbic acid is one of the important antioxidants which is present in appreciable quantities in a large number of vegetables and fruits. A perusal of literature revealed that a few attempts have been made to understand the modulatory effects of ascorbic acid. Guttenplan (1977) reported the inhibitory effects of L-ascorbic acid on mutagenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and dimethylnitrosamine (DMN) in *S. typhimurium*. Toshihiko et al. (1980) reported the reduction of mutagenicity of 1, 4-dinitro-2-methylpyrrole by treatments with ascorbic acid. On the contrary, Shamberger (1984) reviewed the genotoxic effects of ascorbic acid. Keeping this unequivocal nature of the effect of ascorbic acid, the present investigation was planned to unravel the effects of ascorbic acid on sodium azide induced his^+ revertants in *S. typhimurium* and chromosomal aberrations in *A. cepa*.

MATERIALS AND METHODS

Salmonella mutagenicity assay

The basic protocol of Maron & Ames (1983) with a little modification was used to examine the effect of ascorbic acid on sodium azide induced his^+ revertants TA100 and TA1535 (base pair substitution mutagen tester strains) of *S. typhimurium*. Both the plate incorporation and pre-incubation assays were performed. For plate incorporation assay, to 2 ml of molten top agar at 45°C. 0.1ml of overnight grown bacterial tester strains in nutrient broth having a density of 1-2 x 10^9 cells/ml, 0.1 ml of mutagen (sodium azide), 0.1 ml of ascorbic acid were added. In pre-incubation mode of experiment, sodium azide and ascorbic acid were incubated for 30 min at 37°C and from this mixture 0.2 ml was added to a sterile test tube containing 2 ml of molten top agar which was followed by an addition of 0.1 ml of overnight grown culture. The contents of the tube were poured on to minimal glucose agar plates. In order to achieve a uniform distribution of the top agar on the surface of the plate, it is quickly tilted and rotated and then placed on a levelled surface to harden. Then the plates were inverted and kept in B.O.D. incubator at 37°C. Each experiment was run in triplicate and repeated at least once to ensure consistency. Concurrently, the experiments were run to estimate his^+ revertants induced by sodium azide, ascorbic acid alone and negative control where only water was added instead of ascorbic acid/sodium azide. Both the strains i.e. TA100 and TA1535 were kindly supplied by Prof. B.N. Ames, University of California, Berkeley. The genotype of each strain was checked prior to performing the experiments. The strains were kept as frozen permanents in liquid nitrogen for long storage and for routine work master plates were prepared as recommended by Maron & Ames (1983). Scoring of his^+ revertant colonies was made at an interval of 48 h and the antimutagenicity was calculated by applying the following formula:

Antimutagenic (Inhibition) activity (%) = a-b/a-c x 100

where a = Number of histidine revertants induced by positive mutagen (sodium azide); b = Number of histidine revertants induced by mutagen in the presence of ascorbic acid; c = Number of revertants induced by ascorbic acid alone.

In vivo chromosomal aberrations assay in root tips of Allium cepa

The procedure of Grant (1982) with a little modification was followed to evaluate the effect of ascorbic acid on sodium azide induced chromosomal aberrations in root tips of *A. cepa*. Onion bulbs grown in couplin jars with 0.5-1 cm long roots were transferred to another set of couplin jars containing desired concentration of chemicals. The experiments were designed to have the following set:

Control: In such cases, bulbs with emerging root tips were transferred to couplin jars containing distilled water.

Treatment with sodium azide: Couplin jars containing 0.25, 0.50 and 0.75% of sodium azide dissolved in distilled water and the treatment was given for a period of 2 h.

Ascorbic acid treatment: Three types of ascorbic acid treatments i.e., pre-treatment, simultaneous treatment and post-treatment were given.

(a) *Pre-treatment*: In this treatment, root tips were treated with varying concentrations of ascorbic acid i.e., 0.25, 0.50 and 0.75% for a period of 2 h followed by the treatment of sodium azide (0.75%) for the same period. (b) *Simultaneous treatment* (combined treatment): In such cases, *Allium* bulbs were transferred to couplin jars containing varying concentrations of ascorbic acid + 0.75% sodium azide. (c) *Post-treatment*: The root tips in this case were first treated with 0.75% sodium azide and then transferred to varying concentrations of ascorbic acid.

The treated root tips after duly washing with distilled water were either fixed as such in Farmer's fluid [acetic acid - alcohol (1:3)] or treated with colchicine for a period of 2 h prior to fixation. The fixed root tips were strained with Feulgen's stain after hydrolyzing in 1N HCL and were squashed in 45% acetic acid as outlined by Sharma & Sharma (1980). The squashed slides were analyzed for mitotic index, chromosomal aberrations and micronuclei.

Preliminary experiment was conducted employing varying concentrations of sodium azide to select the optimum dose inducing highest frequency of chromosomal aberrations without impairing the course of cell division. That optimum dose was employed to determine the effect of ascorbic acid. As sodium azide is one of the diagnostic mutagens for Ames assay, the dose recommended by Maron & Ames (1983) was used to determine the effect of ascorbic acid in *S. typhimurium*.

RESULTS AND DISCUSSION

It is evident from Table 1 that the effect of ascorbic acid is more in pre-incubation mode of experimentation as compared to plate incorporation method. The effect was dose-dependent and appeared to be quite significant, particularly at higher concentrations, where his^+ revertants were reduced by 73.08%. On comparing the effect between 2 strains, it was observed that the effect was more in TA100 strain. The present results are in agreement with the observation made by Khudoley

TABLE 1 : Effect of ascorbic acid on TA 100 and TA 1535 tester strains of *Salmonella typhimurium*.

Treatment	Dose (µg/0.1 ml/plate)	TA 100		TA 1535	
		Mean ± S.E.	% inhibition	Mean ± S.E.	% inhibition
Spontaneous	1	133.33 ± 2.02	-	16.00 ± 0.57	-
Positive control					
Sodium azide	1.5	1998.33 ± 6.01	-	1485.33 ± 6.77	-
Co-incubation	1	1970.00 ± 25.19	1.51	1210.66 ± 2.33	13.83
	10	1940.00 ± 27.57	3.12	1110.66 ± 0.66	20.94
	100	1820.00 ± 1.75	9.56	961.61 ± 7.27	31.55
	1,000	1765.00 ± 2.89	12.51	858.00 ± 1.51	38.93
	10,000	1758.33 ± 22.07	12.86	712.33 ± 1.45	49.33
	20,000	1745.00 ± 5.13	13.58	440.00 ± 2.89	68.68
Pre-incubation	1	1824.00 ± 7.54	9.34	880.66 ± 1.20	37.31
	10	920.00 ± 1.76	57.81	986.33 ± 9.71	29.79
	100	865.33 ± 3.71	60.75	680.66 ± 2.90	51.55
	1,000	762.33 ± 6.36	66.27	553.33 ± 6.01	60.61
	10.000	662.33 ± 14.83	71.63	548.33 ± 16.93	60.97
	20,000	635.33 ± 4.33	73.08	382.33 ± 5.93	72.78

Per cent inhibition = (Number of histidine revertants induced by positive mutagen (sodium azide) — Number of histidine revertants induced by mutagen in the presence of ascorbic acid) / (Number of histidine revertants induced by positive mutagen — Number of revertants in negative control)

et al. (1981), who reported that ascorbic acid reduced his^+ revertants significantly, induced by N-nitrodiethylamine, N-nitromorpholine and nitroso analogue.Wirth et al. (1980) reported the antimutagenicity of ascorbic acid against phenacetic acid, acetamine and their respective N-hydroxylated metabolites in *S. typhimurium*. The mutagenesis induced by MNNG and dimethyl nitrosoguanidine (DMNG) in *S. typhimurium* TA 1530 was inhibited by ascorbate (Guttenplan 1978). It has been postulated that ascorbate might protect against electrophilic attack on cellular DNA by intercepting electrophiles. However, the mechanism of sodium azide mutagenesis is not well known but it has been postulated that sodium azide is metabolized into L-azidoalanine by condensing with o-acetyl-serine catalyzed by o-acetylserine (thiol) lyase (Rosichen et al. 1980, Owais et al. 1983). Though from the present investigation it cannot be concluded, yet it can be postulated that ascorbic acid may be acting as antimutagen by blocking partially the metabolism of sodium azide into L-azido alanine.

The treatment of sodium azide reavealed that it induced an appreciable frequency of chromosomal aberrations. The chromosomal aberrations were categorized as physiological (the abnormalities which cannot be ascribed to chromatid/chromosome breakage or consequence of the same but most probably arising due to changes in cell milieu or normal functioning of spindle) and clastogenic (ascribed to breakage of chromatid/chromosome or consequences of these). Besides this, micronuclei were also scored. Ascorbic acid did not alter the mitotic index in significant manner,

TABLE 2 : Effect of pre-treatment, post-treatment and simultaneous treatment with different concentrations of ascorbic acid on the number of aberrant cells induced by sodium azide (a known mutagen) in root tip cells of *Allium cepa*.

Types of chromosomal aberrations	Negative control		Sodium azide (0.75%)		Ascorbic acid																											
					Pre-treatment									Post-treatment									Simultaneous treatment								
					0.25%			0.5%			0.75%			0.25%			0.5%			0.75%			0.25%			0.5%			0.75%		
	I	II	I	II	I	II	% inh	I	II	% inh	I	II	% inh	I	II	% inh	I	II	% inh	I	II	% inh	I	II	% inh	I	II	% inh	I	II	%inh
(A)																															
Stickiness	390	2	277	32	219	10	-	230	17	-	269	5	-	247	13	-	286	13	-	269	14	-	285	21	-	238	12	-	227	12	-
Increased chromosome No.	-	-	277	4	219	0	-	230	0	-	269	0	-	247	0	-	286	0	-	269	0	-	285	0	-	238	0	-	227	0	-
Total No. of dividing cells (I) and (A) physiological aberrant cells (II)	390	2	277	36	219	10	76.47	230	17	55.88	269	5	91.00	247	13	67.64	286	13	64.64	269	14	64.70	285	21	44.12	238	12	70.58	227	12	70.58
(B)																															
Breakage	390	0	277	17	219	3	-	230	1	-	269	8	-	247	6	-	286	1	-	269	1	-	285	2	-	238	2	-	227	2	-
Ring chromosome	390	0	277	2	219	0	-	230	0	-	269	0	-	247	0	-	286	0	-	269	0	-	285	0	-	238	0	-	227	0	-
Anaphase bridges	390	0	277	17	219	7	-	230	8	-	269	6	-	247	7	-	286	13	-	269	12	-	285	8	-	238	10	-	227	6	-
Total No. of dividing cells (I) and (B) clastogenic aberrant cells (II)	390	0	277	36	219	10	72.22	230	9	75.00	269	14	61.00	247	13	63.88	286	14	61.11±	269	13	63.88	285	10	72.22	238	12	66.66	227	8	77.77
Total No. of dividing cells (I) and (A+B) physiological as well as clastogenic aberrant cells (II)	390	2	277	72	219	20	74.28	230	26	65.71	269	19	75.71	247	26	65.71	286	27	64.28	269	27	64.28	285	31	58.57	238	24	68.57	227	20	74.28

A = Physiological chromosomal aberrations; B = Clastogenic chromosomal aberrations;
I = Total number of dividing cells; II = Number of aberrant cells of particular chromosomal aberrations.

$$\% \text{ inh} = \frac{\text{Number of aberrant cells observed with sodium azide - Number of aberrant cells remained after ascorbic acid treatment}}{\text{Number of aberrant cells observed with sodium azide - Number of aberrant cells in negative control}} \times 100$$

but it is evident from Table 2 that ascorbic acid reduced sodium azide-induced chromosomal aberrations. It was observed that the pre-treatment was more effective in reducing physiological type of aberrations, but all the 3 modes of treatments were equally effective in reducing clastogenic aberrations induced by sodium azide. It was observed that the pre-treatment was more effective in reducing micronuclei too (Table 3). As micronuclei are attributable to both physiological as well as

TABLE 3 : Effect of different concentrations of ascorbic acid (antioxidant) on sodium azide (a known mutagen)- induced micronuclei in root tip cells of *Allium cepa*

Different treatments	Negative control	Sodium azide	Ascorbic acid								
			Pre-treatment			Post-treatment			Simultaneous treatment		
		(0.75%)	0.25%	0.5%	0.75%	0.25%	0.5%	0.75%	0.25%	0.5%	0.75%
Total number of cells	4000	3671	7000	8000	9000	4000	3328	3172	3859	2904	1460
Total number of micronucleated cells	0	5	1	1	1	2	1	1	2	1	1
Micronucleated cells (%)	0	0.13	0.01	0.01	0.01	0.05	0.03	0.03	0.05	0.03	0.03
Inhibition (%)			80	80	80	60	80	80	60	80	80

clastogenic types of aberrations and the present observation that pre-treatment significantly reduced physiological type of aberration, therefore, the reduction in micronuclei with the pre-treatment is expected. A persual of literature revealed that there is no report of ascorbic acid on the reduction of radiomimetic/chemical induced chromosomal aberrations in plant system. Sram et al. (1983a) reported that prophylactic administration of ascorbic acid led to the reduction in the chromosomal aberrations in peripheral lymphocytes of workers exposed to coal tar. A similar reduction in chromosomal aberrations was noticed by them in the workers exposed to halogenated ethers (Sram et al. 1983b). It has also been reported that ascorbic acid inhibits or reduces leukaemogenic action of p-hydroxyphenyl lactic acid when used simultaneously (Rausenbakh et al. 1982). Ghaskadbi & Vaidya (1989) reported that ascorbic acid reduced di-iodohydroxyquinoline induced micronuclei in Swiss albino mice.

Three hypotheses are usually offered to explain the antimutagenic/anticlastogenic effect of antioxidants viz., (1) that antioxidant directly interacts with mutagen and changing it into nonmutagenic form; (2) that antioxidant inhibits the metabolic conversion of a mutagen to its ultimate mutagenic form which usually covalently reacts with DNA and (3) that antioxidant blocks the enzymatic activity which inhibits the metabolic conversion of a promutagen into ultimate mutagen. As is believed, sodium azide is converted into ultimate mutagen for its activity suggested that ascorbic acid may be blocking partially the conversion to ultimate mutagen.

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MUTAGENIC EFFECTIVENESS AND EFFICIENCY OF CERTAIN MUTAGENS IN *BRASSICA CAMPESTRIS*

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SUMMARY

Mutagenic effectiveness and efficiency of gamma rays, diethyl sulphate (dES) and maleic hydrazide (MH) and a combination of gamma rays (GR) and dES was studied in 2 cultivars of *Brassica campestris* var. sarson Prain. It was observed that GR and MH exhibited maximum effectiveness in TM-17 and dES and MH exhibited maximum effectiveness in TM-21. However, in terms of mutagenic efficiency, GR proved to be the most efficient in both the varieties.

Key Words: Mutagenic effectiveness, efficiency, yellow sarson.

INTRODUCTION

The sensitivity of an organism depends upon the mutagen employed and genetic make-up according to Blixt (1968). The usefulness of any mutagen depends upon not only on its effectiveness but also to a large extent upon its efficiency. Effective mutagenesis is brought about by the production of usefull mutations with minium undesirable changes.

The present report deals with the study of effectiveness and efficiency of gamma rays (GR), diethyl sulphate (dES) and maleic hydrazide (MH) as well as a combination treatment of GR with dES on 2 cultivars of yellow sarson (*Brassica camestris* var. sarson Prain). Though the general effectiveness and efficiency of the mutagens is well established, their specific effect on yellow sarson has, however, not been reported so far.

MATERIALS AND METHODS

Seeds of 2 cultivars of yellow sarson procured from B.A.R.C., Bombay, viz., TM-17 and TM-21 were subjected to physical and chemical mutagens. About 250 seeds (with about 10% moisture content) per dose were subjected to physical mutagen treatment of GR at 20 kR,, 40 kR and 60 kR and pre-soaked for 4 h in distilled water before sowing. For chemical treatments, 250 seeds per concentration were pre-soaked in distilled water for 4 h and treated with 1.5%, 1% and 1.5% concentrations of dES and MH for 1 h. Another set of seeds were subjected to a combination of GR and dES viz., GR (60 kR) + dES (1%). The seeds were first subjected to GR and soaked in water prior to chemical treatment were washed in running tap water for 30 min at the end of the respective treatment. The treated and control seeds were sown in random block design with 3 replications per treatment. Lethality was calculated from seeds kept in petriplates lined with moist filter paper at room temperature (25 ± 2°C). Seedliing height and injury was noted from 10 days old seedlings grown in pots. Root tips were fixed in fixative when they grew to 1 or 2 cm long. Mitotic and meiotic abberrations were scored from root tip meristem cells and immature pollen mother cells of young flower buds (from field grown plants) fixed in Carnoy's I and II fixatives respectively. The material was processed for cytological study by aceto-orcein and acetocarmine techniques for mitosis and meiosis respectively. For pollen sterility, pollen grains from randomly selected plants from each treatment were stained with 1% acetocarmine. Those that remained unstained or had a shrivelled appearance were taken to be sterile. Chlorophyll mutations were scored as percentage of segregating M_2 plant progenies (Gaul 1964). The types of chlorophyll mutants were Chlorina, Xantha, Viridis and Albino (Zareen 1991). Mutagenic effectiveness and efficiency were determined using the formula suggested by Konzak et al. (1965).

RESULTS AND DISCUSSION

The results (Tables. 1-3) suggest that the highest value of effectiveness was obtained in case of 0.5% dES treatment in cultivar TM-21 and the next value was obtained in case of 0.5% MH in cultivar TM-17. Mutation rate (chlorophyll mutations) based on effectiveness shows dES to be the most effective of all the mutagens employed. This was more pronounced in cultivar TM-21. This was followed by MH which was more pronounced in cultivar TM-17 than in cultivar TM-21. Gamma rays were less effective than dES and MH in both the cultivars. Combination treatments were least effective of all showing a marginal difference in effectiveness between the cultivars.

Mutagenic efficiency varied with the different M_1 parameters taken for calculation (e.g. lethality and seedling injury). The highest value of mutagenic efficiency was obtained in 20 kR GR with respect to lethality and in 60 kR GR with respect to seedling injury in cultivar TM-17. Whereas in cultivar TM-21 the highest values were obtained in 20 kR GR with respect to lethality and 40 kR GR with respect to seedling injury. Greatest efficiency based on pollen sterility was obtained in 20 kR GR in both the cultivars.

Several mitotic and meiotic aberrations like stickiness of chromosomes, diagonally oriented metaphases, anaphasic bridges, laggards, micronuclei and asynchronous divisions were observed.

TABLE 1 : Percentage of chlorophyll mutations, mutagenic effectiveness and mutation rate in *Brassica campestris* var sarson.

Treatment Dose/Conc	Cultivar TM-17 % of chlorophyll mutations (MF)	Cultivar TM-17 Effectiveness (MF/Dose of TXC)	Cultivar TM-17 Mutation rate	Cultivar TM-21 % of chlorophyll mutations (MF)	Cultivar TM-21 Effectiveness (MF/Dose of TXC)	Cultivar TM-21 Mutation rate
Control	-	-	-	-	-	-
GR (20 KR)	2.31	0.11		2.20	0.11	
(40 KR)	3.84	0.09	0.10	3.02	0.07	0.08
(60 KR)	5.82	0.09		4.12	0.06	
dES (0.5%)	0.92	0.61		2.93	1.93	
(1.0%)	1.83	0.61	0.68	3.07	1.02	1.34
(1.5%)	3.70	0.82		4.91	1.09	
MH (0.5%)	2.84	1.89		2.28	1.52	
(1.0%)	3.36	1.12	1.23	2.75	0.91	1.19
(1.5%)	3.07	0.68		5.22	1.16	
Combinations						
GR (20 KR) + dES (1%)	1.04	0.01		2.73	0.03	
GR (40 KR) + dES (1%)	3.36	0.02	0.01	3.40	0.02	0.02
GR (60 KR) + dES (1%)	4.05	0.01		4.44	0.01	

TABLE 2 : Relative efficiency of mutagens based on biological parameters in *Brassica campestris* var sarson cultivar TM-17.

Treatment (Dose/Conc)	% of chlorophyll mutation (M2)	% Lethality (L)	Efficiency (M1/L)	Seedling injury (I)	Efficiency (M1/I)	Pollen sterility (S)	Efficiency (M1/S)	% Mitotic abberrations (Mi)	Efficiency (M1/Mf)	% Meiotic abberrations	Efficiency (Me/Mf)
Control	-	-	-	-	-	-	-	-	-	-	-
GR (20 KR)	2.31	13.60	0.16	25.25	0.09	11.25	0.20	1.83	0.79	1.35	0.58
(40 KR)	3.84	37.00	0.10	42.25	0.09	25.25	0.15	3.85	1.00	2.00	0.52
(60 KR)	5.82	43.10	0.13	47.50	0.12	35.30	0.16	5.85	1.00	4.60	0.87
dES (0.5%)	0.92	33.00	0.02	29.25	0.03	20.00	0.04	0.54	0.58	0.09	0.09
(1.0%)	1.83	39.00	0.04	33.00	0.05	25.40	0.07	0.98	0.53	0.23	0.12
(1.5%)	3.70	49.00	0.07	40.50	0.09	41.00	0.09	2.56	0.69	0.85	0.22
MH (0.5%)	2.84	49.00	0.05	37.25	0.07	17.50	0.16	0.71	0.25	0.04	0.01
(1.0%)	3.36	60.00	0.05	46.75	0.07	22.00	0.15	1.06	0.31	0.25	0.07
(1.5%)	3.07	68.50	0.04	53.00	0.05	36.50	0.08	3.07	1.00	0.33	0.10
Combinations											
GR (20 KR) + dES 1%	1.04	53.50	0.01	60.60	0.01	40.00	0.02	2.11	2.02	0.17	0.16
GR (40 KR) + dES 1%	3.36	77.50	0.04	77.00	0.04	53.10	0.06	6.07	1.80	3.30	0.98
GR (60 KR) + dES 1%	4.05	83.50	0.04	85.00	0.04	63.00	0.07	8.05	1.98	3.53	0.87

TABLE 3 : Relative efficiency of mutagens based on biological parameters in *Brassica campestris* var sarson cultivar TM-21.

Treatment (Dose/Conc)	% of chlorophyll mutation (M2)	% Lethality (L)	Efficiency (M1/L)	Seedling injury (I)	Efficiency (M1/I)	Pollen sterility (S)	Efficiency (M1/S)	% Mitotic abberrations (Mi)	Efficiency (M1/Mf)	% Meiotic abberrations	Efficiency (Me/Mf)
Control	-	-	-	-	-	-	-	-	-	-	-
GR (20 KR)	2.20	4.20	0.52	25.25	0.12	11.25	0.21	1.12	0.50	1.00	0.45
(40 KR)	3.02	13.00	0.22	42.25	0.13	25.25	0.12	4.68	1.54	2.90	0.96
(60 KR)	4.12	39.50	0.10	47.50	0.12	35.30	0.13	6.28	1.52	3.90	0.94
dES (0.5%)	2.93	26.60	0.11	29.25	0.10	20.00	0.16	0.12	0.04	0.04	0.01
(1.0%)	3.07	32.50	0.09	33.00	0.05	25.40	0.13	0.40	0.13	0.24	0.07
(1.5%)	4.91	42.50	0.11	40.50	0.07	41.00	0.16	5.24	1.06	0.38	0.07
MH (0.5%)	2.28	49.00	0.04	37.25	0.06	17.50	0.18	0.05	0.02	0.02	0.00
(1.0%)	2.75	55.50	0.05	46.75	0.05	22.00	0.14	1.41	0.51	0.25	0.09
(1.5%)	5.22	76.50	0.06	53.00	0.10	36.50	0.15	4.70	0.90	1.00	0.19
Combinations											
GR (20 KR) + dES 1%	2.73	38.50	0.07	60.60	0.03	40.00	0.07	1.44	0.52	0.90	0.32
GR (40 KR) + dES 1%	3.40	48.40	0.07	77.00	0.04	53.10	0.07	5.03	1.47	1.25	0.36
GR (60 KR) + dES 1%	4.44	72.50	0.06	85.00	0.05	63.00	0.08	8.71	1.96	2.00	0.45

The highest efficiency values based on mitotic aberrations was observed with combination treatments of GR (20 kR) + dES (1%) in cultivar TM-17 and GR (60 kR) + dES (1%) in cultivar TM-21. For mitotic abnormalities, highest value of efficiency was observed with combination treatment of GR (40 kR) + dES (1%) in cultivar TM-17 and 40 kR GR in cultivar TM-21.

The chlorophyll mutation frequency is an indicator to predict the frequency of factor mutations and is thus an index for evaluating genetic effects of mutagens (Gustafsson 1951, D'Amato et al. 1962, Monti 1968, Genebach et al. 1970, Murr & Stebbins 1971, Walles 1973). There was a dose dependent increase in the chlorophyll mutation frequency in the present study (Tables 2, 3). This was supported by Blixt (1964), Blixt et al. (1966), Swaminathan et al. (1962), Swaminathan (1965) and Goud (1967) who indicated that genes effecting chlorophyll mutations accurred near the centric region of the chromosome where recombination occurs very rarely. Vast difference can be noted amongst the values of mutagenic effectiveness and efficiency in the present data both in terms of chlorophyll mutants spectra and mutation frequencies. This may be atributed to difference in genetic make up of the different varieties.

A significant variation in the mutation rate was observed which is based on efficiency and reflects the specific action of the mutagens. The combination treatments produced the maximum mutants rate with respect to all the parameters except the meiotic aberrations where gamma rays produced the maximum effect in TM-17 (Table 4).

TABLE 4 : Mutation rate based on efficiency in *Brassica campestris* var sarson cultivar TM-17.

Treatment	Lethality	Seedling injury	Pollen sterility	Mitotic aberrations	Meiotic aberrations
GR	31.23	38.33	23.93	3.84	2.65
dES	40.33	34.25	28.80	1.36	0.39
MH	59.16	45.66	25.33	1.61	0.20
Combinations	71.30	74.20	52.03	5.41	2.33

TABLE 5 : Mutation rate based on efficiency in *Brassica campestris* var sarson cultivar TM-21.

Treatment	Lethality	Seedling injury	Pollen sterility	Mitotic aberrations	Meiotic aberrations
GR	19.06	23.96	21.70	4.02	2.60
dES	33.86	48.01	23.60	1.92	0.22
MH	60.33	45.06	21.33	2.05	0.42
Combinations	53.13	78.00	46.00	5.06	1.38

In TM-21 (Table5) the maximum mutation rate resulted due to combination treatment with respect to seedling injury, pollen sterility and mitotic aberrations, MH recorded the highest mutation rate with respect to lethality and gamma rays recorded the highest mutation rate with respect to the meiotic aberrations.

According to Blixt (1968), the sensitivity of an organism depends on the mutagen employed and the genetic make up. Sharma & Chatterjee (1960) and Varughese & Swaminathan (1968) are of

the opinion that the difference is due to the amount of DNA and its replication time in the initial stages. It might be due to the physiological stage of the cell, ability to repair the damage or several other physical factors (Brock 1965, Chopra & Swaminathan 1966, Auerbach 1967, Gelin 1968, Ilivea Staneva 1971). The usefulness of any mutagen depends not only in its effectiveness but also to a large extent upon its efficiency. Effective mutagenesis is brought about by the production of useful mutations with minium undesirable changes.

In the present study, gamma rays and maleic hydrazide possessed maximum effectiveness in var. TM-17 and both the chemical mutagens possessed maximum effectiveness in var. TM-21. In both the varieties, however, the gamma rays demonstrated maximum mutagenic efficiency.

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EFFECTS OF MALATHION ON FERTILITY IN SOME OIL CROPS

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SUMMARY

Present work deals with the assessment of the spraying of malathion, one of the most extensively used commercial non-systemic class of organophosphate insecticides, on the fertility of some oil crops, *Brassica juncea, Helianthus annuus*, and *Sesamum indicum*. The parameters assayed included total frequency of meiotic anomalies, disintegration of microspore tetrads, number of pollen grains per anther, pollen sterility, length of inflorescence in *B. juncea*, number of siliqua per plant in *B. juncea* and capsule in *S. indicum*, number of seeds set per M_1 plant and weight of 100 seeds harvested from M_1 and their germination frequency. Spraying evoked several types of anomalies during first and second meiotic divisions, primarily related to aberrant spindle organization and/or function and chromatin agglutination. Degeneration of microspore tetrads was noticed in most of the sprayed sets of *H. annuus* and few sets of *S. indicum*. A general decrease in mean number of pollen grains per anther of sprayed plants was reported in all the presently studied oil crops. Although, pollen sterility was increased in all sprayed sets, it became exorbitant in *B. juncea*. The mean number of capsules per plant declined significantly in all sprayed *S. indicum* sets. Number of seeds set per M_1 plant and their germination declined significantly.

Key Words : Assessment, consequence, malathion, spraying fertility, oil crops.

INTRODUCTION

Malathion (O'O dimethyl phosphorodithioate of diethyl mercaptosuccinate) is one of the extensively used commercial non-systemic class of organophosphate insecticides. A perusal of literature reveals that the cytomorphological influence of a large number of pesticides on crop plants in general, and on oil crops in particular, have not been studied. This is more true for their effects on the attributes of fertility. Further, since, organophosphate insecticides are one of the most widely used pesticides for combating insects and since, oil crops compose one of the highly susceptible crops to diseases, the repercussion of malathion treatments of oil crops have been explored in detail. This communication deals with the assessment of spraying of this insecticide on the magnitude of fertility in some oil crops.

MATERIALS AND METHODS

Malathion (MLT) emulsions, prepared in distilled water, having concentrations, 0.075%, 1.0% and 1.5% were used for spraying. Plants (M_1 plants) of each oil crop, *Brassica juncea* cv. Kranti, *Helianthus annuus* cv. HL 7, and *Sesamum indicum* cv. TC 289, raised in polythene bags having a mixture of soil and manure, were divided into five groups (A-E). Group A was left as control while the groups B-E were subdivided into three groups each (I-III) which were sprayed

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Response coefficient (R.C.) was calculated for appraising the effects of MLT emulsions on various parameters, in relation to control. The positive values stipulate incitement and negative values repression.

$$R.C. = \frac{\text{value of treated set - value of control set}}{\text{value of control set}}$$

TABLE 1 : Effect of MLT on the frequencies of meiotic anomalies, pollen per anther, tetrad disintegration and pollen sterility.

Treatment	*B. juncea*			*H. annuus*				*S. indicum*			
	TMA	PPA	PS	TMA	TD	PPA	PS	TMA	TD	PPA	PS
Control	1.8	2632	0.0	2.6	0.0	4054	2.0	2.4	0.0	1893	0.8
0.075%											
I spray	21.1	1915	31.2	7.9	5.4	3978	41.5	6.6	0.0	1499	51.9
	+10.5	-0	+346.6	+1.9		-0	+19.2	+1.7		-0	+58.6
II spray	17.3	2398	7.9	7.5	3.1	3968	35.4	7.0	0.0	1637	52.2
	+8.4	. -0	+87.6	+1.8		-0	+16.2	+1.8		-0	+59.0
III spray	19.8	1837	5.0	9.4	18.5	3688	37.5	7.5	0.0	1582	54.2
	+9.8	-0	+54.7	+2.5		-0	+17.3	+2.0		-0	+61.3
0.10%											
I spray	22.1	1421	3.2	10.0	21.2	3810	43.6	7.0	0.0	1280	54.9
	+11.0	-0	+34.7	+2.7		-0	+20.2	+1.8		-0	+62.1
II spray	19.3	2023	13.4	9.9	1.8	3304	39.0	9.3	0.7	1396	53.8
	9.5	-0	+148.4	+2.7		-0	+18.0	+2.8		-0	+60.8
III spray	21.0	1433	27.6	11.5	3.1	3692	40.6	9.0	0.0	1158	57.1
	+10.4	-0	+306.5	+3.3		-0	+18.8	+2.6		-0	+64.7
1.0%											
I spray	19.8	1508	56.2	11.3	0.0	2948	50.2	13.7	0.0	1153	67.1
	+9.7	-0	+623.5	+3.2		-0	+23.5	+4.6		-0	+76.2
II spray	24.0	1565	48.2	10.8	2.0	3434	46.2	12.7	0.0	1097	65.4
	+12.0	-0	+535.0	+3.1		-0	+21.5	+4.2		-0	74.2
III spray	21.9	1433	46.6	15.7	25.7	3271	52.6	11.8	6.4	1235	68.3
	+10.9	-0	+517.0	+4.9		-0	+24.7	+3.8		-0	+77.5
1.5%											
I spray	29.0	2240	47.9	21.0	0.0	2995	54.1	16.6	0.0	1143	69.1
	+14.8	-0	+531.4	+6.9		-0	+25.4	+5.8		-0	+78.5
II spray	21.3	2340	44.5	23.5	31.5	2827	54.0	13.3	0.0	991	62.1
	+10.5	-0	+493.5	+7.8		-0	+25.3	+4.4		-0	+70.4
III spray	27.8	1901	57.6	16.8	4.4	3111	54.0	16.5	0.0	864	68.9
	+14.1	-0	+640.0	+5.3		-0	+25.3	+5.7		-0	+78.2

TMA = Total meiotic anomaly (mean per cent). TD = Tetrad disintegration (mean per cent). PPa = Mean number of pollen grains per anther. Ps = Pollen sterility (mean per cent).

TABLE 2 : Effect of MLT on length of inflorescence, silqua per plant, seeds set per plant, weight of 100 seeds and seed germination in *B. juncea*.

Treatment	*Length (cms.) of inflo.	R.C.	*Siliqua/plant	R.C.	*Seeds/plant	R.C.	Seed wt. (mg)	R.C.	% Germination	R.C.
Control	12.6		10.2		78.9		411		76	
	7.9-18.6		6-13		46-121					
0.075%										
I spray	15.0	+0.19	12.0	+0.18	68.5	-0.1	422	+0.0	64	-0.1
	9.5-19.7		9-16		38-100					
II spray	16.5	+0.31	9.7	-0.05	74.5	-0.0	440	+0.0	68	-0.1
	8.5-25.5		6-24		47-128					
III spray	14.4	+0.15	12.0	+0.18	67.9	-0.1	382	-0.0	61	-0.2
	7.0-21.3		7-17		37-102					
0.10%										
I spray	14.2	+0.13	11.2	+0.10	63.5	-0.2	385	-0.0	66	-0.1
	5.0-27.0		16-19		26-147					
II spray	13.37	+0.06	11.4	+0.12	61.7	-0.2	382	-0.0	64	-0.1
	7.5-18.0		6-15		33-85					
III spray	19.1	+0.52	9.8	-0.04	70.2	-0.1	362	-0.1	63	-0.1
	10.5-33.3		7-14		39-109					
1.0%										
I spray	12.9	+0.02	12.5	+0.23	68.7	-0.1	357	-0.1	62	-0.1
	8.2-18.8		8-16		39-114*					
II spray	8.9	-0.29	10.3	+0.01	38.3	-0.5	372	-0.1	57	-0.2
	7.4-15.5		8-15		26-48					
III spray	12.6	0.002	11.1	+0.09	67.4	-0.1	399	-0.0	59	-0.2
	7.0-20.5		7-14		37-118					
1.5%										
I spray	5.1	-0.60	5.0	-51	39.2	-0.5	200	-0.5	56	-0.2
	3.0-10.0		4-7		13-4					
II spray	5.7	-0.54	6.1	-0.40	34.8	-0.5	211	-0.4	63	-0.1
	4.3-8.5		4-8		21-4					
III spray	9.1	-0.27	6.5	-0.36	61.3	-0.2	382	-0.0	55	-0.2
	5.0-12.5		4-8		30-105					

* Upper values refer to mean; lower values refer to range.

OBSERVATIONS

Data related to the presently evaluated parameters are presented in Tables 1-4. Even a single spray with a concentration as low as 0.075% was sufficient to induce alterations in all the currently analysed parameters. In some sprayed sets a certain frequency of anthers were sterile.

The control sets of all the three oil crops had only slightly irregular meiotic course. However, spraying evoked several types of anomalies during first as well as second meiotic divisions, primarily related to aberrant spindle organisation and/or function and chromatin agglutination. The common types of anomalies related to above were retarded movement of chromosome for metaphase congression, chromosomes congression in more than one group at metaphase, lagging of

TABLE 3 Effect of MLT on seeds set per plant and weight of 100 seeds in *H. annuus*.

Treatment	*Seeds/plant	R.C	Seed wt. (mg)	R.C
Control	123.3 111-133		2607	
0.075% I spray	112.4 89-139	-0.09	1244	-0.52
II spray	96.8 35-116	-0.22	1155	-0.56
III spray	94.5 61-125	-0.23	2308	-0.12
0.10% I spray	119.2 35-265	-0.03	1420	-0.46
II spray	92.3 19-185	-0.25	1619	-0.38
III spray	97.0 90-107	-0.21	1031	-0.61
1.0% I spray	110.6 80-135	-0.10	813	-0.69
II spray	78.7 60-108	-0.36	1242	-0.52
III spray	72.0 28-133	-0.42	1470	-0.44
1.5% I spray	50.0 45-57	-0.60	1085	-0.58
II spray	21.7 0-59	-0.82	633	-0.76
III spray	44.7 30-71	-0.64	645	-0.75

* Upper values refer to mean; lower values refer to range.

chromosomes, stickiness of chromosomes, formation of chromatin bridges and micronuclei, etc. Certain other types of meiotic anomalies reported either often or rarely were formation of restitution nuclei, transcellullar migration of chromatin, presence of monads, diads, triads and polyads, etc. Highest proportion of total meiotic irregularity was recorded in *B. juncea* followed by *H. annuus* and *S. indicum* (Fig. 1A). Disintegration of microspore tetrads was noticed in most of the sprayed sets of *H. annuus* and few sets of *S. indicum*.

A general decrease in mean number of pollen per anther of sprayed plants was reported in all oil crops with *H. annuus* showing minimal reduction (Fig. 1B). Although, pollen sterility was higher in all sprayed sets, it became exorbitant in *B. juncea* (Fig. 1C).

Mean length of the inflorescence and number of siliqua per plant of sprayed sets of *B. juncea* were mostly stimulated excluding sets sprayed with 1.5% MLT (Table 2). On the other hand, number of capsules per plant declined significantly in all sprayed sets of *S. indicum* (Table 4).

TABLE 4 Effect of MLT on capsule per plant, seeds set per plant, seed weight of 100 seeds and seed germination in *S. indicum*.

Treatment	* Capsule/ plant	R.C.	*Seeds/ plant	R.C.	Seed wt. (mg)	R.C.	Germination (%)	R.C.
Control	9.5 8-12		61.6 38-84		288		78	
0.075% I spray	2.6 2-3	-0.72	23.6 0-61	-0.62	130	-0.55	56	-0.28
II spray	4.6 2-11	-0.51	20.3 0-33	-0.67	192	-0.33	53	-0.32
III spray	4.0 3-5	-0.58	19.5 0-39	-0.68	200	-0.31	55	-0.30
0.10% I spray	3.5 3-4	-0.63	12.5 0-42	-0.80	196	-0.32	55	-0.30
II spray	4.1 3-7	-0.56	23.0 0-26	-0.63	169	-0.41	62	-0.21
III spray	3.5 2-6	-0.63	19.6 0-42	-0.68	161	-0.44	50	-0.36
1.0% I spray	4.2 2-5	-0.56	21.6 5-73	-0.65	136	-0.53	49	-0.37
II spray	3.4 3-50	-0.64	21.6 0-31	-0.65	169	-0.41	33	-0.58
III spray	3.2 2-5	-0.66	16.4 0-39	-0.73	161	-0.44	52	-0.33
1.5% I spray	2.4 2-4	-0.74	3.8 0-21	-0.94	164	-0.43	47	-0.40
II spray	3.1 2-5	-0.67	11.4 0-58	-0.81	186	-0.35	47	-0.40
III spray	3.6 2-6	-0.62	8.7 0-33	-0.86	187	-0.35	40	-0.49

* Upper values refer to mean; lower values refer to range.

Number of seeds set per plant and weight of seeds harvested from sprayed plants decreased in all sets with reduction being relatively higher in *H. annuus* and *S. indicum* as compared to *B. juncea*. (Fig. 1D, E). Seeds of *H. annuus*, harvested from control as well as sprayed plants did not germinate due to unknown reasons, whereas those of *B. juncea* and *S. indicum* proclaimed limited germination with cutback being higher in *S. indicum* (Fig. 1F).

DISCUSSION

To the best of our knowledge, the present report is first of its kind. Further, the available data clearly confer genotoxicity to this insecticide, since junst a single spray with a concentration as low as 0.075% could induce sufficient setback to sexuality and affiliated traits. However, the response of the crops of MLT was genotype dependent.

Genotoxic effects of certain insecticides on male meiosis have also been evaluated earlier in some plants (Amer & Farah 1968 1976, Amer & michael 1983, Derenne 1953, Grover et al. 1988,

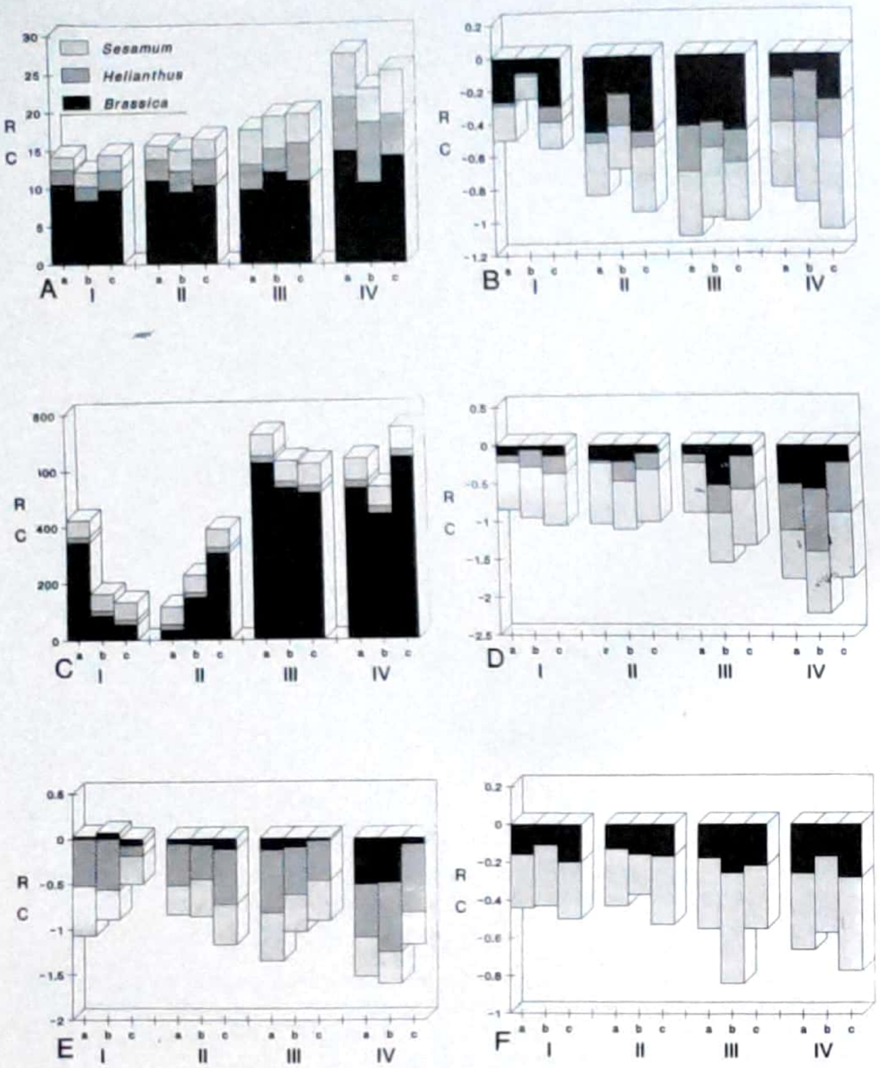

Fig. 1 : Effects of malathion spraying on meiotic course (A), pollen per anther (B), pollen sterility (C), number of seeds set per plant (D), weight of air dried harvested seeds (E) and germination of harvested seeds (F). I = 1.5%, II = 1.0%, III = 0.10%, IV = 0.075%, a = first spray, b = second spray, d = third spray.

Grover & Malhi 1984, Grover & Mittal 1984, Grover & Tyagi 1980, Jain 1988, Jain & Sarbhoy 1988, Kabir & Sultanul 1986, Kaur & Grover 1985, Kumar & Sinha 1989, Lakshmi et al. 1977). Most of these workers reported the induction of meiotic anomalies as well establishing the present finding.

In case of oil crops since seeds are the main harvest decrease in seed set, seed weight and seed germination shall incur financial loss to growers. MLT which is sprayed for combating the insects for increasing the yield can very well commit that financial loss. Decrease in yield of crops after tratments with some insecticides have also been reported by Jain (1988), Lakshmi et al. (1988), Reddy & Rao (1981), and Sharma & Singh (1990). However, the presently reported decrease in the seed set could be due to partial sterility resulting because of instigated irregular course of meiosis in sprayed plants. In support of this, the presence of significant amount of pollen sterility can be asserted. Decrease in seed weight and seed germination could be due to mutations. Only future detailed genetic analyses can provide proper explanation for these.

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IMPROVEMENT OF HEXAPLOID TRITICALES THROUGH HYBRIDIZATION WITH RYE AND WHEAT

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SUMMARY

Three hexaploid triticales, Mapache, Mizar and Monsanto were cytologically confirmed. More number of rod bivalents and lesser number of univalents in hybrids involving Mapache attributed to the presence of R/D substitutions. Improvement in seed set, grain weight and other agronomical characters were noticed in back cross progenies.

Key Words : Hybridization, triticales.

INTRODUCTION

Crossing hexaploid triticales with hexaploid wheat and/or with diploid rye, followed by selfing of the F_1 hybrids or backcrossing of the hybrids with 6*x* triticale allows the introduction of genes from wheat and rye into triticale, thus facilitates the improvement of the triticales (Reddy 1990). In hexaploid wheat, a number of valuable genes are located in the D-genome. Therefore, it is possible that the quality, disease resistance, winter hardiness, and other agronomic characters of hexaploid triticale could be improved by introducing the D-genome chromosomes (Gupta & Reddy 1991). Crosses between triticale x wheat not only helps to select improved triticales, but the segregating progenies may also useful in selecting better wheats. The present paper reports the improvement of hexaploid triticale through hybridization with wheat and rye.

MATERIALS AND METHODS

The seed materials of the study include 3 hexaploid Mapache, Mizar and Monsanto and one variety each of 6*x* rye (Assam rye). Taking triticales as female parent, crosses were made separately between triticale with wheat and rye.

Meiotic studies were made in the F_1 hybrids. Hybrids that were confirmed meiotically were advanced to F_2, F_3 and part of F_1 hybrid seeds were also utilized in a back crossing programme. Using F_1 as female parent, crosses were made between F_1 with respective triticales to produce BC_1 and BC_2 generations. Selected plants were used to raise BC_1S_1 and BC_2S_1 populations. Plants that showed good agronomical characters were selected in F_2, F_3, BC_1S_1 and BC_2S_1 generations.

Data on 5 quantitative characters viz., plant height (cm), number of tiller/plant; number of spikelets/spike, grain yield (g)/plant, 100-grain weight(g) were recorded on the selected plants. 't' test was applied to compare the mean agronomic performance of selected plants with that of controls.

RESULTS AND DISCUSSION

In triticale x wheat crosses, when the tricicale and the resultant F_1 hybrids were used as female parents, the crosses were highly seccessful. Variable degree of success were reported in reciprocal crosses (Varughese et al. 1986). The meiotic studies of F_1 hybrids clearly revealed their

Figs. 1-4 : Cytological and morphological features of the hybrids derived from triticale x rye and triticale x wheat crosses. 1 &2. M I in F_1 Hybrids with $4x = 28$ in triticale x rye cross. 3. Morphology of spikes, triticale (right), hybrid (triticale x rye) and rye (left). 4. M I in F_1 hybrid with $6x = 42$ in triticale x wheat crosses.

hybrid nature (Figs. 1-4, Table 1). Low frequency of quadrivalents in all the hybrids might have resulted from translocations among rye chromosomes or due to homoeologous pairing of wheat chromosomes. The frequency of rod bivalents and univalents were more in Monsanto x rye hybrids and were followed by Mizar x rye and Mapache x rye. Similarly, more number of bivalents and less number of univalents in Monsanto x Sonalika crosses; less number of bivalents and more number of univalents in Mapache x Sonalika crosses were observed. These results could be explained on the basis of presence of two R/D substitutions in triticale Monsanto; one substitution in Mizar and none in Mapache. Associations among univalents observed in most of the hybrids in the present study could be of secondary in nature (Solar et al. 1980).

TABLE 1 : Cytological characteristic features of F_1 hybrids derived from triticale x wheat and triticale x rye crosses (first line is the mean and second line is range).

	IV	II		I	Xta
		Rod	Ring		
Triticale x Rye					
Mapache x rye	0.01 (0-1)	5.31 (0-12)	1.97 (0-6)	13.40 (6-20)	12.44 (5-18)
Mizar x rye	0.01 (0-1)	5.38 (0-12)	1.74 (0-6)	13.72 (6-22)	12.20 (4-17)
Monsanto x rye	0.01 (0-1)	5.44 (0-12)	1.35 (0-6)	14.38 (6-22)	11.12 (4-15)
Triticale x wheat					
Mapache x Sonalika	-	2.06 (1-4)	11.89 (10-13)	14.10 (10-16)	27.84 (26-28)
Mizar x Sonalika	0.05 (0-1)	2.13 (1-4)	12.52 (10-14)	12.47 (10-16)	29.08 (27-31)
Monsanto x Sonalika	0.05 (0-1)	2.04 (1-4)	13.96 (12-15)	9.77 (9-14)	32.25 (31-33)

The seed set was low in triticale x rye crosses compared tó triticale x wheat crosses, indicating low level of crossability of triticale with rye (Table 2). The low crossability can be explained on the basis of presence of dominant Kr_1 and Kr_2 incompatable genes in triticale (Lange & Riley 1973). Soler et al. (1990) observed that advanced progenies of triticale x wheat crosses had varying number of D- and R- genome chromosomes alongwith complete A- and B-genome chromosomes which perhaps were responsible for homoeologous pairing formation leading to structural changes. Various structural changes including quadrivalent formation were also noticed by Dubovets & Bormotov (1989) in hybrids derived from crosses involving hexaploid triticale x rye and were suggested to be responsible for a low seed set. Earlier it has also been shown that low fertility in amphiploid (AARR) derived from *T. monococcum* x *Secale cereale* were partly attributed to cytological irregularities (Sodkiewicz 1988). Seed set in F_2, F_3 were higher than F_1 and similarly seed set was higher in BC_2S_1 as compared to BC_1S_1 (Table 3). This is perhaps due to stablization of meiosis at advanced generations. Significant increase in number of grains/spike, grain yield/plant and 100-grain weight were observed in the present study. Improvement in these triticales can be attributed to transfer of desirable genes from wheat D- genome to triticale and to recombinations

TABLE 2 Details of crosses between triticale x rye and triticale x wheat in different generations.

Sl. No.	Details of corss/Generation	Tirticale x Rye			Triticale x Wheat		
		Mapache x Rye	Mizar x Rye	Monsanto x Rye	Mapache x Sonalika	Mizar x Sonalika	Monsanto x Sonalika
1	2	3	4	5	6	7	8
1.	No. of spikes pollinated	33	31	29	35	36	32
2.	No. of florets pollinated	2431	2264	2096	2516	2618	2244
3.	No. of F_1 seeds obtained	48	34	27	528	624	639
4.	F_1 seed set (%)	1.97	1.50	1.28	20.98	23.83	28.47
5.	No. of F_1 plants raised	31	24	18	348	416	478
6.	F_1 Generation						
	a. No. of spikes selfed	18	14	15	54	61	48
	b. No. of spikes backcorssed	21	16	14	30	30	30
7.	F_2 Generation						
	a. No. of seeds obtained	54	53	58	876	966	793
	b. Seed set (%)	17.64	22.26	22.74	77.24	83.34	86.95
	c. No. of plants raised	52	51	56	869	954	478
8.	BC_1 Generation						
	a. No. of seeds obtained	105	89	109	261	329	358
	b. No. of plants raised	89	61	82	244	316	334
	c. No. of (selected) plants selfed	22	16	18	16	21	14
	d. No. of spikes backcorssed (in selected plants)	16	21	19	35	41	38
9.	F_3 Generation						
	a. No. of plants selected	33	19	14	19	26	31
10.	BC_1S_1 Generation						
	a. No. of plants obtained	102	114	129	269	318	336
	b. No. of plants selected	46	31	38	34	29	17
11.	BC_2 Generation						
	a. No. of plants obtained	98	118	132	326	351	361
	b. No. of (selected) plants selfed	19	21	31	16	28	18
12.	BC_2S_1 Generation						
	a. No. of plants selected	24	18	38	17	19	23

of rye genomes within triticale. Hybrids produced from triticale x wheat crosses were shown to contain improved agronomical characters and high disease resistance (Yao et al. 1988, Sharma & Sethi 1988). Thus the present results, therefore, suggest the importance of wheat and rye genomes in widening the germplasm base of triticale.

TABLE 3 : Data on some quantitative characters in parents and hybrids in triticale x rye and triticale x wheat crosses.

Parents/ cross	Generation	No. of Plants selected	Quantitiative characters				
			Plant height (cm)	No. of tillers/ plant	No. of spikelets/ spike	Grain yield (g) / plant	100-grain weight (g)
1	2	3	4	5	6	7	8
PARENTS							
Mapache	-	10	98.4±0.61 (93-101)	4.38±0.28 (3-8)	24.41±0.13 (15-26)	8.94±0.11 (4-12)	3.92±0.04 (3.2-4.4)
Mizar	-	10	92.6±0.48 (88-100)	4.41±0.18 (3-8)	22.3±0.08 (15-25)	9.31±0.08 (4-12)	3.99±0.08 (3.4-4.6)
Monsanto	-	10	89.9±0.51 (86-94)	4.43±0.21 (3-8)	22.8±0.16 (17-27)	10.29±0.13 (4-12)	4.11±0.06 (3.6-4.8)
Assam Rye	-	10	137.6±0.32 (129-141)	8.6±0.14 (5-13)	29.6±0.14 (25-33)	12.81±0.24 (6-15)	3.66±0.11 (2.9-3.9)
Sonalika	-	10	76.4±0.28 (73-81)	4.8±0.12 (3-7)	21.2±0.12 (19-25)	10.91±0.21 (6-14)	3.94±0.10 (3.6-4.0)
HYBRIDS							
Mapache x Rye	F_1	33	*68.4±0.19 (63-74)	4.34±0.16 (2-6)	*18.2±0.09 (7-23)	7.11±0.04 (2-9)	3.04±0.08 (2.5-3.2)
	BC_1S_1	46	**90.6±0.24 (83-97)	4.40±0.13 (1-6)	24.6±0.11 (9-27)	8.98±0.08 (2-12)	3.88±0.06 (2.8-4.0)
	BC_2S_1	24	*92.3±0.16 (91-99)	4.46±0.14 (2-6)	24.8±0.06 (9-27)	9.16±0.08 (3-13)	3.94±0.06 (3.5-4.2)
Mizar x Rye	F_1	19	*61.3±0.29 (57-68)	4.01±0.16 (1-6)	*18.8±0.11 (7-23)	8.41±0.08 (1-10)	3.90±0.06 (2.5-3.2)
	BC_1S_1	31	**84.6±0.18 (80-88)	4.46±0.11 (1-6)	22.5±0.07 (9-23)	9.51±0.09 (2-12)	3.98±0.08 (3.5-4.2)
	BC_2S_1	18	**85.2±0.17 (80-89)	4.48±0.18 (2-6)	23.6±0.07 (11-25)	9.69±0.09 (6-12)	4.10±0.04 (3.8-4.2)
Monsanto x Rye	F_1	14	*60.2±0.26 (58-64)	4.31±0.16 (2-6)	**19.9±0.07 (9-23)	*8.63±0.09 (1-3)	3.92±0.05 (2.5-4.2)
	BC_1S_1	38	*74.6±0.31 (69-78)	4.54±0.14 (2-6)	23.2±0.10 (9-25)	10.31±0.11 (2-13)	4.10±0.04 (3.4-4.3)
	BC_2S_1	38	*78.4±0.22 (73-84)	4.68±0.21 (2-6)	24.9±0.09 (9-27)	10.88±0.14 (3-14)	4.26±0.08 (3.8-4.5)
Mapache x Sonalika	F_1	19	**94.3±0.12 (81-97)	**7.98±0.11 (2-9)	26.4±0.04 (17-31)	9.89±0.11 (3-12)	**4.11±0.06 (2.8-4.7)
	BC_1S_1	34	96.3±0.11 (84-99)	*8.11±0.09 (2-11)	*28.6±0.06 (19-33)	*13.43±0.12 (4-15)	**4.14±0.05 (3.0-4.9)
	BC_2S_1	17	97.6±0.10 (82-101)	*8.18±0.12 (3-11)	*29.1±0.05 (21-33)	*14.79±0.08 (4-16)	*4.43±0.08 (3.2-5.0)

(Cond.)

TABLE 3 : (Concluded)

1	2	3	4	5	6	7	8
Mizar x Sonalika	F_1	26	**86.9±0.09 (74-98)	*7.76±0.13 (3-10)	24.7±0.06 (17-29)	10.02±0.11 (3-12)	*4.16±0.09 (3.3-4.9)
	BC_1S_1	29	88.4±0.13 (73-94)	*8.38±0.11 (2-12)	**25.3±0.07 (19-31)	**11.89±0.13 (4-14)	*4.21±0.07 (3.5-5.0)
	BC_2S_1	19	89.6±0.07 (72-98)	*8.41±0.09 (3-12)	*27.4±0.06 (19-33)	*14.26±0.10 (5-16)	*4.56±0.11 (3.6-5.2)
Monsanto x Sonalika	F_1	31	**84.6±0.13 (77-96)	**6.91±0.12 (2-9)	24.6±0.08 (19-27)	10.88±0.09 (3-13)	**4.31±0.08 (3.8-5.0)
	BC_1S_1	17	87.4±0.11 (74-99)	*8.18±0.09 (3013)	**25.3±0.09 (19-29)	**12.98±0.08 (5-15)	**4.37±0.06 (3.8-5.3)
	BC_2S_1	23	88.5±0.09 (71-98)	*8.24±0.11 (3-13)	*26.8±0.05 (19-33)	*15.14±0.11 (6-17)	*4.58±0.08 (3.9-5.4)

*, ** = Significant at 1% and 5% respectively.

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MITOCLASTIC AND CLASTOGENIC PROPERTIES OF ANALGIN

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SUMMARY

Analgin (phenyl dimethyl pyrazolone), a commonly used analgesic drug was found to induce in *Allium cepa* root tip cells a drastic lowering of the mitotic index and arrest of mitosis together with a wide spectrum of mitotic abnormalities and a few structural aberrations of chromosomes. These included endoreduplication, early splitting of chromosomes, chromosome inflammation and dissolution, stickiness, kinetochore inactivation,neocentric activity of telomeres, tropokinesis, unequal distribution of chromosomes at anaphase, double stranded chromosomes at anaphase, unipolar movement at anaphase, chromatid and chromosome breaks, nuclear pycnosis and nuclear polymorphism. These abnormalities were also observed in materials given a 24 h recovery time after drug treatment suggesting that these are stable cytogenetic effects of analgin.

Key Words : Analgin, *Allium* test, mitoclastic effects.

INTRODUCTION

Although several adverse side effects of some therapeutic drugs have been known since a long time (Gorrod 1979, Griffin & D'Arcy 1979, Gilman et al. 1991), cytogenetic hazards posed by the frequent use of these drugs by human beings were realised only recently (Wilson 1950, Wilson & Bowen 1951, Vig 1976, Montesano et al. 1976, Nesnow et al. 1987). Humdreds of new drugs introduced into market every year especially in the developing countries are not put to routine toxicological evaluations insisted by the World Health Organisation. Hence, the screening of therapeutic drugs for mutagenic, clastogenic and carcinogenic properties has been undertaken in this Department and the results from the *Allium* test (Levan1949) on analgin, a widely used analgesic drug are summarised in this paper. Gorrod (1979) had pointed out its harmful side effects which included hypersensitivity reactions, granulocytopenia and agranulocytosis.

MATERIALS AND METHODS

Fresh bulbs of *Allium cepa* (2n = 16) were planted in moist sand and when roots emerged after 2 or 3 d, the rooted bulbs were washed in distilled water and transferred to vials containing 0.1 µg/ml, 1µg/ml and 10 µg/ml solutions of analgin (phenyl dimethyl pyrazolone), manufactured by Hoechst India Ltd., Bombay, prepared in glass distilled water along with controls. The experiments were done in duplicate. Root tips from one batch of materials were fixed immediately after treatment and the rooted onion bulbs from the other batch were washed in water after drug treatment and were kept for another 24 h in distilled water in respective vials and then the root tips were fixed. Fixing was done in a 3 : 1 mixture of ethyl alcohol and acetic acid at room temperature. The following day, the root tips were hydrolysed by a quick dip in a 1 : 1 mixture of conc. HCL and ethyl alcohol,

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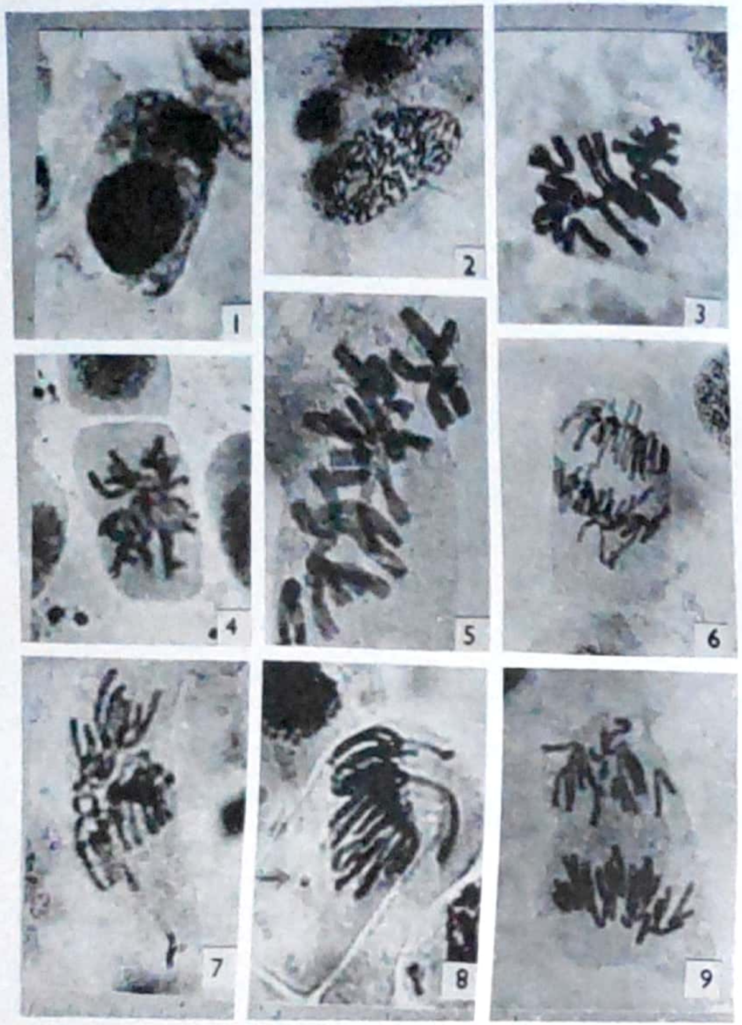
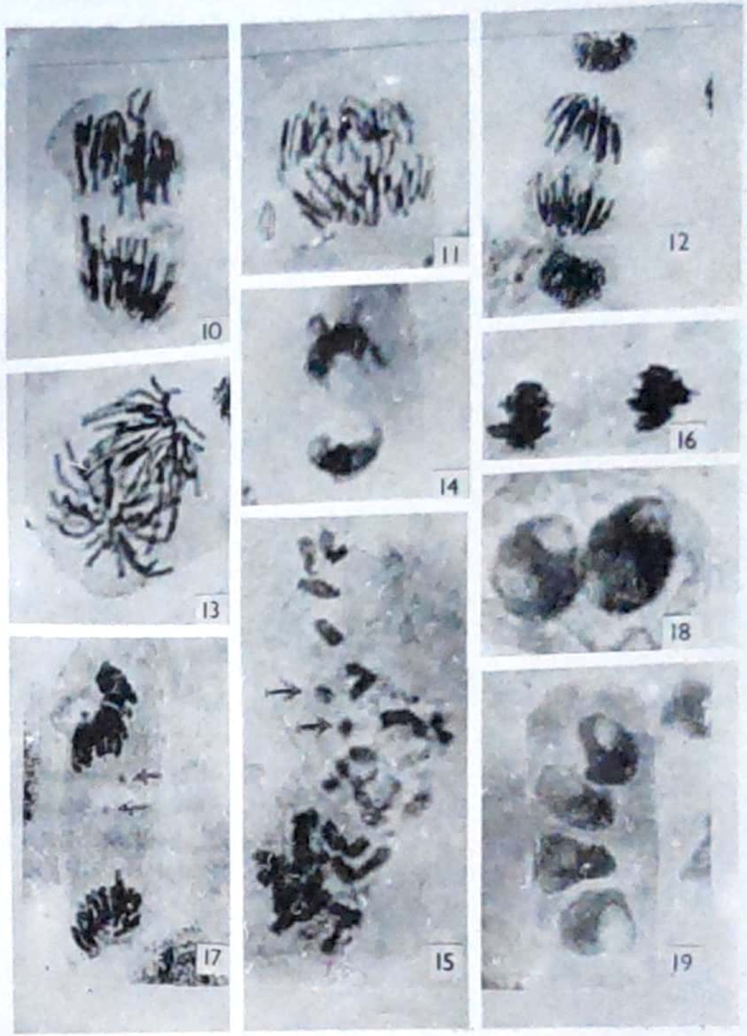


Figs. 1-9: Cytological abnormalities induced by analgin in *Allium cepa* root tip cells. 1. Nuclear lesion in an interphase cell. 2. A polyploid cell at prophase. 3. Late prophase showing precocious longitudinal splitting of chromosomes. 4. Stickness and clumping of chromosomes at prophase. 5. Tropokinesis at metaphase with distinctly split chromosome. 6. Tropokinesis at anaphase. 7. Metaphase showing kinetochore inactivation and neocentric activity of telomeres; some degree of stickiness is also evident. 8. Unipolar movement of chromosome along with tropokinesis; a lone fragment is seen at the equator. 9. Unequal distribution of chromosomes at anaphase (All x 750 except Fig. 5 x 1500)



Figs. 10-19: Cytological abnormalities induced by analgin in *Allium cepa* root tip cells. 10. Anaphase with two chromosomes moving ahead of others at one pole. 11. Aneuploid number of chromosomes at anaphase. 12. Anaphase with double stranded chromosomes. 13. Double star anaphase. 14. Mitotic arrest at anaphase resulting in irregular crescent shaped chromatin masses. 15. Metaphase with supercondensed chromosomes showing chromatid and chromosome breaks and fragments. 16. Mitotic arrest at early telophase resulting in irregular daughter nuclei with projecting chromosomes. 17. Disorientation of one anaphase pole; two fragments are seen near the equator. 18. A binuclear cell with nuclear lesions. 19. A tetranucleate cell with atypical nuclei showing nuclear lesions (All x 750 except Fig.15 x 1500)

TABLE 1: Summary of cytogical effects of analgin on *Allium cepa* root tip cells.

Conc. of analgin	No. of cells examined	No. of dividing cells	Mitotic index	Percentage of cells in Normal interphase	Interphase with abnormalities	Normal prophase	Prophase with abnormalities	Normal metaphase	Metapphase with abnormalities	Normal anaphase	Anaphase with abnormalities	Normal telophase	Telophase with abnormalities
Treatment 1 : Materials fixed immediately after drug treatment for 24 h.													
10 µg/ml	3449	80	2.32	56.62	40.85	0.03	0.15	0.12	0.12	0.03	0.09	0.09	2.11
1 µg/ml	3291	160	4.86	78.06	17.07	1.09	0.08	0.88	0.18	1.03	0.73	0.64	0.24
0.1 µg/ml	4187	132	3.15	84.81	12.04	0.48	0.06	0.64	0.45	0.43	0.11	0.53	0.48
Treatment 2 : Materials fixed after 24 h recovery following 24 h drug treatment													
10 µg/ml	4248	82	1.93	64.00	34.01	0.12	Nil	0.35	0.09	0.14	0.19	0.21	0.85
1 µg/ml	3368	154	4.57	83.90	11.53	0.18	Nil	0.36	0.06	0.03	0.18	0.38	3.38
0.1 µg/ml	3193	63	1.97	84.97	13.06	0.22	Nil	0.41	0.28	0.13	Nil	0.03	0.90
Control	1221	76	6.22	93.3	0.5	2.45	Nil	1.55	Nil	1.39	Nil	0.81	Nil

washed in the fixative and squashed in 1% acetocarmine. The slides were examined under the microscope and the frequencies of different types of abnormalities per treatment were recorded. Photomicrographs were made from fresh preparations.

OBSERVATIONS

Mitoses were normal in the control materials kept in distilled water. On the other hand, mitotic abnormalities mere encountered even in materials treated with the lowest concentration (0.1 µg/ml) of analgin. These abnormalities were suggestive of mitoclastic rather than clastogenic properties of the drug. Analgin also prevented interphase cells from entering into the M-phase thereby lowering the mitotic index in treated cells. It was also capable of arresting mitosis at any stage.

Among the various cytological abnormalities induced by analgin, nuclear lesions (Figs. 1,18,19) were the most frequent. Endopolyploidy (Fig.2), early splitting of chromosomes (Fig.3) clumping and stickness of chromosomes (Fig.4), tropokinesis (Figs. 5,6), kinetochore inactivation and stickness together with neocentic activity of telomeres in some chromosomes (Fig.7), unipolar movement of chromosomes (Fig.8), anaphases with unequal distribution of chromosomes (Fig.9), asynchronous movement of chromosomes (Fig.10), aneuploid number of chromosomes (Fig.11) and double stranded chromosomes (Fig.12), double star anaphase resulting in irregularly crescent shaped chromatic masses (Fig.14) and irregular nuclei with projecting chromosomes (Fig.16), disorientation of chromosomes at one pole at anaphase (Fig.17), absence of cytokinesis at telophase leading to the formation of binucleate (Fig.18) and multinucleate cells (Fig.19) were the other common mitotic abnormalities encountered in the drug treated materials. The nuclei in multinucleate cells were often unequal in size, varied in shape and were with prominent lesions as in Fig.19. Structural aberrations of chromosomes were found to be manifested as chromatid and chromosome breaks and fragments as seen in Figs. 8, 15 and 17. In Fig. 15 the metaphase chromosomes appear supercondensed and split except at çentromeric regions as in C-metaphase. All these abnormalities were encountered in materials given a recovery time of 24 h, after drug treatment. The results are summarised in Table 1.

DISCUSSION

The above results point out that analgin is a strong mitoclastic agent interfering with the progression of cell cycle at any stage. The drastic lowering of the mitotic index in the treated materials as compared to the untreated controls is a direct proof for its antimitotic property. Of the 3 concentrations of the drug used, the intermediate concentration (1 µg/ml) was found to be less effective in mito-depression, although the mitotic indices in cells treated with this concentration was lower than that in the untreated control. On the other hand, the highest incidences of mitotic abnormalities were encountered in materials treated with this concentration of analgin. Hence it may be presumed that analgin at this concentration is capable of keeping more number of cells in M-phase than at interphase and that it caused the arrest of M-phase at various sub-stages. This was coupled with spindle abnormalities. These results are in conformity with the results of other authors with other antimitotic chemicals (Biesele 1958, Deysson 1968, Kihlman 1966).

Although metaphases with diplochromosome were not observed, anaphases with double stranded chromosomes were frequently encountered in analgin treated materials in the present study suggesting the occurrence of endoreduplication at S-phase prior to the onset of M-phase. Such double stranded chromosomes at anaphase have been reported in *A. cepa* root tip cells treated with the marine toxin Holothurin (Santhakumari & Stephen 1988). According to Nagl (1970), endoreduplication in plant cells occurs as a result of blockage of the cell cycle at G2 phase. However, the present observations clearly indicate that analgin is capable of inhibiting cell cycle not only at G2 but also in the substages of the M-phase. Mitotic arrests at prophase, metaphase, anaphase and even early telophase were noticed in this study. Whereas double-stranded chromosomes at anaphase might have resulted from endoreduplication, the endopolyploid prophase within intact nuclear envelope as in Fig.2 is suggestive of endomitosis and the well split chromosomes at late prophase (Fig.3) and at metaphase (Fig.5), and supercondensed split chromosomes at metaphase (Fig.15) are suggestive of C-mitosis. In Nagl's (1970) opinion endomitosis results from mitotic blockage at prophase and C-mitosis from blockage at metaphase.

The various types of spindle abnormalities, kinetochore inactivation and neocentric activity of telomeres presently observed have striking similarities with those induced by several antimitotic substances (Biesele 1958, Deysson 1968). Endopolyploidy frequently observed in treated materials may be due to endoreduplication, endomitosis or C-mitosis. Endopolyploidy is a common cytological feature of cancer cells (Levan 1969). Binucleate and multinucleate conditions presently reported are also found in cancer cells (Graham 1963, Oksala & Therman 1974).

Structural aberrations of chromosomes such as chromosome inflammation, stickness, chromatid and chromosome breakage, fragments etc. presently observed have been reported to be induced by several clastogenic and carcinogenic substances,(Kihlman 1966, Montesano et al. 1976, Nesnow et al. 1987). Nuclear lessions were found to be induced by adriamycin in *A. cepa* root tip cells (Mercykutty & Stephen 1980).

On the whole, the present results show that analgin is both mitoclastic and clastogenic and hence it is advised to use this drug only under medical surveillance. The above discussed cytological abnormalities may be responsible for granulocytopenia and agranulocytosis reported by Gorrod (1979) in patients administered with analgin. These results also prompt further research on the mutagenic and carcinogenic potentials of this drug using in vitro and in vivo systems as outlined by Montesano et al. (1976). Harmful effects of some other analgesic drugs have already been reported by Mazrooei & Kabarity (1984).

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COMPARISON OF CLASTOGENIC EFFECTS OF TWO ARSENIC SALTS ON PLANT SYSTEM IN VIVO

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SUMMARY

The clastogenic effects of 2 water soluble inorganic salts of arsenic, sodium arsenite and sodium arsenate were studied on plant systems in vivo. Seeds of *Hordeum vulgare* were soaked in solutions of the salts in 5 concentrations ranging from 1 to 10,000 ppm and then sown. Effects were screened from root tips of seedlings at intervals of 24 h up to 120 h, following the usual pretreatment-fixation-aceto-orcein squash schedule. Endpoints screened were chromosomal aberrations and mitotic index. Data were analysed statistically following 2-way ANOVA. The frequency of abnormal cells was directly proportional to the dose of the chemical and inversely proportional to the duration of recovery. The frequency of dividing cells (mitotic index) was reduced proportionately with the concentration used and increased with the increase in duration of recovery after exposure. Mainly, spindle distrubances were recorded, indicating that the effects of these salts were possibly related to their known affinity for thiol groups.

Key Words : Arsenic, clastogenic effects, plants in vivo.

INTRODUCTION

In nature, arsenic is present in a number of minerals, including arsenides of copper, nickel, iron or as arsenic sulfide or oxide. In water, it is usually found in the form of arsenite or arsenate (Ferguson & Gavis 1972). Interest in arsenic contamination was increased following the estimation of high levels of arsenic in drinking water from tube wells in several districts of West Bengal. Plants grown on arsenic contaminated soil were observed to contain higher levels of arsenic, especially in the roots (Grants & Dobbs 1977). Earlier reports have shown that arsenic can inhibit DNA, RNA and protein synthesis (Nakamuro & Sayoto 1981). However, it was not able to induce gene mutations in *Salmonella typhimurium* TA 102 strain, unlike other heavy metals (Lofroth & Ames 1978).

As compared to the vast amount of information available on the toxic effects of inorganic arsenic compounds on different test systems (Merian 1991) data on their effects on chromosomes or on cell division in higher organisms, is relatively meagre (Sharma 1984, 1985). The present investigation was, therefore, undertaken to study the clastogenic effects of 2 inorganic salts of arsenic namely, sodium arsenite and sodium arsenate, on *Hordeum vulgare* L. in vivo to compare their relative effects and to determine the threshold values.

TABLE 1 : Effects of exposure to arsenic salts on cell division.

(A)	(B)	Sodium arsenite MI Mean ± SD	Sodium arsenite % of Aberration ± SD Gr I	Gr II	Gr III	Sodium arsenate MI Mean ± SD	Sodium arsenate % of Aberration ± SD Gr I	Gr II	Gr III
Control	24	4.22 ± 1.60				4.22 ± 1.60			
	48	3.72 ± 1.10				3.72 ± 1.10			
	72	3.46 ± 0.77	Nil			3.46 ± 0.77			
	96	3.12 ± 0.51				3.12 ± 0.51			
	120	2.98 ± 0.39				2.98 ± 0.39			
10^4	24					3.46 ± 1.16	3.76 ± 1.48	0	
	48					3.10 ± 1.20	2.4 ± 1.9	1.1 ± 2.2	0.0
	72	lethal				2.16 ± 0.59	3.1 ± 3.09	0	0.0
	96					2.00 ± 0.96	0.58 ± 1.29	2.35 ± 3.2	
	120					1.68 ± 0.59	1.42 ± 3.17	0.8 ± 1.78	0.0
10^3	24	2.06 ± 0.65	5.18 ± 1.59	1.42 ± 3.19		3.70 ± 1.13	2.49 ± 1.85	0.44 ± 0.98	0.0
	48	3.28 ± 2.10	3.54 ± 3.39	0.38 ± 0.85	.33 ± .75	3.56 ± 0.79	2.3 ± 1.56		0.86 ± 1.92
	72	3.32 ± 0.67	3.04 ± 1.8			3.42 ± 0.83	2.2 ± 2.09		
	96	3.02 ± 0.99	1.97 ± 1.9			2.70 ± 0.98	2.12 ± 2.13		
	120	2.20 ± 0.94	1.52 ± 3.39			1.68 ± 0.48	2.26 ± 3.16		
10^2	24	3.56 ± 0.93	3.67 ± 2.5			4.08 ± 0.93	1.96 ± 1.23		
	48	3.38 ± 0.42	2.62 ± 1.51		.35 ± 1.23	3.58 ± 1.72	1.5 ± 1.61		
	72	3.36 ± 1.19	2.68 ± 1.9			3.18 ± 1.00	1.25 ± 2.7		
	96	3.02 ± 1.69	1.78 ± 2.4			3.04 ± 1.28	1.37 ± 3.08		
	120	1.92 ± 0.60	.8 ± 1.8			2.02 ± 0.43	1.64 ± 2.26		
10	24	3.66 ± 1.27	2.94 ± 0.39	0.42 ± 0.94		4.18 ± 0.57	1.5 ± 2.5		
	48	3.40 ± 1.60	2.8 ± 1.7			3.66 ± 0.73	1.16 ± 1.54		
	72	3.40 ± 0.91	2.5 ± 1.6			3.40 ± 2.00	0.94 ± 1.29		
	96	2.72 ± 0.66	1.7 ± 2.3			2.92 ± 0.90	0.5 ± 1.1		
	120	2.30 ± 1.71	.52 ± 1.17			2.32 ± 0.36	0.0 ±		
1	24	3.92 ± 0.76	2.03 ± 1.17			4.02 ± 1.23	0.44 ± 0.98		
	48	3.70 ± 0.96	1.16 ± 2.3			3.22 ± 0.82	0.0		
	72	3.28 ± 0.68	1.3 ± 1.9			3.14 ± 0.61	0.0		
	96	3.10 ± 0.68	0.9 ± 2.01			3.00 ± 0.739	0.0		
	120	2.87 ± 0.69	0			1.84 ± 0.69			

A= Concentration used; B = Duration in recovery in h; MI = Mitotic index; Grs I, II, III, = Different types of aberrations; Total cells scored per set = 5000

TABLE 2 : Comparison between the effects of different doses and different durations of sodium arsenite and sodium arsenate on cell division.

Duncan's multiple range tests :						
Sodium arsenite :						
I.	Between doses					
	Doses in ppm	10^3	10^2	10	1	C
	Sample means	13.85	15.24	15.48	16.87	17.5
II.	Between duration					
	Duration in hours	120	96	72	24	48
	Sample mean	12.27	14.98	16.98	17.42	17.48
Sodium arsenate :						
	Doses in ppm	10^3	1	10^2	10	C
	Sample means	15.04	15.22	15.90	16.72	17.5
II.	Between duration					
	Duration in hours	120	96	72	48	24
	Sample means	11.02	14.78	16.64	17.74	20.2

Underlines denote insignificant differences among the means at $p = 0.05$.

MATERIALS AND METHODS

Aqueous solutions of sodium arsenate and sodium arsenite were prepared in different concentrations namely, 1, 10, 100, 1000, 10,000 ppm. Healthy 100% viable seeds of *H. vulgare* were soaked in each concentration overnight. After treatment the seeds were washed and allowed to germinate in sawdust and sand mixture. Control was kept in distilled water.

In each set and its control, 10 root tips were excised at intervals of every 24 h up to 120 h. The tips were washed thoroughly and pretreated in colchicine (0.2%), for 1 h, fixed in acetic ethanol (1:3) for 1 h and then stained according to the standard acetic-orcein technique and squashed in 45% acetic acid (Sharma & Sharma 1980).

The end points scored were frequencies of dividing cells, chromosomal aberrations and spindle disturbances. Approximately 5000 cells were observed for each sampling period per concentration. The observations induced and recorded in chromosomes were categorised into 3 groups namely, group I with spindle disturbances such as laggards, multipolarity, sticky bridge and early separation, group II with chromosome breaks and fragments and group III with micronuclei. Statistical analysis was done following 2-way ANOVA and Student's test.

RESULTS AND DISCUSSION

The results have been given in Tables 1 - 3. Seeds failed to germinate after soaking in the highest concentration of sodium arsenite. Mitotic index was reduced significantly by the higher doses of both salts, the degree of reduction being directly proportional to the concentration used. The frequency of dividing cells increased proportionately with the period of recovery ofter exposure in all cases. 10 ppm induced a lower degree of reduction in mitotic index as compared to the two highest doses.

The frequencies of chromosomal abnormalities induced were directly proportional to the concentration used and were significantly high when exposed to higher doses. As seen from Table 1, abnormalities (Gr. I) like stickiness, clumping, laggards, unequal separation were common after 24, 48, 72 h and to some extent even 120 h of recovery. Abnormalities (Gr. II) such as break and fragments were found in low frequencies after exposure to higher doses at 24 and 48 h of recovery. However, micronuclei (Gr. III) were found after 48 h of recovery. The frequency of abnormalities induced by sodium arsenite was higher than that induced by sodium arsenate but not to significant level (Tables 1-3).

TABLE 3 : Comparision between the effects of Na-arsenite and Na-arsenate after 24 h exposure followed by recovery

Doses	Salt	Duration	M.I.	t value	AB%	t value
10^3 ppm	A	24	2.06		6.50	
	B	24	3.70	2.81*	2.90	1.77
	A	48	3.28		4.20	
	B	48	3.56	0.279	3.26	0.55
	A	72	3.32		3.04	
	B	72	3.42	0.21	2.20	0.68
	A	96	3.02		1.97	
	B	96	2.70	0.514	2.12	0.118
	A	120	2.20		1.52	
	B	120	1.68	1.10	2.26	0.36
10^2 ppm	A	24	3.56		3.67	
	B	24	4.08	0.885	1.96	1.37
	A	48	3.38		3.17	
	B	48	3.58	0.252	1.50	2.18
	A	72	3.36		2.68	
	B	72	3.18	0.258	1.25	0.97
	A	96	3.02		1.78	
	B	96	3.04	0.021	1.37	0.23
	A	120	1.92		0.80	
	B	120	2.02	0.303	1.64	0.67
10 ppm	A	24	3.66		0.836	
	B	24	4.18	0.836	1.50	1.23
	A	48	3.40		2.80	
	B	48	3.66	0.331	1.16	1.59
	A	72	3.40		2.50	
	B	72	3.40	0	0.94	1.70
	A	96	2.72		1.70	
	B	96	2.92	0.40	0.50	1.05
	A	120	2.32		0.52	
	B	120	2.30	1.23	0	1.0
1 ppm	A	24	3.93		2.03	
	B	24	4.02	0.06	0.44	2.44*
	A	48	3.70		1.16	
	B	48	3.22	0.88	0	1.04
	A	72	3.28		1.30	
	B	72	3.14	0.335	0	1.53
	A	96	3.10		0.90	
	B	96	3.00	0.22	0	1.01
	A	120	2.87		0	
	B	120	1.84	2.37*	0	0

A = Na-arsenite, B = Na-arsenate.

The significance of this work is in the high levels of arsenic recorded in well water in several areas of West Bengal and the subsequent increase in plant parts. Natural As levels in plants seldom exceed 1 mg/kg, but the leaf content may be higher if arsenic pesticides have been used. From their use as pesticides, plant defoliants and herbicides, inorganic and organic As compounds have been found to accumulate in soils and plants (Merian 1991). Bioaccumulation of As is also very high in plankton, sea weed and algae (NRCC 1978, Nriagu 1994, US EPA 1980). Phytotoxicity of arsenite is higher than that of arsenate when present in soil. The present investigations indicate that this trend is also reflected in the action of As salts on plant cell division. The maximum frequency of chromosomal aberrations involved spindle disturbances and were mainly of group I type (Table 1). Such effects may be attributed to the known affinity of arsenic salts for sulphur containing compounds, mainly thiols, involved in spindle formation.

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KARYOMORPHOLOGICAL STUDIES IN TWO ORNAMENTAL PLANTS OF LAMIACEAE

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SUMMARY

Karyomorphological analysis conducted on *Salvia splendens* (2n = 44) and *Teucrium plectranthoides* (2n = 32) show that both are tetraploids with a homogeneous, primitive karyotype showing diminution in chromatin length. Polyploidy and micro-morphometrical change of chromosomes seem to be involved in the evolution of these taxa.

Key Words : *Salvia, Teucrium*, chromosomes, karyotype, polyploidy.

INTRODUCTION

Lamiaceae comprises 220 genera and about 4000 species of medicinal, aromatic and ornamental plants with a global distribution (Hedge 1992). In South India, the family is represented by 134 species, distributed in about 29 genera (Rani & Mathew 1983). *Salvia splendens* Sello ex R. & S. (Scarlet sage) and *Teucrium plectranthoides* Gamble (Wood germander) are garden plants or 'garden escapes', found growing wildly in the western ghats. Previous cytological studies on these taxa are restricted to chromosome counts and detailed karyomorphological analysis has not been conducted yet. So the present study is intended to reveal their chromosome constitution.

MATERIALS AND METHODS

Materials were collected from different South Indian gardens and high ranges of Kerala (Thekkady). Voucher specimens are herbarized at our Institute. Mitotic studies were made on young and healthy root tips pretreated with cytostatic chemicals. For this, a mixture of saturated aqueous p-dichlorobenzene with a trace of aesculine and saponin at 0-5° for 5 min. and 10-14°C for 2 - 2½ h were found to be suitable. They were then fixed in Carnoy's fluid, followed by the aceto-orcein squash techniques (Sharma & Sharma 1980). Leitz Biomed Photomicroscope and Kodak high contrast copy film served for photography. Karyotype drawings were made with a camera lucida. In both karyotypes, the mean centromeric index value (TF%) was determined after Huziwara (1962). The variation coefficient in the karyotypes were also calculated (Verma 1980). The chromosomes were graded according to their nature and size. Karyotype formula was constructed as follows : Type A or SAT - chromosomes - short (2.2-1.8 μm) with 2 constrictions; Type B-chromosomes with a nearly median to nearly submedian primary constriction (2.2-2.0 μm); Type C-very short chromosomes that are nearly metacentric (<2.0 μm).

RESULTS AND DISCUSSION

The somatic chromosome number was found to be 2n = 44 in *S. splendens* (Fig.1) and 2n = 32 in *T. plectranthoides* (Fig.2). Previous chromosome counts on these taxa confirm the present

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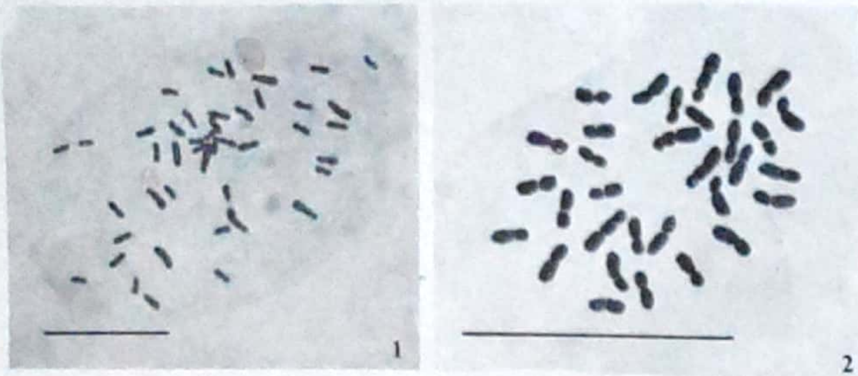

Figs. 1 & 2: Photomicrographs of somatic chromosomes. 1. *Salvia splendens* (2n = 44). 2. *Teucrium plectranthoides* (2n = 32) (Scale = 10 μm).

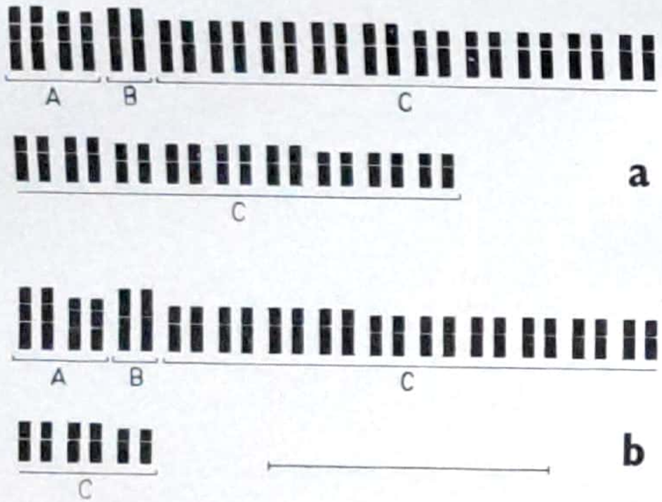

Fig. 3: Idiograms of a. *Salvia splendens*, b. *Teucrium plectranthoides*; (A,B,C indicate the chromosome types.) (Scale = 10 μm).

records (Bhattacharya 1978, Gajapathy 1962). Both taxa were found to be tetraploids with the basic chromosome number being 11 in *S. splendens* and 8 in *T. plectranthoides*. The secondary base number of 11, found in *S. splendens* might have either derived from the primary base numbers 5 and 6 through amphipolyploidy (Grant 1981) or by ascending or descending dysploidy (Love et al. 1957). Previous records reveal the existence of polyploidy in *S. splendens* (Bhattacharya 1978). The tetraploid chromosome number of *T. plectranthoides* might have derived from the primary number through autopolyploidy (Stebbins 1971). The presence of this base number in *Teucrium* is confirmed by a previous report (Gajapathy 1962). Polyploidy was prevalent in both taxa, which provides increased possibilities for new gene combinations, which are of considerable importance in speciation and evolution (Göttschalk 1985).

In *S. splendens* 2 pairs of chromosomes are with secondary constriction, 10 pairs are with nearly median primary constriction and 10 pairs with nearly submedian primary constriction. The variation coefficient and total forma % in this taxa was found to be 17.07 and 41.19 respectively. The karyotype of *T. plectranthoides* possess 2 pairs of SAT-chromosomes, 9 pairs of nearly metacentric chromosomes and 5 pairs of nearly submetacentric chromosomes. The variation coefficient was found to be 16.13 and total forma % to be 42.27.

The karyotypes of both plants were characterized by a steady diminution in chromatin length (Fig.3). The total chromatin length was found to be 71.6 μm in *S. splendens* and 46.6 μm in *T. plectranthoides*. Presence of 4 SAT-chromosomes is a remarkable feature observed in these tetraploid plants. It has been established that each basic set of chromosome has at least one chromosome with a secondary constriction (Sharma 1976). Both taxa are also characterized by a high TF% and low VC values. A high mean centromeric index and a low variation coefficient value are characteristic of a symmetrical karyotype, which corresponds to the primitive status in the evolution of flowering plants (Stebbins 1971). Cytological studies conducted on *S. splendens* and *T. plectranthoides* reveals that polyploidy and minute structural alterations of chromosomes are 2 principal factors associated with the evolution of these taxa.

ACKNOWLEDGEMENT

The authors thank the Principal, Sacred Heart College, Thevara, Cochin for providing the facilities.

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CHROMOSOME MOSAICS IN THE GERMLINE OF *ERYSIMUM PEROFSKIANUM*

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SUMMARY

Erysimum perofskianum Fisch. et Mey. an ornamental cultivated for its beautiful yellow flowers has 2n = 40 chromosomes. This is a new count for this species. Plants of this species collected from 2 different populations differed in their meiotic details. While in one population, M I chromosomes exhibited stickiness followed by abnormal A I segregation, the plants of other population had varying chromosome numbers (2n = 38 - 40). The plants of the former population set healthy fertile seeds and the seeds of the second population failed to germinate.

Key Words : Chromosome, mosaics, *Erysimum*.

INTRODUCTION

E. perofskianum Fisch. et May. (Brassicaceae) commonly known as 'Wall flower', is an annual ornamental plant characterised by bright yellow showy inflorescences. During the course of our studies in Brassicaceae, we came across 2 populations of *E. perofskianum* which differed from each other with respect to their cytological details. In the present communication, cytogenetical evaluation of these 2 populations and the possible reasons for cytological aberrations have been reported.

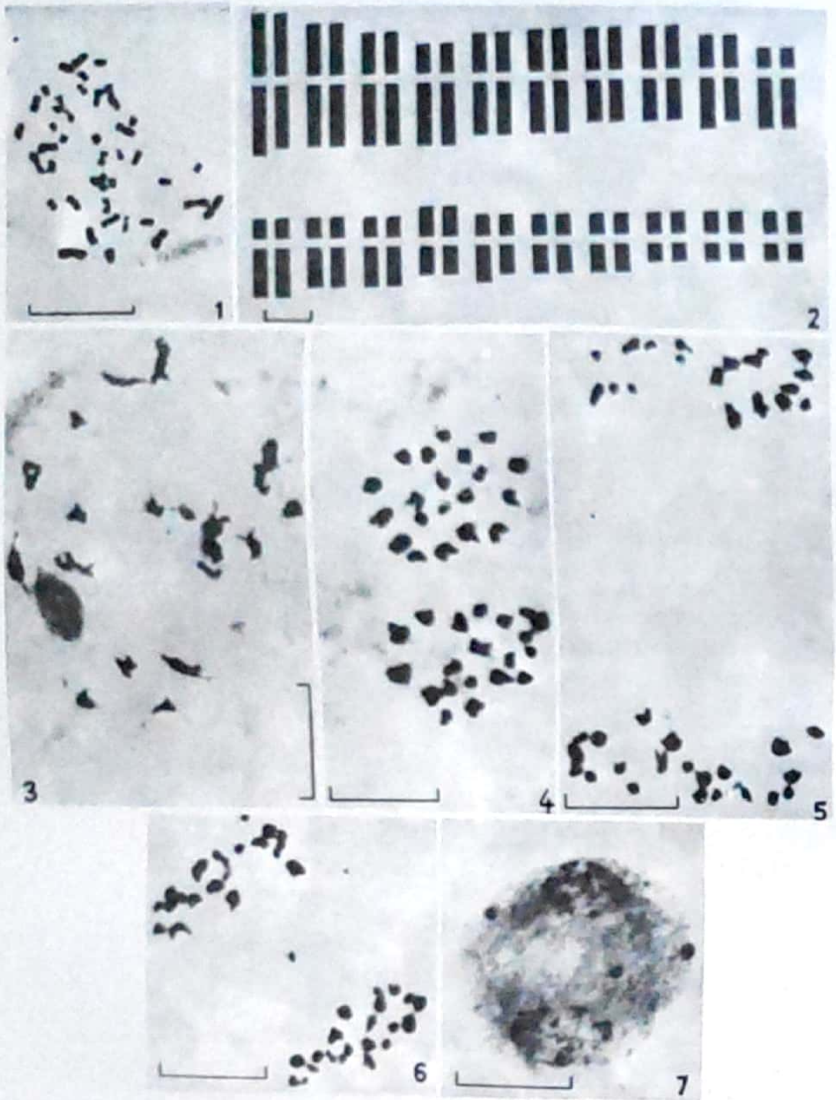
MATERIALS AND METHODS

Two populations of *E. perofskianum* growing at Shalimar Garden (Pop. 1) and Kashmir University Botanic Garden (Pop. 2) were studied in detail. Karyotypic studies were made from nucellar cells. Young growing ovaries pretreated in cold water (with traces of Aesculin) at 3-4°C for 24 h were fixed in acetic-alcohol solution in the ratio of 1 : 2. Young inflorescences were fixed in modified Carnoy's fixative (ethyl alcohol, glacial acetic acid and chloroform in 1 : 1 : 1). Fixed ovaries and inflorescences were stored in 70% ethanol. Mitotic chromosomes were studied from nucellus following the usual Feulgen technique and meiotic chromosomes were studied by squashing anthers in 1% propionocarmine.

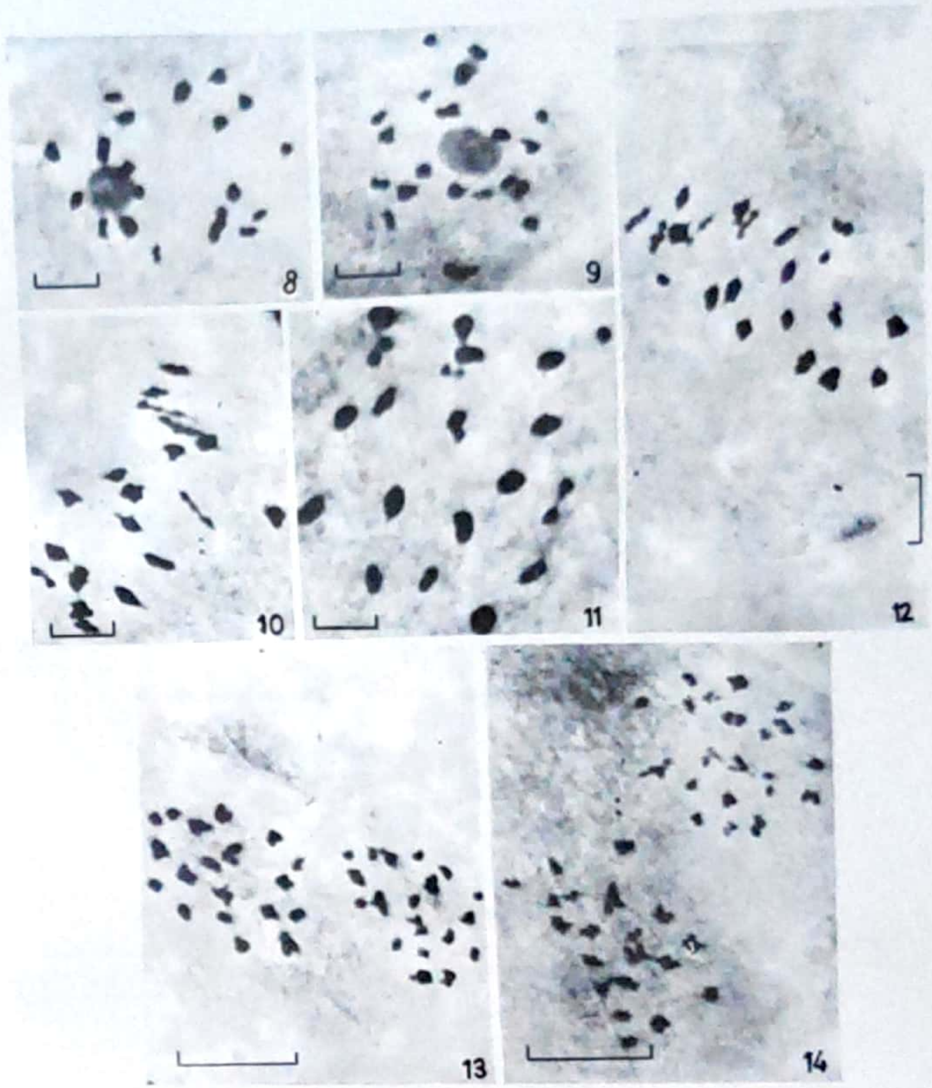
OBSERVATIONS

Karyotypes

In all well spread preparations made from the nucellar cells (Pop. 2), 40 chromosomes were counted in each cell. These comprised 14 median and 26 submedian chromosomes (Figs. 1,2). The chromosome size in this species ranges from 0.84 µm to 2.62 µm. Total and mean chromatin lengths of the complement are 60.06 µm and 1.5 µm respectively. The ratio between the size of the longest and the smallest chromosome of this complement is 3.11. Except for a few minor differences, the 40 chromosomes can be arranged into 20 pairs. In none of the chromosomes could a satellite or a secondary constriction be discerned.



Figs. 1-7: *Erysimum perofskianum* (pop. 1) (2n = 40) 1. Somatic chromosomes. 2. Idiogram 3. Diplotene. 4. AI with 20 : 20 segregation. 5. A I with 21 : 19 segregation. 6. A I with 20 : 19 segregation and one laggard. 7. T I with micronuclei. (scale = 10 µm).



Figs. 8 - 14: *Erysimum perofskianum* (pop. 2) 8. Diakinesis with 19 bivalents. 9. Diakinesis with 20 bivalents. 10. M I with 19 bivalents. 11. M I with 19 bivalents and a Univalent. 12. M I with 19 bivalents and 2 Univalents. 13. A I with 19 : 19 segregation. 14. A I with 20 : 20 segregation. (Scale = 10 µm).

Meiosis

In Pop. 1, 20 bivalents were observed in all the pollen mother cells (PMCs) at diplotene (Fig. 3). Most of these cells had 1 or 2 nucleoli with 1-3 bivalents attached to them. On an average, the total number of chiasmata per cell at this stage was 44.95 with 14.8 interstitial and 30.15 terminal. The cells at metaphase I (MI) were not suitable for study due to clumping of bivalents. Anaphase I (AI) segregation was irregular in some cells. Out of 30 cells observed at this stage, 18 cells (60%) had normal 20 :20 segregation, 9 (30%) had 21 : 19, 2 (6.67%) had 21 and 18 chromosomes at 2 poles respectively with one laggard while a single cell (3.33%) had 20 and 19 chromosomes at 2 poles respectively with a laggard (Figs. 4-6). Micronuclei were observed in most of the PMCs at telophase I and II (Fig. 7). The pollen fertility was 83.7%.

In pop. 2, meiosis was characterised by the presence of chromosomal mosaics with varying number of bivalents in different PMCs of the same anther. Out of 117 PMCs studied at prophase I (PI) and MI, in 50 cells (42.7%) there were 19 bivalents in each cell (Figs. 8, 10), in 25 cells (21.3%) a univalent was seen in addition to 19 bivalents in each PMC (Fig. 11) and in 42 (35.9%) cells 20 bivalents were observed in each cell (Figs. 9, 12). This difference was noticed at all the stages of meiosis and these cells appeared intermixed with each other without forming groups/ patches of cells with one particular chromosome number.

At diplotene and diakinesis, the size difference in the bivalents was quite prominent with 2 or 3 bivalents standing apart from the rest in being larger than the rest. At diplotene, usually one large and 2 small nucleoli were observed in each cell. Invariably, 2-4 bivalents were found attached to these nucleoli. In a single cell, however, 6 bivalents were found attached to the nucleoli. One of the very interesting features about this cytotype was the presence of a single euchromatic bivalent both at diplotene and MI. This bivalent was the smallest.

The difference in chromosome number observed at PI and MI was further confirmed from the study of cells at AI. Out of 31 PMCs observed at this stage, 13 (41.93%) cells had normal 20 : 20 segregation (Fig. 14) while 7 (22.58%) had 20 : 19, 7(22.58%) had 19 : 19 (Fig. 13) and 4 (12.9%) had 20 : 18 chromosomal segregation. In this population, despite the chromosome mosaicism observed at earlier stages of meiosis, no laggards were found at AI and TI. This becomes all the more important in view of the presence of cells with odd number of chromosomes, which could have remained as laggards in at least some of the cells. The pollen fertility is 84.2%. These plants set abundant seeds which, however, failed to germinate.

DISCUSSION

The present report of 2n = 40 in *E. perofskianum* is at variance with the earlier count of 2n = 32 - 36 (Manton 1932) in this species. This indicates that *E. perofskianum* exists in nature as more than one cytotype.

A perusal of available literature reveals that *Erysimum* is a tribasic with x = 7, 8, 9 (Manton 1932, Darlington & Wylie 1955, Mukherjee 1975). However, the present observation of 2n = 40 in *E. perofskianum* indicates that another base number (x = 10) is also operating in this genus. The earlier report of 2n = ca. 40 in *E. purpureum* (Manton 1932) supports this view. As such the base numbers in this genus form a series of 7, 8, 9 and 10. Keeping the earlier contention in view, it seems that the present stock of *E. perofskianum* (2n = 40) is a tetraploid. The very fact that the 40 chromosomes of this species pair perfectly to form 20 bivalents only is an indication of its genomic allotetraploid nature. The stickiness of bivalents after diplotene could be due to some late acting gene.

As pointed out earlier, meiosis studied from 2 populations was found to be abnormal. In pop. 1, the behaviour of chromosomes up to diakinesis was normal with the formation of bivalents only. However, beyond this stage meiosis did not follow a normal course and in the cells at MI, bivalents exhibited clumping. This clumping of bivalents perhaps disturbs the segregation of chromosomes at AI. In about 40% of cells at AI, the segregation was characterised by unequal number of chromosomes or the presence of lagging chromosomes; the latter forming micronuclei. These abnormalities did not drastically affect the fertility of the plants of this species and these plants set abundant viable seeds. Although, a somewhat similar situation has been reported in *Fagopyrum cymosum* (Gohil et al. 1983) the difference is that while *E. perofskianum* plants set enough healthy seeds, the plants of *F. cymosum* are totally sterile.

Meiosis in the plants of pop. 2 was quite interesting due to the presence of different numbers of chromosomes. The number ranged from 2n = 38 - 40. In a random sampling of 117 PMCs, only 35.9% had 20 bivalents while the rest (64.1%) had either 19 bivalents or 19 bivalents and one univalent in each PMC. This phenomenon of chromosomal mosaicism can be explained on the basis of either cytomixis or chromosomal instability during premeiotic mitosis. Of these, cytomixis does not seem to be the reason of this mosaicism in the present case since no supporting evidence (like cytoplasmic connections) was observed. Moreover, if it is due to cytomixis, there should have been cells with more than 40 chromosomes which is not the case. The hypoploidy is perhaps due to abnormalities in the premeiotic mitosis wherein some chromosomes are lost during division. According to Sachs (1952), the chromosomal mosaics probably arise by spindle abnormalities in the premeiotic mitosis. Chromosomal mosaics have also been reported in hybrids of *Solanum nigrum* complex (Venkateswarlu & Rao 1969), *Achillea millifolium* (Ehrendorfer 1959), *Medicago sativa* (Murray & Craig 1964), *Gloriosa superba* (Narain 1980) and inbred lines of *Raphanus sativus* var. *radicola* (Dayal 1979).

The chromosomal mosaicism seems to affect the fertility of the plants exhibiting this phenomenon. Although the plants set enough seeds, these failed to germinate either in laboratory or under natural conditions. This sterility can be attributed to the failure of chromosomes to segregate evenly at AI in majority of cells (60%). But since about 40% cells at AI had normal segregation, some degree of fertility should have been there. But it is likely that in them too there must have occurred some disjunctional problems like homologous chromosomes going to the same pole or like that which ultimately resulted in seed sterility. This observation is at variance with the opinion expressed by Venkateswarlu & Rao (1969) that factors controlliong chromosomal mosaicism and fertility of the plants exhibiting mosaicism are independent of each other and mosaicism does not reduce the fertility of such individuals. The present investigation reveals that the mosaicism does affect the fertility of seeds.

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KARYOTYPE STUDIES OF SEVEN SPECIES OF *TEPHROSIA* FROM SOUTH INDIA

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SUMMARY

Karyotypes of 7 species of *Tephrosia* namely, *T. maxima* (L.) Pers., *T. pulcherrima* (Baker) Gamble, *T. purpurea* (L.) Pers., *T. spinosa* (L.f.) Pers., *T. tinctoria* (L.) Pers., *T. villosa* (L.) Pers. and *T. vogelii* Hook. f. are reported. The chromosome numbers in *T. pulcherrima* and *T. spinosa* and karyotypes of *T. maxima, T. pulcherrima, T. spinosa* and *T. vogelii* are reported for the first time. *T. pulcherrima*, considered by some authorities as part of the variable species *T. tinctoria* is shown to have a distinct karyotype so as to warrant a separate species status. It is also suggested that the trend in karyotype evolution in the genus is from symmetry to asymmetry.

Key Words : *Tephrosia*, karyotype, evolution.

INTRODUCTION

Tephrosia Pers. (Subfamily Papilionoideae of Leguminosae) is a large genus of over 400 species, mostly African (Geesink 1981). Cytology of about 14% of the Tephrosias has already been reported (Darlington & Wylie 1955, Bolkhovskikh et al. 1969, Goldblatt 1981, 1984, 1988, Kumar & Subramaniam 1986). But most of these works are limited only to determinations of chromosome number and further details of karyotypes are known only in 9 species (Bhatt 1974, Singh et al. 1976, Sareen & Trehan 1977, Krishnappa & Basavaraj 1978, Agarwal & Gupta 1983, Kumari & Bir 1990). Results of karyotype studies on 7 species of *Tephrosia* from South India are reported here.

MATERIALS AND METHODS

Plant materials were collected from different localities in the States of Kerala and Tamil Nadu. The places of collection and the voucher numbers are given in Table 1. Voucher specimens are deposited in KUBOT. Chromosome numbers were determined both by meiotic and mitotic counts. Flower buds and root tips were fixed in ethanol-acetic acid mixture (3 :1). Root tips were treated in 0.002M 8-hydroxyquinoline for 3 h at 4°C. The chromosomes were stained in 2% acetocarmine. Photomicrographs were taken from temporary preparations. Centromere positions in chromosomes were determined following Leven et al. (1964) and karyotypes were categorized according to Stebbins (1958).

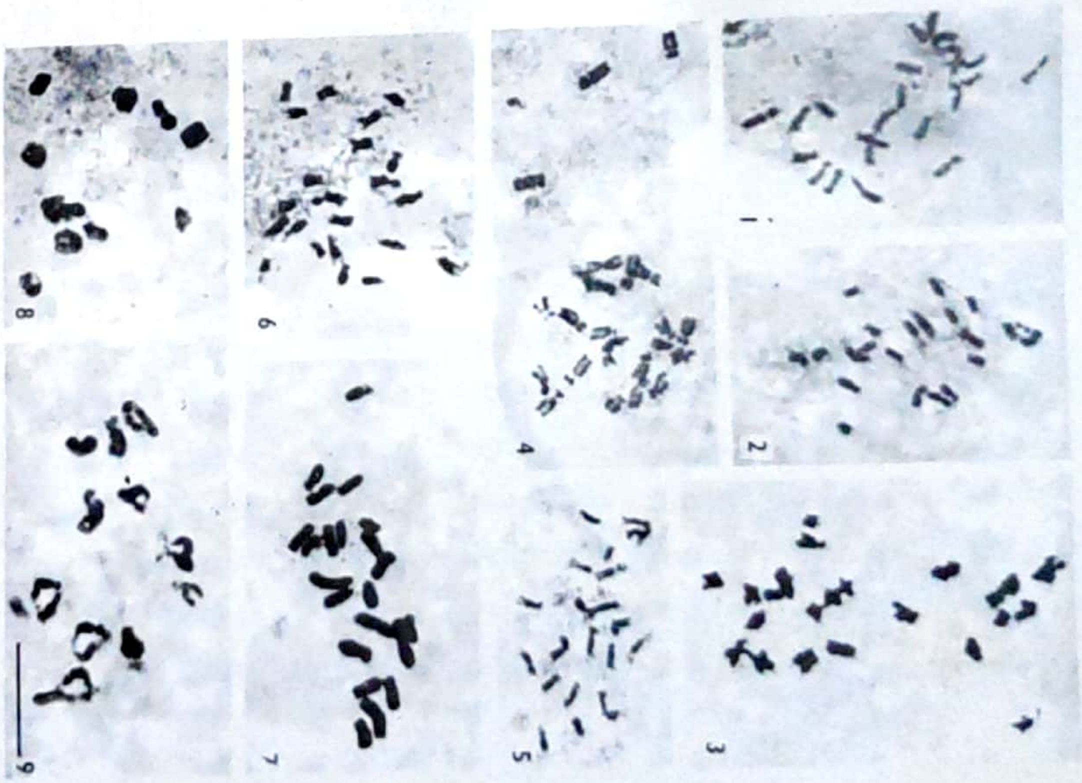
OBSERVATIONS

The 7 species of *Tephrosia* reported here showed $2n = 22$ chromosomes in root tip cells (Figs. 1-7). Chromosome numbers in *T. pulcherrima* and *T. spinosa* are reported here for the first time and meiotic behaviour in these were also observed. Both these species showed $n = 11$ bivalents in each PMC (Figs. 8, 9). Other details of the karyotypes of the 7 species and their idiograms are given in Table 1 and Figs. 10-16. Secondary constrictions and satellites could be clearly seen only in *T. tinctoria* and *T. villosa*. The long arms of the first and third pairs of chromosomes in the

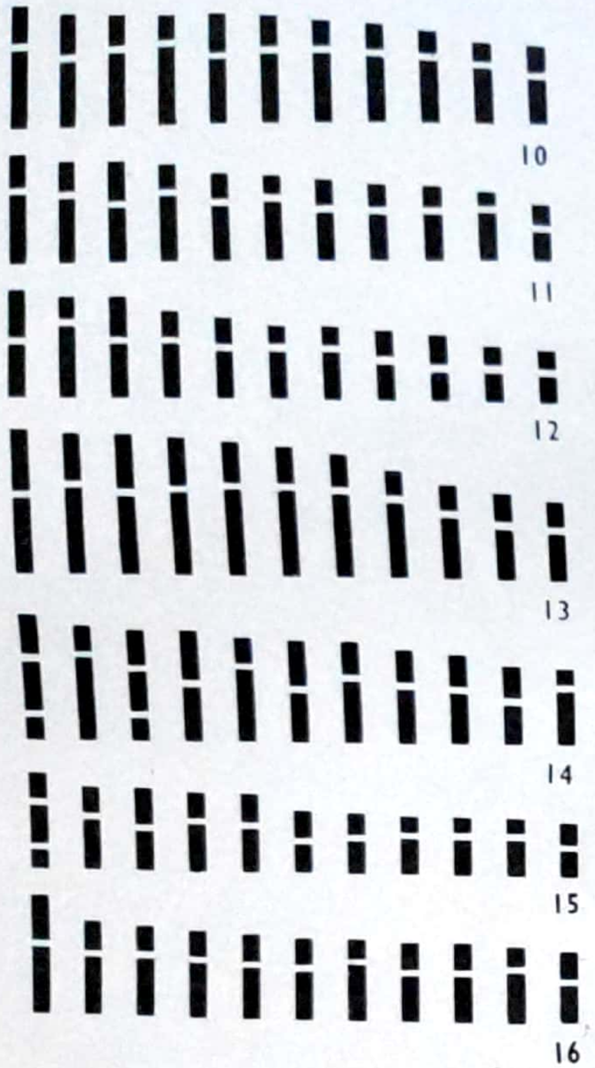
TABLE 1: Karyomorphological data on species of *Tephrosia* from South India.

Species with source and voucher number	Karyotype formula	n. f. value	TCL (µm)	Range of size and ACL(µm)	L/S	% of chromosomes with arm ratio>2:1	Karyotype category
T. maxima Kariavattom : 10014	2n=22=6m+16sm	28	52.66	1.75-3.00 (2.39)	1.71	72.73	3A
T. pulcherrima Silent Valley : 10024	2n=22=4m+16sm+2st	26	42.88	1.25-2.59(1.95)	2.07	54.55	3B
T. purpurea Kariavattom : 10015	2n=22=2M+8m+10sm+2st	32	39.70	1.17-2.50(1.80)	2.14	27.27	2B
T. spinosa Vadakkankulam: 10183	2n=22=6m+16sm	28	58.68	1.82-3.33(2.67)	1.83	45.45	2A
T. tinctoria Kodaikanal : 10194	2n=22=8m+12sm+2st	30	51.42	1.75-2.92(2.34)	1.67	27.27	2A
T. villosa Thiruvananthapuram:10116	2n=22=10m+12sm	32	35.12	1.21-2.15(1.60)	1.78	36.36	2A
T. vogelii Munnar : 10071	2n=22=8m+14sm	30	42.74	1.59-2.80(1.94)	1.76	27.27	2A

n.f., fundamental number of chromosome arms: TCL, Total chromosome length of diploid complement: ACL, Average chromosome length: L/S, Ratio of longest to shortest chromosome.



Figs. 1-9: 1 - 7. Metaphase chromosomes. 8 & 9. PMCs. 1. *T. maxima* (2n=22), 2. *T. pulcherrima* (2n=22), 3. *T. purpurea* (2n=22), 4. *T. spinosa* (2n=22), 5. *T. tinctoria* (2n=22), 6. *T. villosa* (2n=22), 7. *T. vogelii* (2n=22), 8. *T. pulcherrima* (n=11), 9. *T. spinosa* (n=11). (Scale Bar = 3 µm).



Figs. 10-16: Idiograms of species of *Tephrosia*. 10. *T. maxima*. 11. *T. pulcherrima*. 12. *T. purpurea*. 13. *T. spinosa*. 14. *T. tinctoria*. 15. *T. villosa*. 16. *T. vogelii*.

former and the first pair in the latter were satellited. *T. maxima*, *T. spinosa*, *T. villosa* and *T. vogelii* have only m and sm chromosomes. The remaining 3 species have 2 st chromosomes each besides m and sm chromosomes in the karyotype. Karyotypes of the species investigated were, in general, symmetrical, but those of *T. pulcherrima* and *T. maxima* belonging to 3A and 3B category were asymmetrical than the rest.

DISCUSSION

Morphological characters such as silvery oblong or obovate leaflets, axillary racemes and bearded styles are common to both *T. pulcherrima* and *T. tinctoria*. On the basis of this, Rudd (1991) treated these as elements of a single variable species. However, Gamble (1918) had elevated var. *pulcherrima* to specific status, characterized by leaves with a few leaflets and with terminal leaflets much longer than the laterals. Britto (1983) also supported this treatment. The present study showed that *T. pulcherrima* has a more asymmetrical karyotype with 2n=22=4m+16sm+2st than that of *T. tinctoria* with 2n=22=8m+12sm+2st chromosomes. Besides, the 2 taxa showed remarkable differences in the n.f. value, TCL and ACL also. These cytological differences between the two seem to support a separate species status to *T. pulcherrima*.

The karyotypes of *T. maxima*, *T. spinosa*, *T. vogelii* and *T. pulcherrima* are reported here for the first time. The only previous report on the karyotype of *T. tinctoria* (Krishnappa & Basavaraj 1978) revealed 2n=22=4m+12sm+6st chromosomes with n.f. =26. This is markedly different from the present report of 2n=22=8m+12sm+2st with n.f.=30. However, it may be seen that during the present study, a karyotype of 2n=22=4m+16sm+2st with n.f.=26 was found in *T. pulcherrima*, which is merged by Rudd (1991) into *T. tinctoria*. It seems very likely that Krishnappa & Basavaraj (1978) have also taken *T. pulcherrima* as part of the variable species *T. tinctoria*, and reported its karyotype under the name *T. tinctoria*.

The only previous work on karyotype evolution in *Tephrosia* is that of Kumari & Bir (1990) who reported moderately to highly symmetrical karyotypes in 5 species of the genus. But, they have also observed that on the basis of symmetry index (SI) values in the 5 species the trend in karyotype evolution in the genus is towards more symmetry and that when gradient index (GI) values are taken into account, the trend is towards asymmetry. It need not be explained that the above trends cannot be independent of each other and that independent consideration of these will only create confusion. As has been suggested by Jones (1978) the trends of karyotype evolution have to be inferred by a synthetic approach. As mentioned above *T. pulcherrima* and *T. tinctoria* are morphologically very close to each other and provide an ideal set to determine the derived nature of one or the other by a study of features other than chromosomes.

Polhill (1981) and Goldberg (1986) have identified a set of morphological features, which are primitive in the Papilionoideae and other dicotyledons, and their contrasting derived features. An analysis of 7 primitive morphological features such as shrubby habit, sparse indument, 7-foliolate leaves, axillary short racemose inflorescence, white or yellow flowers, regular calyx and woody fruit valves and their derived characters such as herbaceous habit, copious indument, a few or 1 leaflet, lax terminal racemose inflorescence, other flower colours, subequally lobed calyx and membraneous fruit valves, indicating evolution in *Tephrosia* was made. It was found that other features being similar *T. tinctoria* is more derived than *T. pulcherrima* in having membraneous fruit valves while *T. pulcherrima* is more advanced than *T. tinctoria* in having only 3-5 leaflets and subequally lobed calyx, confirming overall advancement of *T. pulcherrima* over *T. tinctoria*. The karyotype of *T. pulcherrima*, belonging to 3B category, is more asymmetrical than that of *T. tinctoria* which belongs to 2A category. This much of correlation between morphologically derived nature and greater asymmetry of karyotype in *T. pulcherrima* may indicate that asymmetry of karyotype is the derived condition in the genus. A comparison of the fundamental number of chromosome arms in these and other species support this inference and provide some insight into the mechanism of karyotype evolution in the genus. Stebbins (1950, 1971) and Jones (1978) have shown that increase in asymmetry is achieved by unequal translocations or pericentric inversions which results in a reduction in the fundamental number of arms without change in chromosome number. The n.f. value = 26 in *T. pulcherrima* is considerably lower than that of the n.f. = 30 in *T. tinctoria*. It may also be seen that among the species reported *T. maxima* with n.f. value = 28 has more asymmetrical karyotype than *T. villosa* and *T. purpurea* with n. f. value = 32 and *T. vogelii* with n. f. value = 30. This, notwithstanding the occurrence of n.f. = 28 and comparatively symmetrical karyotype in *T. spinosa*, indicates a positive correlation between reduction in the number of chromosome arms and increase in karyotype asymmetry, which provides further evidence for a symmetry to asymmetry trend in karyotype evolution in the genus. It also suggests that structural alterations of chromosomes such as pericentric inversions and unequal interchanges have played an active role in evolution of the genus.

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ISOZYME VARIATION IN RESPONSE TO ENVIRONMENTAL CHANGES IN THE *STELLARIA LONGIPES* COMPLEX (CARYOPHYLLACEAE)

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SUMMARY

Identical genotypes of *Stellaria* species representing 4 ploidy levels were grown under 4 combinations of temperature and photoperiod to examine the changes in isozyme patterns in response to environmental conditions. Four of the 5 enzymes tested showed variation for 7 activity zones, between the contrasting environments. Considerable variation was observed both within and among the 4 ploidy levels and between treatments. Diploid populations showed slightly lesser variation between treatments than did the polyploids. The ability to alter enzyme structure under different climatic conditions probably infers a possible adaptive strategy of this successful and highly polymorphic species.

Key Words : *Stellaria*, isozymes, environmental conditions.

INTRODUCTION

Stellaria longipes Goldie (Caryophyllaceae) is a circumpolar, highly polymorphic species. It occurs in a wide range of climates, in diverse forms and exhibits extensive phenotypic plasticity (Chinnappa & Morton 1984). These features have made this species amenable for cytogenetic, ecophysiological and molecular studies (Chinnappa 1985, Macdonald et al. 1988, Emery et al. 1994a, Zhang & Chinnappa 1994).

Identical genotypes of *S. longipes* grown under different environmental conditions show dramatic alterations in phenotype morphology (Macdonald et al. 1984). The habit of a single genotype varies from dwarf plant with a single flower and ovate leaves, to tall plant with branched cymes and lanceolate leaves. Temperature and wind have been shown to exert a major influence on stem elongation, as compared to photoperiod (Emery et al. 1994b). It has been established that plants of *S. longipes* originating from different habitats differ in their amount and patterns of plasticity (Macdonald et al. 1988, Emery et al. 1994a). In recent years, studies have demonstrated alterations in leaf enzymes in response to induced changes in the environment (Simon et al. 1989, Savich & Tazhibaeva 1988, Chou et al. 1991). Several studies provide evidence for adaptation and acclimation at the level of enzyme (Simon 1979, Simon et al. 1983). Isozyme variation has been suggested as an important factor which aids the plant in responding to various environmental factors (Salisbury & Ross 1992). One of the methods employed to study isozyme variation is electrophoresis (Nevo et al. 1986). In the light of the above studies, we investigated whether changes in temperature or photoperiod had any effect on the expression of isozymes of *S. longipes*. Here, we discuss our results based on 5 enzyme systems, of which, the genetics of 3 of them has already been reported by Cai & Chinnappa (1989a).

MATERIALS AND METHODS

Populations used in the study along with their chromosome numbers, collection numbers and origin are given in Table 1. One genotype from each of the 30 populations of *S. longipes* and a diploid progenitor species, *S. longifolia*, listed in Table 1, were used throughout the study. These plants originated from a wide range of habitats distributed across North America. Plants were collected as clones and have been maintained in the greenhouse of the University of Calgary.

TABLE 1: List of genotypes used in the study along with their chromosome numbers and origin.

Species	Chrom. No (2n)	Accession No	Origin
S. longifolia	26	CC 818	Hawk Hills, North West Territories, Canada
	26	CC 1184	Bragg Creek, Alberta, Canada
	26	NA 4734	Waterton Park, Alberta
	26	NA 7013	White Mountain, Arizona, USA
	26	NA 5803	Windsor, Ontario
	26	NA 10654	Alger, Michigan, USA
S. longipes	52	AD 1	Thompson Bay, Saskatchewan, Canada
	52	CC 817	Hawk Hills, North West Territories
	52	NA 1537	Mosanee, Ontario
	52	CC 530	Exshaw, Alberta
	52	NA 4766	Yellowstone National Park, Wyoming USA
	52	CC 223	Calgary, Alberta
	52	CC 569	Longview, Alberta
	52	CC 366	Plateau Mountain, Alberta
	52	NA 13935	Ogilvie Mountains, Yukon, Canada
	78	NA 4566	Dawson Bay, Manitoba, Canada
	78	NA 14364	Fort Resolution, North West Territories
	78	SIB	Sibley Peninsula, Ontario
	78	CC 249	Canmore-Exshaw, Alberta
	78	CC 315	Manning Prov. Park, British Columbia, Canada
	78	CC 335	Aspen Grove, British Columbia
	78	CC 648	Elbow River, Alberta
	78	NA 3867	Cypress Hills, Alberta
	78	CC 1270	Pink Mountain, British Columbia
	104	NA 4183	Carlton, Quebec, Canada
	104	NA 7277	Wasatch National Forest, Utah, USA
	104	CC 617	Kamloops, British Columbia
	104	NA 7332	Rocky Mountain, Wyoming
	104	NA 7294	Medicine Bow Mountains, Wyoming
	104	NFL	Quirpon, Northern Peninsula, New Foundland, Canada

Clones were grown in 10 cm pots with a sterilized potting mixture of Terragreen:sand:peat (2:1:1). Plants were held in a Conviron growth chamber under short-day-cold (SDC) conditions (winter growth conditions) for 120 days prior to the experiment. Immediately before the experiment, the plants were transferred to growth chambers under the conditions defined in Table 2. These growth chamber conditions were consistent with those of previous studies on plasticity (Chinnappa & Morton 1984, Macdonald et al. 1988, Emery et al. 1994a). The light source consisted of Sylvania Gro-lux Lifeline fluorescent tubes and G.E. Shadowban 75 W bulbs. One clone from each of the 30 populations was treated under 4 specified conditions (total of 120 plants). Aerial portions of the plant were harvested, frozen immediately in liquid nitrogen and stored at -70° C until use for electrophoresis.

All procedures were carried out at 4° C. Plant material was ground in a mortar in a ratio of 0.3 g fresh weight to 0.7 ml 0.1 M Tris-HCl extraction buffer, pH 7.5 (Bayer & Crawford 1986). Twenty g of PVP were added to each sample at the time

TABLE 2: Description of the four environmental conditions used in the present study.

Condition	Temperature		Photoperiod (h)
	Day	Night	
LDW	24	14	21
SDW	20	16	8
LDC	11	5	21
SDC	7	5	8

of grinding. The homogenate was centrifuged for 25 min in a Sorvall RC-5B refrigerated superspeed centrifuge at 15,000 g. The supernatant was removed and stored at -70°C until electrophoresis.

A discontinuous polyacrylamide gel electrophoresis system described by Maurier & Allan (1972) was used. The buffer for the separating gel (7.5% acrylamide) was 0.3 M Tris-HCl (pH 8.8) and the buffer for the stacking gel (4% acrylamide) was 0.19 M Tris-HCl (pH 6.8). The electrode buffer consisted of 0.025 M Tris, 0.19 M glycine, pH 8.3. Bio-Rad plates of 10.1 x 8.2 cm dimensions were used to make the 0.075 cm thick gels. Thawed supernatent samples of 15µl with 2-3 drops of 0.025% bromophenol blue were loaded into each well. Gels were run at 6 mA per gel for approximately 2 h. Gels were assayed for glutamate dehydrogenase (Gdh), phosphoglucomutase (Pgm), alcohol dehydrogenase (Adh), shikimate dehydrogenase (Skdh) and 6- phosphogluconate dehydrogenase (6-Pgd), according to the procedure of the Soltis et al. (1983). The 5 different enzymes were scored according to their banding patterns on the electrophoresed gels. Shannon-Weaver diversity index (H') (Shannon & Weaver 1949) was used to determine the diversity occurring at each zone of enzyme activity. Means of H' were estimated according to the method of Pielou (1974). Of the 5 enzyme systems studied, the genetics of only 3 is known. Hence, the diversity was calculated based on the enzyme phenotypes, rather than genotypes.

RESULTS AND DISCUSSION

A single zone of activity, designated as Gdh A, was observed for glutamate dehydrogenase in plants at all 4 ploidy levels. This zone was most variable in the diploid populations, which exhibited 3 phenotypes. Two phenotypes were identified for the tetraploids and octoploids and single monomorphic band was evident for the hexaploids. The observed banding patterns were the same for all the 4 treatments.

Two distinct zones of activity were exhibited for Pgm in all the populations. The slowly migrating cathodal zone (Pgm-A) was invariant over the 4 treatments and a single monomorphic band was observed in all diploids, hexaploids and octoploids. However, one tetraploid genotype exhibited 2 bands in this zome. The anodal zone (Pgm-B) showed some variation among the 4 temperature and light regimes, although diploid plants expressed a single band following each of the 4 treatments. Hexaploids generally showed 2 phenotypes at this zone, single bands which differed in their migration distances. For the hexaploids, the pattern remained constant over LDW, LDC and SDC conditiions, while an additional band appeared under SDW conditions. Octoploids showed 2 distinct singlebanded phenotypes under SEW and LDW conditions and a 2-banded phenotype under LDC and SDC. Tetraploids showed the maximum variation at this zone. Three phenotypes were identified for SDW and LDW plants. Two of these were single-banded and the third double-banded. Alcohol dehydrogenase exhibited a single zone of activity, designated Adh-A. All diploid genotypes under LDW and SDW exhibited a single band. Three of the six genotypes, under LDC showed 2 banded patterns, while one genotype in SDC condition expressed 2 bands at this zone. Tetraploid genotypes exhibited 2 Adh-A phenotypes, 1 single-banded and 1 double-banded phenotype under SDW, LDC and SDC. One tetraploid at LDW showed a double-banded pattern while a single band was observed under the other 3 conditions. Three phenotypes

TABLE 3: Estimates of diversity for enzyme phenotypes in 30 populations of four different ploidy levels of *Stellaria* and four environmental treatments combined

Enzyme phenotype	Ploidy level 2n=26	2n=52	2n=78	2n=104
Gdh-A	0.43	0.20	0.00	0.18
Pgm-A	0.00	0.20	0.00	0.00
Pgm-B	0.00	0.42	0.35	0.36
Adh-A	0.20	0.30	0.44	0.44
Skdh-A	0.00	0.00	0.21	0.15
Skdh-B	0.39	0.36	0.38	0.39
6-Pgd-B	0.36	0.42	0.47	0.41
Mean	0.21	0.28	0.27	0.29

TABLE 4: Proportion of enzyme phenotypes exhibiting differences under the four test conditions. Each number represents the proportion of total phenotypes that varied between two conditions for a particular level.

Condition	Ploidy	Growth condition SDW	SDC	LDW	LDC
SDW	2n=26	-	0.23	0.13	0.17
	2n=52	-	0.37	0.20	0.13
	2n=78	-	0.40	0.40	0.20
	2n=104	-	0.42	0.37	0.11
SDC	2n=26	-	-	0.23	0.30
	2n=52	-	-	0.33	0.23
	2n=78	-	-	0.43	0.28
	2n=104	-	-	0.34	0.34
LDW	2n=26	-	-	-	0.23
	2n=52	-	-	-	0.20
	2n=78	-	-	-	0.25
	2n=104	-	-	-	0.31
LDC	2n=26	-	-	-	-
	2n=52	-	-	-	-
	2n=78	-	-	-	-
	2n=104	-	-	-	-

TABLE 5: Average proportion of enzyme phenotypes of *Stellaria* species that exhibited differences under the four test conditions. Ploidy levels are averaged together (based on data in Table 4).

Growth condition	Condition SDW	SDC	LDW	LDC
SDW	-	0.36	0.29	0.16
SDC	-	-	0.34	0.31
LDW	-	-	-	0.25
LDC	-	-	-	-

were identified in the hexaploids, with single, double and triple bands. Hexaploids under LDW exhibited doublets while in other treatments, they showed single bands. The 3 phenotypes observed for the octaploid genotypes varied with the environment. Two octoploids exhibited single band in the SDW condition and double bands in the other 3. One octoploid showed a single band in LDS, and a double banded pattern under LDC and SDW and a triple band in SDC. Two zones of activity, namely, Skdh-A and Skdh-B were identified for shikimate dehydrogenase. The fast zone, (Skdh-A) varied in the hexaploids and octoploids. Only a single band was observed in diploids and tetraploids whereas, the hexaploids and octoploids showed both a single as well as a double-banded phenotype. Skdh-B was extremely variable in plants from all the 4 ploidy levels. A number of diploids lacked activity in this zone under LDW (2 of 6 populations) and SDC (5 of 6 populations) conditions, so did 2 populations of tetraploids under these conditions. Tetraploids possessed variable banding patterns, however, the loss or gain of bands was not consistent among them. Four of the 9 hexaploids also exhibited null Skdh-B activity under LDW and SDC conditions. Octoploids lacked activity for Skdh-B under SDC conditions as well. However, this zone was active for all plants in LDW. Two zones of activity were identified for 6-phosphogluconate dehydrogenase (6-Pgd-A and B). Five phenotypes were observed in diploids, 2 in tetraploids and octoploids and 3 in hexaploids for the slow migrating zone (6-Pgd A). The fast migrating zone (6-Pgd-B) exhibited 2 phenotypes for all ploidy levels. SEW and LDC treated plants of all ploidy levels were conserved at this zone. One diploid genotype was null under SDC, while at all other treatments it exhibited 2 phenotypes. Tetraploids lacked activity at 6Pgd-B in 4 populations under SDC and in one under LDW conditions. Three octoploid populations and all hexaploids under SDC lacked activity at 6-Pgd-B as did one hexaploid population at LDW.

Considerable variation, both within and among the 4 ploidy levels was observed among the 8 zones of activity : Gdh-A, Pgm-A, Pgm-B, Adh-A, Skdh-A, Skdh-B, 6-Pgd-A and 6-Pgd-B. Table 3 illustrates the variation within each ploidy level for 7 zones. Values of H^1 were calculated based on the frequency of occurence of each phenotype in a particular genotype. In general, diploid populations showed the least diversity, exhibiting fewer phenotypes as well as the lowest average degree of diversity (0.21) (Table3). Our observations are consistent with previous reports that diploids often contain fewer number of isozymes than related polyploids (Gottlieb 1981). The presence of additional isozymes in polyploid taxa has been attributed to gene increases resulting as a direct consequence of chromosome doubling or fertilization of unreduced gametes during polyploid evolution (Gottlieb 1981). The 3 polyploid levels of *S. longipes* showed similar degrees of variation, both in the average number of isozyme phenotypes and in the degree of diversity, which was also reported by Cai & Chinnappa (1991). Another aspect of interest is that for the same enzyme, certain zones were more diverse than others. Skdh-B, for instance, displayed relatively high H^1 values. Similarly, all other zones, except 6-Pgd-A showed high diversity in the environmental conditions. The high diversity values, reflected as different enzyme phenotypes, could be a consequence of the degree of change that occurred in plants in the different environments. At least for a few enzymes such as Skdh and 6-Pgd, gene duplication has been reported to be involved in the evolution of tetraploid *Stellaria* (Cai & Chinnappa 1989b). When genes are duplicated, the selectional constraints acting on different loci are independent and could be the reason why for an enzyme, one locus is more diverse than the other loci (Goodman 1984). As Roose & Gottlieb (1980) suggested, gene duplication might play a major role in the evolution of new metabolic capabilities. This is an area that needs further investigation.

The proportion of the enzyme phenotypes that varied under the different environments is presented (Tables 4 & 5), comparing 2 conditions at a time. Table 4 provides the comparisons at each ploidy level and Table 5 averages the 4 ploidy levels together. Interestingly, some variation occurred between all environments and within all the ploidy levels. The highest proportion of diversity occurred between SDW and SDC raised plants. This seems to imply that temperature has greater effect on the expressed phenotype than does day length. However, the least amount of variation was observed between SDW and LDC plants. These are again plants grown in the extreme temperatures and photoperiods. Hence, there appears to be a complex interaction between temperature and photoperiod determining enzyme phenotypic expression. The highest proportion of variable phenotypes all involve SDC raised plants (SDC vs. SDW, SDC vs. LDW and SDC vs. LDC). It is under these conditions that bands were often observed to be missing. Intuitively, this seems reasonable since SDC conditions would correspond to cold winter conditions in nature. Plants in these conditions are generally inactive and metabolically slower. Enzyme systems would seem to be less active in winter than in the active growing summer season. This corresponds with the resultls of Macdonald et al. (1984) in which long days with warm conditions caused maximum stem elongation in *Stellaria*. These workers found temperature to have the greater effect on stem elongation than photoperiod but together they have a synergistic effect.

It was one of the intents of the study to examine if particular genotypes produced different enzyme patterns in response to the various environmental conditions. It appears that certain enzymes are genetically predisposed to respond to environmental changes and would be more plastic in their response, although the exact extent of response is difficult to measure. Five of the 8 enzyme zones varied under the different environmental conditions. It was difficult, however, to draw any conclusion since the number of representative genotypes varied considerably (only two of the 30 populations were from tundra habitat whereas 11 were from montane). Throughout the study, every population investigated showed variation in at least one of the enzyme activity zones, regardless of ecotype. Studies investigating enzyme changes in response to environmental conditions so far, have involved only a few enzymes in a limited number of species (Simon 1979, Gaudereault & Tyson 1986). They have all reported some change occurring with varying conditions but the extent of this variation has not been estimated. A few studies (Schott & Brusven 1979, Savich & Tazhibaeva 1988, Nakanishi & Fujii 1992) have shown a change in the number of enzymes expressed between warm and cold acclimated organisms, and an absence of electrophoretic bands in the cold acclimated species.

Qualitative changes in enzymes might serve as an adaptive strategy for an organism (Hormaza & Herrero 1992, Nevo et al. 1993). Organisms that are plastic in their response, as *Stellaria*, occur naturally in a wide diversity of environments. Qualitative changes in enzymes might be more adaptive if variants of the enzyme have different temperature optima (Zhang & Li 1991, Kurokawa & Nakano 1991). Simon (1986), Simon et al. (1983, 1989) found this to be the case for mitochondrial isozymes of NAD-malate dehydrogenase in 3 species. Presumably, plants with a large temperature range would adapt themselves to climatic variation better than others. Additionally, it might be more efficient for an organism to produce several isozymes which function in different temperature regimes than to expend vast amounts of energy producing large quantities of a single enzyme (Schott & Brusven 1979). This would seem more reasonable for those plants naturally

occurring over diverse habitats than those occurring in a stable environment. Studies comparing distinct, stable, non-plastic species with those that are phenotypically variable and plastic would be of interest.

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EFFECT OF CHEMICAL MUTAGENS ON *AGERATUM CONYZOIDES* L.

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SUMMARY

Seeds of *Ageratum conyzoides* L. (4x) were treated with different concentrations of chemical mutagens such as Diethyl sulphate (DES), Ethyl methane sulphonate (EMS) and Hydrazine hydrate (HZ). This study indicated that lower concentrations of DES and HZ promoted vegetative growth and yield than control. Whereas, higher concentrations were found to be harmful to the plant. Results obtained from EMS treatment were not encouraging with respect to growth and yield. Meiotic abnormalities were observed in all the treated plants.

Key Words : *Ageratum conyzoides*, chemical mutagens, growth, cytological abnormalities.

INTRODUCTION

Although much work has been done during the recent years on radiosensitivity of seeds to ionizing radiations and chemical mutagens, there is no report of effect of chemical mutagens on *Ageratum conyzoides*. Germination, survival and seedling growth are widely used as an index in determining the biological effects of various mutagens (Reddy et al. 1993). The chemical mutagens have advantages of higher efficiency and relatively greater specificity of mutation than physical mutagens. Efficient treatments are essential for economical use of mutagens as tools for the induction of heritable changes in qualitative and quantitative characters of plants. Chromosomal aberrations induced by various chemical mutagens differ in their frequency and time depending on the genotype and the potency of the mutagenic agent. The objective of the present investigation was, therefore, to find out the comparative effect of chemical mutagens like diethyl suslphate (DES), ethyl methane sulphonate (EMS) and hydrazine hydrate (HZ) on the frequency and types of meiotic chromosomal aberrations and the differential responses of *A. conyzoides* (4x).

MATERIALS AND METHODS

Healthy seeds of *A. conyzoides* (4x) were pre-soaked in distilled water at room temperature for 12 h. The pre-soaked seeds were treated with various concentrations of DES, HZ (0.05 %, 0.1 %, 0.15 % and 0.2 %) and EMS (0.1%, 0.2%, 0.3% and 0.4%) prepared in 0.1 M phosphate buffer (pH 7.0) for 6 h at room temperature. During the treatment the solutions were intermittently stirred. After the treatment period the seeds were rinsed several times with distilled water. For each treatment, 100 seeds were taken. In case of control, seeds were treated with distilled water for the same duration. The seeds were sown in pots. Effect of these 3 mutagens was observed on seed germination, growth of plant and yield of seeds. Meiotic anomalies were also noted.

RESULTS AND DISCUSSION

Lower concentrations (0.05 % and 0.1 %) of DES and HZ showed early germination whereas, higher concentrations (0.15 % and 0.2 %) treated with EMS took maximum number of days (37 d) for germination. With lower concentrations of EMS, a stimulation effect on germination has been reported in the white variety of *Allium cepa*, while red variety showed a decline with increasing concentrations (Bhamburkar & Bhall 1980). Survival percentage of the seedlings was found to be maximum in 0.05% HZ as compared to the other mutagenic treatments. The lower germination and survival percentage in higher concentration of DES and EMS may be due to disturbances caused at the physiological level of cells and/or chromosomal damage (Sinha & Godward 1972).

The average height and number of branches of the plants raised from seeds treated with 0.1 % of DES and HZ were more than that of the control (Table 1). Higher concentrations of DES, HZ and EMS showed stunted growth and less number of branches. A stimulatory effect of lower doses

TABLE 1: Effect of DES, EMS and HZ treatments on seed germination, growth and yield of *A. conyzoides* (4x).

Doses of treatment	No. of days taken for germination	% of seed germination	% of seedling survival	Height of plant (cm.)	Number of branches per plant	Length of leaves (cm.)	Breadth of leaves (cm.)	Number of days taken for flowering	Number of capitula per plant	Wt. of seeds per plant (gm.)
Control	8	98.0	96.5	23.5 ±0.65	6.0 ±0.15	4.5 ±0.16	3.5 ±0.13	42 ±2.32	19.3 ±0.76	3.0 ±0.23
DES 0.05%	6	97.2	96.0	25.0 ±0.74	8 ±0.20	4.5 ±0.15	3.0 ±0.09	40 ±2.14	25.8 ±0.72	4.30 ±0.38
0.1%	7	96.1	92.0	32.0* ±0.89	10* ±0.22	4.6 ±0.16	2.4 ±0.06	42 ±2.38	32.1* ±0.63	5.56* ±0.45
0.15%	15	72.4	61.4	15.0* ±0.72	2 ±0.05	1.5* ±0.03	0.9* ±0.03	58* ±2.68	16.4 ±0.51	2.50 ±0.20
0.2%	28	54.5	36.3	8.3* ±0.38	1* ±0.07	1.2* ±0.02	0.8* ±0.04	63* ±2.81	8.2* ±0.33	1.42* ±0.12
EMS 0.1%	10	91.3	89.4	19.0 ±0.60	6 ±0.17	2.4 ±0.08	1.2 ±0.04	42 ±2.28	12.3 ±0.42	2.36 ±0.19
0.2%	18	78.0	63.7	17.4 ±0.52	4 ±0.14	2.0 ±0.04	1.2 ±0.04	53 ±2.52	8.5* ±0.31	1.40* ±0.12
0.3%	32	59.6	32.6	16.2 ±0.48	2 ±0.08	1.8 ±0.07	0.9* ±0.02	65* ±2.85	6.5* ±0.25	1.00* ±0.08
0.4%	37	33.8	12.8	6.5* ±0.25	1* ±0.07	1.3* ±0.02	0.9* ±0.02	68* ±2.93	3.7* ±0.18	0.45* ±0.03
HZ 0.05%	6	98.6	97.0	29.7 ±0.83	10* ±0.25	4.8 ±0.17	3.2 ±0.12	35* ±1.9	22.6 ±0.69	3.80 ±0.28
0.1%	7	96.5	93.5	42.5* ±0.82	12* ±0.28	6.0* ±0.25	4.2 ±0.18	38 ±2.1	39.1* ±0.98	6.10* ±0.54
0.15%	12	80.3	71.0	21.0 ±0.57	4 ±0.12	2.2 ±0.06	1.7* ±0.04	53 ±2.54	19.2 ±0.75	2.89 ±0.22
0.2%	20	76.4	53.8	15.0* ±0.73	2 ±0.09	1.5* ±0.04	0.8* ±0.02	61* ±2.79	16.4 ±0.53	2.45 ±0.20

*Significant at 5% level.

on seedling height has been reported by Shull & Mitchell (1953). This was suggested to be due to increased activity of auxins (Ehrenberg 1955). Reason for the stunted growth of plant at the higher doses of mutagen treatment may also be attributed to genetic loss due to chromosomal aberrations. It is quite likely that the inhibition of phytohormones responsible for the normal growth might have been affected at the pre-synthesis level (DNA-RNA level). Excessive branching might have occurred as a result of formation of more axillary buds. This may be due to the physiological changes such as disturbances in auxin formation and distribution. Increase in the average length and breadth of leaf was observed in plants obtained from seeds treated with 0.1% HZ (Table 1). The plants obtained from seed treatment showed variations in size and shape of the leaves. Several morphological deformities like, fusion of the opposite leaves, bifurcation of the leaves, curling of the leaves were observed. Tarar & Dnyansagar (1974) have observed similar effects in *Turnera ulmifolia*. Reduction in the size and various deformities of the leaves could be due to chromosomal aberrations and to the disturbances in phytohormones induced by the chemical mutagens. One plant obtained from the seeds treated with 0.2% EMS showed change in the phyllotaxy of the leaves. Johnson (1936) noticed altered phyllotaxy and deformed leaves in *Zinnia* after X-ray irradiation. Early flowering was noticed in the plants obtained from the seeds treated with 0.05 % and 0.1% HZ than control. Plants treated with 4% EMS took maximum number of days (68 d) for flowering. According to Jha & Sinha (1977), EMS provides more genic changes and produces greater inhibition in vegetative and reproductive growth and highest frequencies of lethality.

Meiosis in the control plants was normal, but the plants obtained from the seeds treated with the different chemical mutagens showed various meiotic anomalies (Table 2). At diakinesis and metaphase I, besides bivalents, there were formations of univalents, multivalents, association of variable number of chromosomes in groups at various concentration of mutagen. Laggards were also observed at metaphase I in all the treatments. Clumping of chromosomes has been reported

TABLE 2: Frequencies of meiotic abnormalities at various doses of treatments with DES, EMS and HZ on *A. conyzoides* [4x]

Doses of treatment	Percentage of PMCs with abnormalities at									
	Metaphase I		Anaphase I			Metaphase II		Anaphase II		
	Clumping of chromosomes	laggards	laggards	bridges	Irregular distribution	Clumping of chromosomes	laggards	laggards	bridges	Irregular distribution
Control	-	-	-	-	-	-	-	-	-	-
DES 0.05%	3.1	2.8	1.6	2.4	0.5	2.1	-	1.1	-	-
0.1%	4.2	3.2	2.8	3.1	1.4	2.7	1.2	1.0	0.8	-
0.15%	9.3	8.4	7.2	6.7	2.6	2.0	1.8	2.0	-	0.4
0.2%	10.5	13.6	10.8	9.5	3.9	5.8	2.5	0.6	1.8	0.8
EMS 0.1%	4.9	5.2	3.3	1.8	2.1	5.2	2.4	1.0	1.4	1.2
0.2%	6.4	7.0	4.2	3.6	3.0	7.0	3.8	2.8	2.2	-
0.3%	7.0	9.3	5.8	6.0	3.7	6.8	3.0	2.5	2.0	1.8
0.4%	12.6	15.5	6.3	7.2	5.3	8.0	4.2	2.4	2.2	2.0
HZ 0.05%	3.9	3.8	3.2	3.9	1.2	5.0	0.5	2.0	1.2	-
0.1%	6.8	7.2	4.8	5.3	2.0	6.7	0.9	2.3	2.0	-
0.15%	7.4	9.1	5.7	6.1	3.4	7.5	1.5	3.0	2.8	0.6
0.2%	9.7	12.8	8.0	7.5	4.8	7.0	2.2	3.6	2.8	1.5

following treatments using radiations, chemical mutagens, pesticides and a variety of other agents (Abraham & Cherian 1978). Precocious movement and unoriented chromosomes at metaphase were observed. It could be interpreted as arising from disturbance in the formation of the spindle. At anaphase I, various anomalies like laggards, unequal separation of chromosomes, formation of bridges, micronuclei were observed in all the treatments of DES, EMS and HZ, in varying frequencies. The above abnormalities were less frequent in the second meiotic division. Similar re- sults were also reported by Bose & Saha (1970) in tomato with DES treatment. The bridges with- out fragments seem to have been formed from failure of terminalization in bivalent and the chro- mosomes having stretched between the poles or stickiness of the chromosome ends (Sudhakaran 1971).

In the present study, reduction of seed setting was observed in plants obtained from treated seeds. Ramulu (1970) in *Sorghum*; Fowler & Stefansson (1972) in *Brassica* noted reduction in yield of seeds due to the effect of EMS. It is likely that incidence of chromosomal aberrations may be the major factor responsible for low seed setting. It has been observed that EMS causes high pollen sterility. Similarly, these mutagens affect the normal course of megasporogenesis leading to the sterility of the female gametophyte. Low seed setting in the present plants may be due to the sterility of the male or female gemetophyte or both.

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KARYOMORPHOLOGICAL STUDIES IN *HIPPEASTRUM*

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SUMMARY

Karyomorphology of 13 taxa (6 species, 5 hybrids and 2 varieties) of the genus *Hippeastrum* has been studied. In addition to the predominant basic number of x = 11, variants such as x = 10 and 12 also occur. The different taxa also exhibited high incidence of intraspecific karyotypic variations. Cytological evidence is in favour of placing *Hippeastrum* and *Crinum* in the same tribe Hippeastreae.

Key Words : Amaryllidaceae, *Hippeastrum*, karyomorphology.

INTRODUCTION

Hippeastrum belongs to the Tribe Hippeastreae of the family Amaryllidaceae (*Sensu* Hutchinson 1973). This genus often referred to as "*Amaryllis*" to which they are closely related but differ in having hollow stalk and presence of scales between the filaments in the flower. It has about 75 species which are mostly tropical and subtropical in distribution. They are grown as garden ornamentals for their unassuming flowers ranging from velvet-red to white. Members of this genus were subjected to cytological investigations by many authors like Inariyama (1937), Sato (1942), Mookerjea (1955), Naranjo & Andrada (1975), Narain & Khoshoo (1968, 1977), Guha (1979), Khaleel et al. (1991). However, the quest for size and colour of flower has led to indiscriminate use of hybrids and wild species in breeding, and the evolution of cultivars warrants their screening both cytologically and karyomorphologically. This has promopted the present authors to take up this study. The present study deals with detailed karyomorpohology of 6 species, 5 hybrids and 2 varieties of the genus *Hippeastrum*.

MATERIALS AND METHODS

The materials for the present investigation were collected from different localities of South India. For somatic chromosome studies, young root tips were fixed in acetic-alcohol (1 :3) after a pretreatment with 0.002M 8-hydroxyquinoline at 4°C for 3 to 4 h. The chromosomes were stained in 2% acetocarmine. The karyomorphological analysis was carried out following systems proposed by Stebbins (1958), Levan et al. (1964) and Huziwara (1962).

OBSERVATIONS

Hippeastrum vittatum Herb.

Root tip cells revealed 24 chromosomes (Fig. 4) which ranged in length from 12.5 µm to 5 µm. The ACL was 8.45 µm, while TCL was 205.0 µm and TF% was 29.5. The karyotype (Fig. 10 h) belongs to the category 2B and consists of 2M-, 11m-, 3sm- and 8t-types of chromosomes. The chromosome '3' exhibited heteromorphism with its homologue in the position of the centromere. The karyotype was fairly symmetrical.

***Hippeastrum vittatum* (Hybrid)**

Root tip cells at metaphase showed 22 chromosomes (Fig. 9) which ranged in length from 27.0 µm to 12.0 µm. The ACL was 19.0 µm, TCL was 418.00 µm and TF% was 26.07. The

Figs. 1-9: (All Photomicrographs) Somatic chromosomes of *Hippeastrum*. 1. *H. (Leopoldii Hybrid)* 'Claret'. 2. *H. (Leopoldii Hybrid)* 'Giant white' (No. 2). 3. *H. (Leopoldii Hybrid)* 'Giant white' (No. 1). 4. *H. vittatum*. 5. *H. reciculatum* var. *striatifolium* (Hybrid). 6. *H. reginae*. 7. *H. reticulatum* (Pink flowered). 8. *H. reticulatum* var. *striatifolium* (No. 1). 9. *H. vittatum* (Hybrid). Figs. 1-4, 7-9 (x 915); 5 & 6 (x 685).

karyotype (Fig. 10 i) belongs to the category 3B and it consists of 4 pairs of m-, 2 pairs of sm-, 4 pairs of st- and 1 pair of t-types of chromosomes.

***Hippeastrum reticulatum* Herb. (Pink flowered)**

Root tip cells showed 20 chromosomes (Fig. 7) which ranged in length from 14.5 µm to 5.0 µm. The ACL was 8.9 µm, TCL was 178.0 µm and TF% was 30.89. The karyotype (Fig. 10 e) was fairly symmetrical 3B and consists of 6 M-, 2 m-, 6 sm- and 6 st-types of chromosomes. The chromosomes 1 and 5 showed size heteromorphism with their respective homologues.

***Hippeastrum reticulatum* Herb. (Red flowered)**

Root tip cells showed 22 chromosomes which ranged in length from 10.0 µm to 3.50 µm. The ACL was 6.57 µm, TCL was 144.50 µm and TF% was 28.37. The karyotype (Fig. 10 m) belongs to the category 3B and consists of 4 pairs of m-, 5 pairs of sm- and 2 pairs of st-types of chromosomes. The largest pair of chromosomes showed heteromorphism with regard to their length and arm ratio.

***Hippeastrum reticulatum* Herb. (Orange-flowered)**

Root tip cells showed 22 chromosomes which ranged in length from 9.50 µm to 3.50 µm. The ACL was 6.14 µm, TCL was 135.0 µm and TF% was 29.62. The karyotype (Fig. 10 l) belongs to the category 3B and consists of 4 pairs of m-, 3 pairs of sm-, 2 pairs of st- and 1 pair of t-types of chromosomes.

***Hippeastrum reticulatum* Herb. (White-flowered)**

Root tip cells showed 22 chromosomes which ranged in length from 25.50 µm to 7.50 µm. The ACL was 13.14 µm and TCL was 289.0 µm and TF% was 35.64. The karyotype (Fig. 10 g) belongs to the category 3B and consists of 2 pairs of M-, 3 pairs of m-, 3 pairs of sm-, 2 pairs of st- and 1 pair of t-types of chromosomes.

***Hippeastrum reticulatum* var. *striatifolium* Herb.**

Population 1

Root tip cells showed 22 chromosomes (Fig. 8) which ranged in length from 12.0 µm to 4.0 µm. The ACL was 7.95 µm, TCL was 175.0 µm and TF% was 27.42. The karyotype (Fig. 10 j) belongs to the category 3B and consists of 1 pair of M-, 1 pair of m-, 4 pairs of sm- and 5 pairs of st-types of chromosomes.

Population 2

Root tip cells showed 22 chromosomes which ranged in length from 7.50 µm to 2.50 µm. The ACL was 4.73 µm, TCL was 104.0 µm and TF% was 27.40. The karyotype (Fig. 10 f) belongs to the category 3B and consists of 3 pairs of m-, 5 pairs of sm- and 3 pairs of st-types of chromosomes.

***Hippeastrum reticulatum* var. *striatifolium* (Hybrid)**

Root tip cells showed 22 chromosomes (Fig. 5) which ranged in length from 14.5 µm and 5.0 µm. The ACL was 8.27 µm, TCL was 182.06 µm and TF% was 28.7. The karyotype (Fig. 10 k)

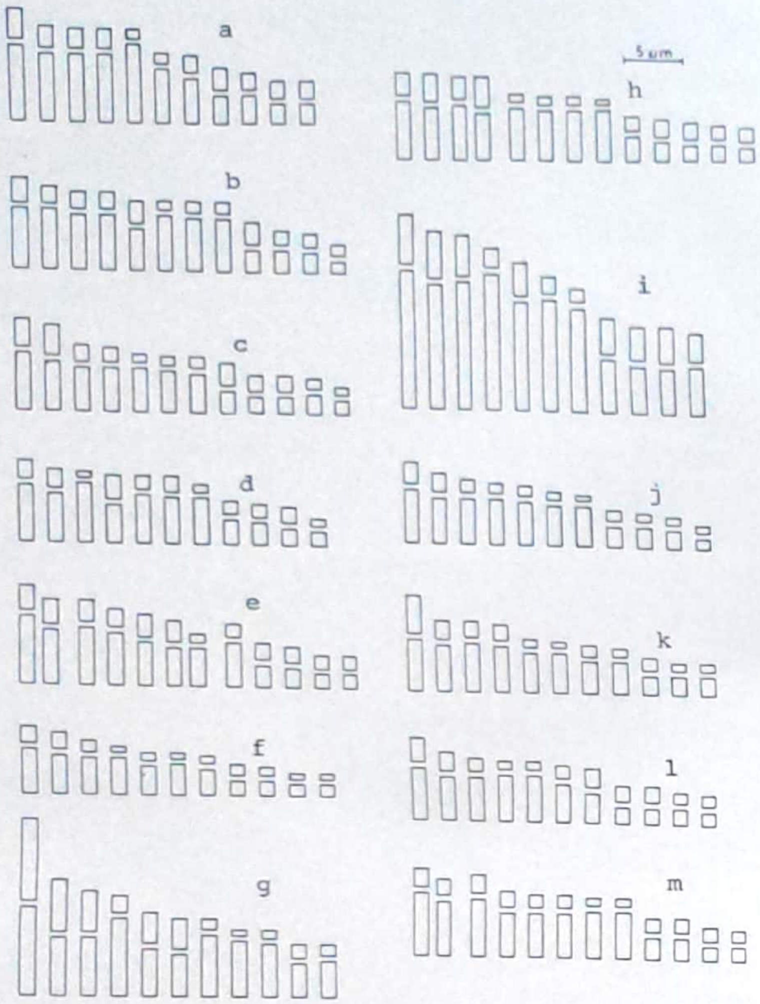

Fig. 10 a-m: Idiograms of *Hippeastrum*. a. *H. reginae*. b. *H. (Leopoldii Hybrid)* 'Giant white' (No. 1). c. *H. (Leopoldii Hybrid)* 'Giant white' (No. 2). d. *H. (Leopoldii Hybrid)* 'Claret'. e. *H. reticulatum* (Pink flowered). f. *H. reticulatum* var. *striatifolium* (No. 2). g. *H. reticulatum* (White flowered). h. *H. vittatum*. i. *H. vittatum* (Hybrid). j. *H. reticulatum* var. *striatifolium* (Hybrid). k. *H. reticulatum* var. *striatifolium* (Hybrid). l. *H. reticulatum* (Orange flowered). m. *H. reticulatum* (Red flowered).

belongs to the category 3B and consists of 4 pairs of m-, 4 pairs of sm- and 3 pairs of st-types of chromosomes.

***Hippeastrum reginae* Herb.**

Root tip cells showed 22 chromosomes (Fig. 6) which ranged in length from 16.0 μm to 6.0 μm. The ACL was 10.5 μm, TCL was 231.0 μm and TF% was 27.65. The karyotype (Fig. 10 a) belongs to the category 3B and consists of 1 pair of M-, 3 pairs of m-, 3 pairs of sm-, 3 pairs of st- and 1 pair of t-types of chromosomes.

***Hippeastrum (Leopoldii Hybrid)* 'Giant white' Dombr.**

Population 1

Root tip cells showed 24 chromosomes (Fig. 3) which ranged in length from 13.0 μm to 4.0 μm. The ACL was 9.10 μm, TCL was 220.0 μm and TF% was 26.36. The karyotype (Fig. 10 b) belongs to the category 3B, and consists of 2 pairs of M-, 2 pairs of m-, 3 pairs of sm- and 5 pairs of st-types of chromosomes.

Population 2

Root tip cells showed 22 chromosomes (Fig.2) which ranged in length from 13.0 μm to 4.0 μm. The ACL was 8.13 μm, TCL was 179.0 μm and TF% was 26.25. The karyotype (Fig. 10 c) belongs to the category 3B and consists of 2 M-, 5 m-, 8 sm-, 5 st- and 2 t-types of chromosomes.

***Hippeastrum (Leopoldii Hybrid)* 'Claret' Dombr.**

Root tip cells showed 22 chromosomes (Fig. 1) which ranged in length from 12.5 μm to 4.5 μm. The ACL was 8.8 μm, TCL was 194.0 μm and TF% was 29.38. The karyotype (Fig. 10 d) belongs to the category 3B, and consists of 1 pair of M-, 4 pairs of m-, 2 pairs of sm- and 4 pairs of st-types of chromosomes.

DISCUSSION

The present study included 13 taxa, all diploids, with 10 taxa (4 species, 2 varieties and 4 hybrid) showing 2n = 22 chromosomes based on x = 11, 2 taxa (1 species and 1 hybrid) with 2n = 24 chromosomes apparently based on x = 12 and 1 species showing 2n = 20 chromosomes probably based on x = 10. Chromosome data on the genus reported here infer the possibility of the genus harbouring multibasic constitution of x =10, x = 11 and x = 12, of which x = 11 is the most frequent. Variants such as 2n = 24 and 2n = 20 have been observed in this genus and it has been suggested that x = 11 could be the earlier evolved basic number from which 2n = 20 and 2n = 24 have evolved by descending and ascending aneuploidy. Available chromosome data show that a polyploid series exists based on x = 11 in the genus at various levels (2x, 4x, 6x and 7x).

Narain & Khoshoo (1968) identified a basic karyotype to be composed of 2 median, 5 submedian and 4 sub-terminal chromosomes, while Naranjo & Andrada (1975) suggested a basic karyotype of 4 metacentric (medium) 2 sub-metacentric (submedian) and 3 acrocentric (sub-terminal) chromosomes in the genus *Hippeastrum*.

All the taxa presently investigated showed variations not only from the above suggested basic karyotypes but differ from one another in the proportion of different chromosome types. They showed high incidence of intrakaryotypic variations with members of the homologues showing heteromorphism in the orientation of centromere or the length of the arms. In *H. vittatum* chromosome '3' is heteromorphic, chromosomes '1' and '5' are heteromorphic in *H. reticulatum* while in *H. leopoldii* (population 2) chromosome '7' showed heteromorphism. *H. vittatum* (hybrid) has the highest chromatin content (TCL : 418.00 µm and ACL : 19.0 µm) as against the lowest in *H. reticulatum* var. *striatifolium* (TCL : 104.00 µm and ACL : 4.73 µm). In the finer details of the individual chromosomes too, they show recognisable degree of differences.

On the whole, the chromosome morphology of the genus *Hippeastrum* is conspicuous with their large-sized chromosomes and clear centromere position. Structural alterations of chromosomes are evident with respect to arm ratio and variation within the karyotype. Also differences in chromosome size, ACL, TCL and karyotype formulae are obvious. Stebbins (1950) considered such intrakaryotypic variability as a well established and important evolutionary mechanism which is often an initial step to speciation. The karyomorphological differences observed among different taxa presently studied apparently evince the cytological basis for their apparently altered plant morphology as well as provide an insight into the probable pattern of intrakaryotypic variation within the species, while their gross karyomorphological similarity suggests their close relationship. The apparently high incidence of karyomorphological variation among the different taxa (present work) in their finer aspects is suggestive of significant role played by structural alterations of chromosomes in the evolution of the species complex.

Bentham & Hooker (1883) placed *Hippeastrum, Haemanthus* and *Zephyranthes* together with *Crinum* in the subtribe Genuinaeae of the tribe Amarylleae. Engler & Prantl (1930) treated them in their subfamily Amarylloideae. Hutchinson (1973) and Traub (1963) treated them under 4 separate tribes (Hippeastreae, Haemantheae, Zephyrantheae and Crineae). Dahlgren et al. (1985) have kept *Crinum* in the tribe Amaryllideae, *Hippeastrum* and *Zephyranthes* together in Hippeastreae and Haemanthus in the Haemantheae. Morphologically these four genera differ from one another. *Hipeastrum* and *Crinum* show similarity in floral structures, and both of them are based on x = 11. Hence, their inclusion together in the same tribe as done by Bentham & Hooker (1883) and Engler and Prantl (1930) appears justifiable.

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CYTOLOGICAL STUDIES IN *TABERNAEMONTANA DIVARICATA*

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SUMMARY

Cytological studies on 4 varieties of *Tabernaemontana divaricata* (L.) R. Br. ex R. & S. have been carried out. The varieties 1, 2 and 3 are diploids (2n = 22) and var. 4 is an autotriploid (2n = 33). The chromosomes are relatively small and all the 4 varieties belonged to 2A karyotype category. An autotriploid variety with 14-15 corolla lobes is being reported for the first time from South India. The presence of a high frequency of univalents during meiotic division in all these varieties resulted in a high percentage of pollen sterility (98%). Factors responsible for the formation of univalents and sterility have been suggested.

Key Words : *Tabernaemontana divaricata*, meiosis, karyotype.

INTRODUCTION

Tabernaemontana divaricata (Apocynaceae) is an evergreen hedge ornamental, distributed throughout India (Bailey 1949, Duthie 1960). The different varieties of this species are sterile and are being propagated through vegetative means. Collections from various localities of South India showed morphologically different taxa. Raghuvanshi & Chauhan (1969) reported highly anomalous meiosis in 4 varieties of *T. divaricata* from North India. They attributed the meiotic irregularities in these varieties to a high temperature. During the course of cytological investigations on South Indian Apocynaceae, the present authors studied karyomorphology and meiosis in 4 varieties of *T. divaricata* in detail and the results are reported here.

MATERIALS AND METHODS

For meiotic studies, young buds were fixed in a mixture of ethyl alcohol, acetic acid and chloroform (3 : 1 : 1) at 9 a.m. For somatic chromosomes, healthy roots raised from stem cuttings were fixed in ethyl alcohol-acetic acid (3 : 1) mixture. The root tips were pretreated with 0.002 M 8-hydroxyquinoline for 3 h at 8°C. Aceto-orcein (2%) stain was used for cytological studies. Pollen sterility estimation was done by keeping pollen grains in acetocarmine-glycerine (1 : 1) mixture for 30 min. The well stained and completely filled grains were considered as fertile and less stained and shrivelled grains as sterile.

OBSERVATIONS

During the present investigation, karyomorphological and meiotic studies on 4 distinct varieties of *Tabernaemontana divaricata* have been carried out.

Variety 1

Leaves are small ranging in length from 11-12 cm and 3.5-4.5 cm in breadth and are glossy. Flowers, small, white with 5 petals, single-whorled with pointed lobes (Fig. 1).

The root tip cells showed 22 chromosomes at metaphase (Figs. 7, 16). The length of the chromosomes varied from 2.75-3.75 µm. All chromosomes were sm-type except the eighth pair which is m-type. The F% ranged from 26.66-39.02. The TF% was 34.53. The karyotype category was 2A.

The pollen mother cells (PMCs) showed varying number of univalents (3-5 in each PMC) and bivalents at metaphase I. Very rarely, a ring of 4 chromosomes and 9 bivalents in each PMC were noticed at metaphase I (Fig. 5). During anaphase I and II, lagging chromosomes were noticed in appreciable frequency (80-90%) (Fig. 6) and hence, an almost complete pollen sterility. The pollen sterility was estimated to be 98.26%.

Variety 2

The leaves are small, about 13 cm long and 4 cm broad. Flowers, white, larger and possess round corolla lobes (Fig. 2).

Root tip cells showed 22 chromosomes at metaphase (Figs. 10,17). The length of the chromosomes ranged from 2.75-4.25 µm. There were one m- and 10 sm- type chromosomes in the haploid complement. The F% varied from 30.76-57.14. The karyotype belonged to 2A category.

The PMCs showed irregular meiotic divisions due to the occurrence of a high frequency of univalents (3-5 in each PMC) at diakinesis and metaphase I (Figs. 8, 9). Pollen sterility was estimated to be about 98%.

Variety 3

The plants are more stouter and leaves are larger than the above 2 varieties. The leaf size ranged in length from 14-14.5 cm and 5-5.5 cm in breadth. Flowers are white. Petals are arranged in 2 whorls each with 5 lobes (Fig.3).

Mitotic studies from root tip cells showed 22 chromosomes at metaphase (Figs. 12,18). The length of the chromosome ranged from 2.75-4.75 µm. All chromosomes are of sm- type except the eighth pair which is of m- type. The F% varied from 26.66-39.02. The TF% was 34.39. The karyotype category was 2A.

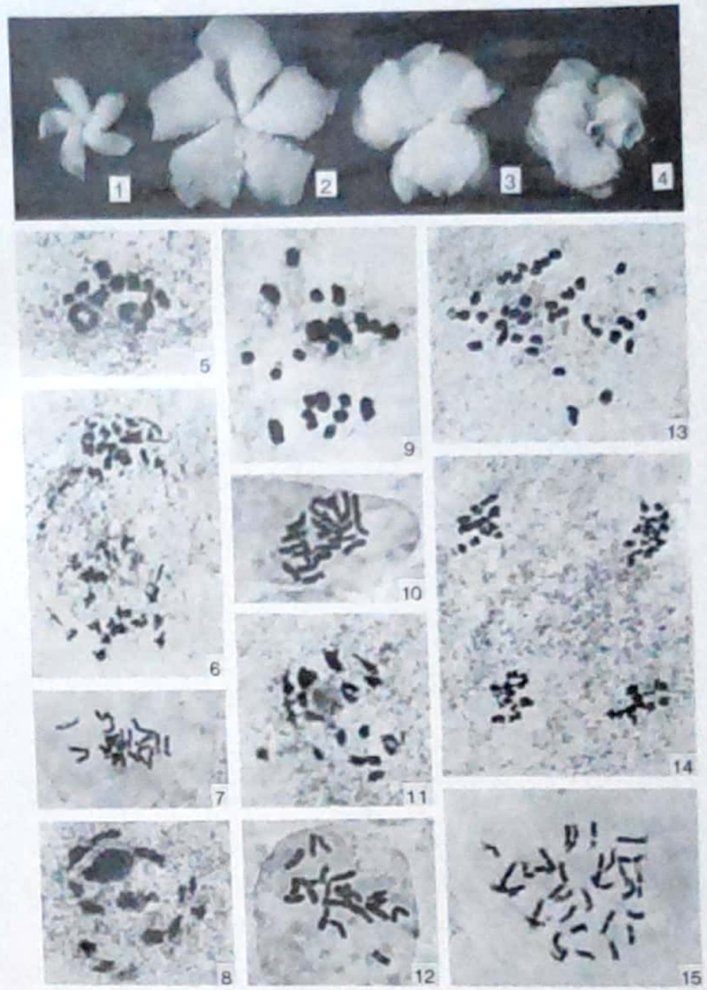
The PMCs showed varying number of univalents (5-10 in each PMC) and bivalents at diakinesis (Fig. 11). The pollen grains were almost completely sterile (98%).

Variety 4

In general appearance, this variety is almost similar to var.3 except in size of the leaves and number of petals. The leaf size ranged in length from 17-17.5 cm and 6-6.5 cm in breadth. Flowers, white. Petals, more than 10 (14-15) and arranged concentrically (Fig. 4).

The root tip cell showed the presence of 33 chromosomes at metaphase (Figs. 15,19). The length of the chromosome ranged from 2.75-3.75 µm. There are 3 m-type and 30 sm-type chromosomes in the somatic complement. The F% ranged from 26.6-39.02. The TF% was 34.39. The karyotype belonged to 2A category.

Meiotic studies showed varying number of univalents (25-29 in each PMC) and bivalents (1-2 in each PMC) at metaphase I (Fig. 13). Irregular separation of univalents resulted in unequal segregation of chromosomes at anaphase II (Fig. 14). The pollen sterility was 98.87%.



Figs. 1-15: 1-4. Flowers of *Tabernaemontana divaricata*. 1. variety 1, 2. variety 2, 3. variety 3, 4. variety 4. 5-15, Cytology of *T. divaricata*. 5-7. var. 1. 5. PMCs showing a ring of 4 chromosomes and 9 bivalents at metaphase I. 6. PMCs showing lagging chromosomes at anaphase I. 7. Somatic chromosomes (2n = 22). 8-10. var. 2. 8. PMCs showing univalents and bivalents at diakinesis. 9. PMCs showing univalents at metaphase I. 10. Somatic chromosomes (2n = 22). 11 & 12. var. 3. 11. PMCs showing univalents and bivalents at diakinesis. 12. Somatic chromosomes (2n = 22). 13-15. var. 4. 13. PMCs showing univalents and bivalents at metaphase I. 14. PMCs showing 4 groups of chromosomes of unequal numbers at anaphase II. 15. Somatic chromosomes (2n = 33) (x 745).

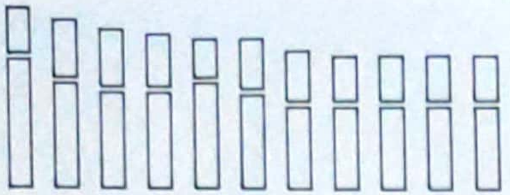


FIG.16

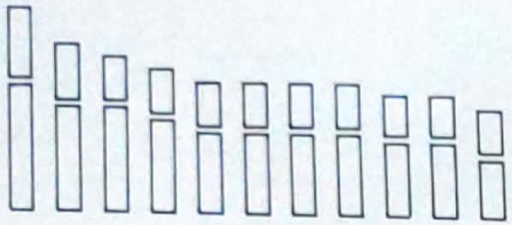


FIG.17

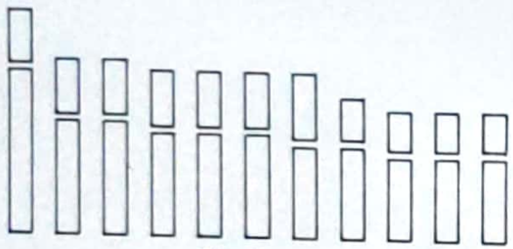


FIG.18

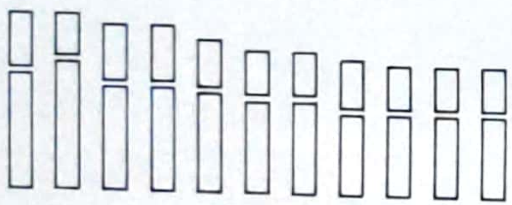


FIG.19

Figs. 16-19: Idiograms of *T. divaricata*. 16. var. 1, 17. var. 2, 18. var. 3, 19. var. 4 (one set out of 3 in the somatic complement).

DISCUSSION

Out of 4 varieties of *T. divaricata* investigated, the varieties 1, 2 and 3 are diploids ($2n = 22$) and var.4 is a triploid ($2n = 33$) on the basic chromosome number of $x = 11$. The karyomorphological evidences indicate that the genome is represented thrice in the variety 4, and, hence, it is an autotriploid. The haploid complements show sm- and m-type chromosomes in all the varieties. All the varieties belong to the same karyotype category (2A). This is indicative of the relatively symmetrical nature of the karyotype. However, on critical analysis it is noticed that the chromosomes of these varieties differ in their arm ratios. The difference noticed in the arm ratio, ACL and TCL among these varieties might have resulted from chromosomal structural changes. In the light of chromosome data so far available in the varieties of the species, and of the karyomorphological information of 4 varieties studied here, it appears that both numerical and gross chromosomal structural alterations have played some role in the origin of different varieties in this species.

During cytological studies in 4 varieties of *T. divaricata*, it is seen that meiosis is highly abnormal. The occurrence of a high frequency of univalents at diakinesis and metaphase I, irregular anaphase separation of univalents resulted in complete pollen sterility in all varieties investigated here.

While studying the chromosomal basis of evolution in Apocynaceae, Raghuvanshi & Chauhan (1969) studied the cytology of 4 varieties of *T. divaricata* from North India. According to them var. 1 (single-flowered, pointed corolla lobes) and var. 2 (single flowered, rounded corolla lobes) are diploids, var. 3 (single large flowered, petals 14-15) and var. 4 (double flowered, petal in two whorled each with five corolla lobes) are triploids.

In contrast to their findings, the present study indicates that var. 3 (double whorled with ten petals) is diploid like that of var. 1 and 2, and the somatic chromosome number of $2n = 22$ is reported here for the first time in this variety. The triploid condition is seen only in the var. 4 and is being reported for the first time in this variety from South India.

Irregular meiotic behaviour including the univalent formation have been reported in a large number of plants and the possible reasons have been advocated by several workers. Specific genes are known to control chromosome pairing in *Pisum* (Gottschalk & Baquar 1971). Smith (1966) and Riley (1966) suggested that chiasma formation and the whole process of meiosis is under genetic control. While studying the cytogenetics of different species of *Phaseolus*, Sarbhoy (1977) reported high frequency of univalents in the PMCs. He suggested that univalent formation is due to either asynapsis, desynapsis or precocious separation of chromosome of some of the bivalents.

Nakahara & Komoto (1957) have shown that high temperature has an effect on the occurrence of univalents. At high temperature the terminalization of chiasma occurs at a faster rate than at low temperature. Celarier (1955) and Ross et al. (1960) have suggested that reduced chiasma frequency may be resulted in desynapsis.

It may be noted that there is no such temperature effect on the present materials, since they were subjected to detailed cytological observation throughout the year. It is suggested that cryptic structural changes or the effect of gene or gene system could have resulted in high frequency of univalent formation and complete pollen sterility.

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KARYOLOGY OF FIVE SPECIES OF WEEVILS (CURCULIONIDAE: COLEOPTERA)

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SUMMARY

Five species of short-snouted weevils were karyologically analysed: *Myllocerus blandus* Faust, *M. viridanus* Fabricius, *Tanymecus feae* var. *plumens* Faust, *Hypomeces squamosus* Fabricius and *Longulus agrestis* Faust. All the species possess 2n=22 and meioformula $10+Xy_p$ except *M. blandus* which has 2n=23 ($10+Xyy_p$). The autosomes are meta-, submeta- and acrocentric. The X is invariably metacentric whereas y is the smallest dot shaped chromosome. Total chromosome length varies from 38.89μm to 52.68μm. A high range in chiasma frequency, from 12-29 per nucleus was recorded during metaphase I.

Key Words : Curculionidae, karyotype, meioformula, chiasma frequency.

INTRODUCTION

Family Curculionidae is one of the large families of Coleoptera having more than 6000 taxonomically described species (Richards & Davies 1979) including 1700 species from Indian fauna (Sharma & Pajni 1981). Cytologically, about 550 species are known. Of them, 153 are native species (Singh 1993). On the basis of the structure of mouth organs and the length of the rostrum, Lacordaire (1863) divided Curculionidae into 2 major groups, Adelognathi and Phanerognathi. All short snouted weevils belong to sub-families Otiorrhynchinae, Brachyderinae, Eremninae and Sitoninae. They are quite stable subfamilies as majority of the species possess 2n=22 and meioformula $10+Xy_p$. Thus they show deviation from the Polyphagan modal diploid number of 20 chromosomes. Karyological investigations on 5 species of short-snouted weevils are described in this paper.

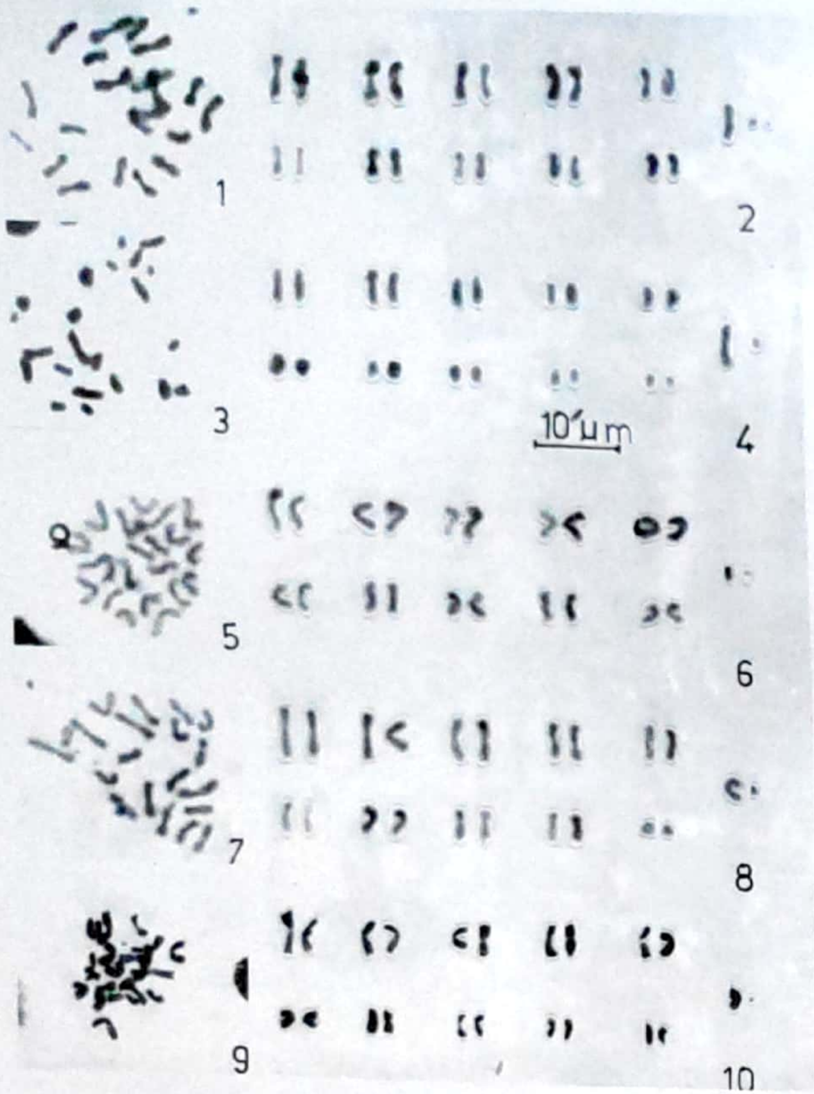
MATERIALS AND METHODS

The adult males of five species of weevils viz. *Myllocerus blandus* Faust, *M. viridanus* Fabricius (s.f. Otiorrhynchinae), *Tanymecus feae* var. *plumens* Faust, *Hypomecus squamosus* Fabricius (s.f. Brachyderinae) and *Longulus agrestis* Faust (s.f. Eremninae) constituted the materials. The weevils were collected from wild bushes and grass from the environs of Pant Nagar, Ram Nagar (Uttar Pradesh) and Kurukshetra. Karyological preparations were made by air-drying technique (Yadav & Lyapunova 1983).

RESULTS AND DISCUSSION

Myllocerus blandus

Spermatogonial metaphase revealed a diploid complement of 23 chromosomes (Fig. 1). The karyotype is constituted by 7 pairs of metacentric (pairs 1-3, 5-8), 3 pairs of sub-metacentric



Figs. 1-10: Spermatogonial metaphases and karyotypes. 1, 2. *Myllocerus blandus*. 3, 4. *M. viridanus*. 5, 6. *Tanymecus feae* var. *plumes*. 7, 8. *Hypomeces squamosus*. 9, 10. *Longulus agrestis*.

(pairs 4, 9 and 10) autosomes, a metacentric X and 2 dot-shaped y chromosomes (Fig. 2). All the chromosomes show a gradual decrease in size. Total chromosome length (TCL) is 50.07 µm, the X and y chromosomes measure 5.90 µm and 0.95 µm respectively. The X occupies fourth position in order of size.

Earlier Sobti & Singla (1986) reported the karyotype of this species from Chandigarh. The karyotype comprised 6 pairs of metacentric and 4 pairs of submetacentric autosomes. The X chromosome was the largest element in the karyotype while the y chromosome was dot-shaped.

Metaphase-I revealed 10 dumb-bell shaped autosomal bivalents and the sex chromosomes formed typical sex parachute represented as Xyy_p (Fig. 11). Chiasma frequency at metaphase I was 29 per nucleus. Meioformula is $10AA+Xyy_p$. Two types of metaphase-II cells were encountered, one with X chromosome (Fig. 11a) and other with two y chromosomes (Fig. 11b), in addition to 10 autosomes. Sobti & Singla (1986) encountered only one y chromosome during metaphase I and II.

Myllocerus viridanus

The spermatogonial metaphase exhibited 22 chromosomes (Fig. 3). It is in agreement with earlier reports (Dasgupta & Basile 1966, Gill *et al.* 1990). There are 6 pairs of metacentric and 4 pairs of submetacentric autosomes. Due to over condensation the centromeric positions were not clear at this stage. The X is the largest element of the karyotype and metacentric in nature (Fig. 4). The y is a dot-shaped chromosome. TCL is 38.89 µm, the X and y measure 5.16 µm and 1.76 µm respectively.

Metaphase I consisted of 10 autosomal bivalents and a sex parachute, Xy_p (Fig. 12). Chiasma frequency at this stage is 12 per nucleus. Meioformula is $10AA+Xy_p$. Metaphase II cells with X and with y chromosomes, in addition to 10 autosomes, were observed (Fig. 12a).

The diploid chromosome numbers of 27 species of *Myllocerus* are known (Singh 1993). These include 18 species from India. They all possess 2n=22 except *M. blandus* (2n=23) which carried an extra y chromosome in the present study. Two parthenogenetic species from Japan, *M. nipponicus* and *M. fumosus* possessed 2n=33 (Takenouchi 1972a,b).

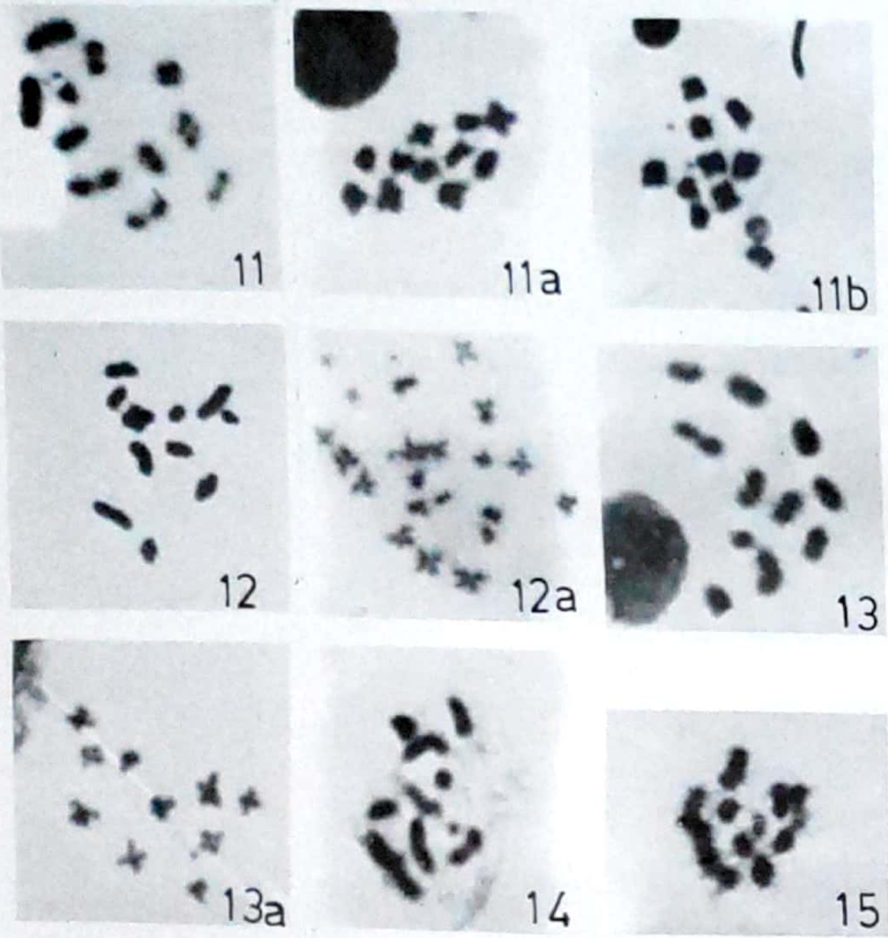
In Otiorrhynchinae 2n varies from 20 in *Hypermias* sp. (Sharma *et al.* 1980) to 44 in *Chrysocomus* sp. (Smith & Virkki 1978). However, majority of species, 71 out of 75, possess 2n=22.

Tanymecus feae var. *plumens*

The diploid chromosome number is 22 in spermatogonial metaphase (Fig. 5). The karyotype comprises 6 pairs of metacentric (pairs 2,4-7, 9), 4 pairs of submetacentric (pairs 1,3,8,10) autosomes (Fig. 6). The morphology of X was obscured due to overcondensation. All the autosomes depicted a gradual decrease in size. The X chromosome is smaller than the last pair of autosomes and y is the smallest element of the karyotype. TCL is 47.40 µm, size of X and y is 1.98 µm and 0.82 µm, respectively.

Metaphase I consists of 10 dumb-bell shaped autosomal bivalents and a Xy_p sex pseudobivalent (Fig. 13). Chiasma frequency at this stage is 22 per nucleus. Meioformula is $10AA+Xy_p$. Metaphase II cells showed 10 autosomes and a sex chromosome either X or y (Fig. 13a).

Cytologically, 9 species of *Tanymecus* are known (Singh 1993). This genus appears to be conservative with regard to diploid complement as all the species possess 2n=22. However,



Figs. 11-15: *Myllocerus blandus*. 11. Metaphase I. 11a. Metaphase II with X. 11b. Metaphase II with two Ys. 12, 12a. *M. viridanus*. 12. Metaphase I. 12a. Metaphase II. 13, 13a. *T. feae* var. *plumens*. 13. Metaphase I. 13a. Metaphase II. 14. Metaphase I of *H. squamosus*. 15. Metaphase I of *L. agrestis*.

T. sciurus is dimorphic (2n=22 and 23) and possesses an extra y chromosome (Sharma & Pal 1983).

Hypomeces squamosus

The diploid chromosome number is 22 in spermatogonial metaphase (Fig. 7). The karyotype is composed of 6 pairs of metacentric (pairs 1-6) and 3 pairs of submetacentric (pairs 7-9) autosomes. Autosome pair 10, however, appeared to be acrocentric (Fig. 8). The X is metacentric in nature while y is dot-shaped. TCL is 52.68 µm, the size of X and y is 4.20 µm and 1.25 µm, respectively.

At metaphase I autosomal bivalents acquired the form of rod and ring-shaped elements while the X and y formed a sex parachute (Fig. 14). Chiasma frequency is 12 per cell.

The diploid number tallies with other reports (Yadav et al. 1987, Gill et al. 1990).

In Brachyderinae, 2n varies from 16 in *Amystax fasciatus* (Takenouchi 1980) to 24 in *Leptomias bipustulatus* (Singh 1993). Of the 61 species, 41 possess 2n=22.

Longulus agrestis

Spermatogonial metaphase revealed 22 chromosomes (Fig. 9). The karyotype comprises 7 pairs of metacentric (pairs 1-4, 6, 7, 9), 3 pairs of submetacentric (pairs 5, 8, 10) autosomes, while X is metacentric and y dot-shaped (Fig. 10). All autosomes showed gradual decrease in size. The X occupies ninth position in order of size. TCL is 48.96 µm, the size of X and y is 3.50 µm and 1.39µm, respectively.

Metaphase I carries 10 rod and ring shaped autosomal bivalents along with Xy_p sex parachute (Fig. 15). Chiasma frequency is 15 per nucleus at this stage. Meioformula is $10AA+Xy_p$.

In Eremninae, 2n varies from 20 in *Phytoscaphus tenuirostris* and *Parascaphus* sp. to 22 in about 27 species (Sharma et al. 1980).

In the present species, the chiasma frequency (X) showed a wide range from 12 to 29 per nucleus. In beetles with uniform 2n the X may be used directly to compare the extent of recombination. Usually a high X releases variability and low value conserves it (Zarchi et al. 1992). The species with high X such as *Myllocerus blandus*, are likely to have greater variation both within and between individuals because higher X is more difficult to control.

Many authors have reported polymorphism and parthenogenesis amongst the European and Japanese species (Smith & Virkki 1978) but no such phenomenon has been observed in the Indian species under report. However, to determine the cases of parthenogenetic and ployploid races in Indian weevils there is need to analyse more species, both male and female, especially from climatically diverse zones.

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EFFECT OF MUTAGENS ON CHIASMA FREQUENCY IN *BOMBYX MORI* L. (LEPIDOPTERA: BOMBYCIDAE)

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SUMMARY

Effect of mutagens (MC, EMS and X-rays) was studied on chiasma frequency in 3 races (Nistari, G and Pure Mysore) of silkworm *Bombys mori*. The difference in chiasma frequency caused by the mutagens was found to be significant with respect to race, drug concentration/dose and their interaction in most of the cases. Overall decrease in the chiasma frequency was recorded with increase in the dose of mutagens. But in case of X-ray treatment, formation of multivalents tend to increase the frequency of chiasmata while appearance of univalents and decrease in the frequency of ring bivalents reduced the same making the total impact non-descernible.

Key Words : *Bombyx mori*, mitomycin C, ethyl methanesuslfonate, X-ray, chiasma.

INTRODUCTION

Almost all the physical and chemical agents which have been found to be mutagenic are reported to affect the rate of chiasma formation (Muller 1954, Aurbach 1956). These agents possibly inhibit the chiasma formation by inhibiting the protein and DNA synthesis (Singh 1982). The present investigation was undertaken to study the effect of mitomycin C, ethyl methanesulfonate and X-rays on the frequency of chiasma formation in silkworm, *Bombyx mori*.

MATERIAL AND METHODS

Three multivoltine races viz., Nistari, G and Pure Mysore of domesticated mulberry silkworm, *Bombyx mori* constituted the experimental material for the present investigation. These races were reared in laboratory conditions at room temperture (24 - 26° C) and were fed with the leaves of Kanva-2 mulberry variety. The methods on the use of different mutagens (MC, EMS and X-ray) have been discussed earlier (Sinha et al. 1993). The male silkworms of fifth instar first day were injected with MC and EMS in 3 concentrations viz., 0.05, 0.10 and 0.15 %. A dose of 0.04 ml of solution was injected into each individual under all treatments. To control batches 0.85 % of saline solution in equal volume (0.04 ml) was injected. For X-ray treatment, male silkworms (V-1) were irradiated with 500, 1000, 2000, 3000 and 5000 R. A control of untreated batch was also maintained. The method adopted for cytological preparations was a modification of Geimsa air-dry technique suggested by Imai (1974). For each treatment 5 male silkworms were selected randomly. The larvae from treated and control batches were sacrificed after 24 h and testes were dissected out for cytological preparations. From each individual one slide was prepared utilizing one testis. From each slide, 10 well spread diplo-diakinetic cells were screened under Carl Zeiss binocular microscope. The diplo-diakinetic cells were observed for chromosome associations, such as, types of bivalants (chain and ring bivalent), formation of uni- and multivalents etc. and chiasma frequency was calculated on the basis of their number per cell.

OBSERVATIONS

The number of chiasmata per bivalent ranged from 1 2. The rod or chain like appearance of bivalent represented one chiasma per bivalent while ring-shaped configuration suggested the formation of 2 chiasmata per bivalent. Multivalents and univalents were completely absent in the normal cell. In control cells, the mean chaisma frequency per nucleus ranged from 33.44 to 33.80 in Nistari, 33.44 to 35.20 in G and 32.48 to 33.00 in Pure Mysore (Table 1). The difference in chiasma frequency caused by MC, EMS and X-ray is significant with respect to race, drug concentration/ doses and their interaction, except for EMS where interaction of race and drug was found to be nonsignificant (Table 2).

In all the races, treatment of germ cells with MC, EMS and X- rays lowered the mean value of chiasma frequency per cell and a dose-dependent gradual decrease was also recorded in this value. In all the treatments, it was found that the frequency of ring bivalents gradually decreased and that of chain bivalents increased with the increase in the concentration/ dose of the mutagens.

TABLE 1 : Effects of mutagens on chiasma frequency in *Bombyx mori*

Race	Conc. %	Mean chiasma per cell (No. ± SE) MC	EMS	Dose (R)	Mean chiasma per cell (No. ± SE) X - RAY
Nistari	Cont.	33.80 ± 0.09	33.44 ± 0.16	Cont.	33.52 ± 0.21
	0.05	33.76 ± 0.07	32.60 ± 0.18	500	32.92 ± 0.72
	0.10	32.16 ± 0.15	31.06 ± 0.17	1000	32.52 ± 0.19
	0.15	30.56 ± 0.15	29.68 ± 0.22	2000	32.04 ± 0.17
				3000	29.96 ± 0.17
				5000	29.72 ± 0.21
C.D. at 5%		0.36	0.53		0.38
G race	Cont.	35.20 ± 0.14	34.44 ± 0.17	Cont.	34.88 ± 0.19
	0.05	35.16 ± 0.26	32.66 ± 0.21	500	34.66 ± 0.07
	0.10	32.56 ± 0.17	31.56 ± 0.18	1000	32.60 ± 0.21
	0.15	31.24 ± 0.26	29.44 ± 0.28	2000	30.40 ± 0.25
				3000	29.60 ± 0.45
				5000	28.84 ± 0.19
C.D. at 5%		0.65	0.61		0.47
Pure Mysore	Cont.	32.76 ± 0.12	33.00 ± 0.26	Cont.	32.48 ± 0.24
	0.05	32.72 ± 0.19	32.20 ± 0.26	500	31.92 ± 0.19
	0.10	31.96 ± 0.26	31.44 ± 0.15	1000	31.44 ± 0.28
	0.15	29.72 ± 0.23	29.36 ± 0.18	2000	30.88 ± 0.19
				3000	30.48 ± 0.15
				5000	29.92 ± 0.12
C.D. at 5%		0.62	0.60		0.62

The formation of quadrivalents and univalents was recorded with the increase in the concentrations of the MC and EMS. The G race was found to be most sensitive to MC with regard to the formation of chiasma. Nistari and Pure Mysore were comparatively less sensitive in this regard. For EMS, the G race was found to be most sensitive with regard to the formation of chiasmata.

Chain hexavalents in addition to ring and chain quadrivalents were induced upon X- irradiation. Hence, it can be said that the X- rays are comparatively more effective in altering the chiasma frequency. Like other mutagens, X-rays also reduced the formation of ring bivalents and increased the incidence of univalents. As a consequence of all these increase and decrease, the mean chiasma frequency per cell could not change significantly. However, the overall decrease in

TABLE 2 : Results of analysis of variance for chiasma frequency in mutagen treated experiments.

Treatment	Source of variation	S.S.	df.	M.S.	F - value
MC	Between sets	156.20	11	14.20	81.77**
	Race	32.07	2	16.03	92.33**
	Drug conc.	117.23	3	39.08	225.02**
	Race * drug conc.	6.90	6	1.50	6.62**
	Within set	8.34	48	0.17	-
	Total	164.54	59	-	-
EMS	Between sets	143.22	11	13.02	53.58**
	Race	3.31	2	1.65	6.80**
	Drug conc.	136.33	3	45.44	187.01**
	Race * drug conc.	3.59	6	0.60	2.46NS
	Within set	11.66	48	0.24	-
	Total	154.89	59	-	-
X - rays	Between sets	278.79	11	25.34	119.15**
	Race	6.70	2	3.35	15.74**
	Drug conc.	226.78	3	75.59	355.37**
	Race * drug conc.	45.31	6	7.55	35.50**
	Within set	16.59	78	0.21	-
	Total	295.38	89	-	-

** significant at 0.01 level; NS - not significant

chiasma frequency with increase in the dose could be recorded. It was also found that for the effect of X-rays on chiasma frequency the G race is most sensitive followed by Nistari and Pure Mysore.

Negative and significant correlation (r= - 0.953) has been recorded between dose rate of MC and chiasma frequency in Nistari. In G and Pure Mysore, the associationship of chiasma frequency with the dose-rate was negative, though nonsignificant. For EMS, significant and negative correlation exist between dose rate and chiasma frequency in Nistari (r= - 0.954) and G (r= -0.984). Under

TABLE 3 : Correlation value and regression data on mutagen conc./dose and chiasma frequencies.

	Correlation coefficient (r)	Regression parameter slop (b)	intercept	Calculated value of "t" (a)	Table value of 't' P=0.05	P=0.01
MC						
Nistari	- 0.953 *	- 23.17	34.37	- 4.506*	4.303	9.925
G race	- 0.948 NS					
Pure Mysore	- 0.900 NS					
EMS						
Nistari	- 0.954 *	- 23.84	33.71	- 4.549 *	4.303	9.921
G race	- 0.984 *	- 31.60	34.42	- 8.041 *		
Pure Mysore	- 0.984 *	- 23.36	33.25	- 5.214 *		
X - rays						
Nistari	- 0.721 NS					
G race	- 0.741 NS					
Pure Mysore	- 0.741 NS					

* Significant at 5% level; NS - not significant

X- ray treatment the relationship between dose rate and chiasma frequency was not found to be significant. This deserves to be mentioned here that the formation of multivalents under X- ray treatment increased the frequency of chiasma on one hand but the the appearance of univalents and decrease in the frequency of ring bivalents, reduced the same to make the total impact nondescernible (Table 3).

DISCUSSION

It is quite obvious that the chemical mutagens viz., MC and EMS cause to reduce the chiasma frequency with the increase in the concentration of the drugs. MC has been reported to be inhibitor of the DNA synthesis in the meiotic cells (Mishra & Patnaik 1986). It has also been established that DNA synthesis is required during pachytene for repair of the breaks associated with crossing over (Westerman 1967). Similarly, X-rays are also reported to inhibit the DNA synthesis (Westerman 1967). They induce reduction in chiasma frequency when premeiotic cells are irradiated while in meiotic cells (early prophase) they cause increase in the chiasma frequency in case of *Schistocerca gregaria* (Westerman 1967). In *B. mori* such an increase has not been recorded, however, nonsignificant increase has been recorded. Like other chemicals (Yefremova & Filippova 1974), EMS may be inhibiting DNA synthesis in the meiotic cells and causing decrease in the chiasma frequency or may be inhibiting protein synthesis. Since protein synthesis is also reported to occur throughout meiotic cycle and required to repair the breaks associated with the crossing over (Mishra & Patnaik 1986).

Chiasma frequency has a very positive correlation with the recombination of genetic material, a well known source of genetic variability. These mutagens on one hand increase the overall mutagenial type of genetic load, and on other hand deprive the population of the genetic variation of the recombinational nature. Thus, the populations are thus put to double disadvantage.

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Short Communication

CYTOLOGY OF *LANTANA*

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SUMMARY

Cytological investigations of *Lantana crenulalta* L. and *L. trifolia* L. (2n=22) performed here show x=11 as the basic chromosome number for the genus. The chromosome counts of *L. crenulata* (n=11, 2n=22) and *L. trifolia* (n=11, 2n=22) recorded here constitute the first reports for these species. The karyotype analysis of *L. trifolia* and *L. crenulata* has been undertaken. For both the species the karyotype is asymmetric.

Key Words : *Lantana*, chromosome, karyotype.

The genus *Lantana* (Verbenaceae) with tropical American origin occurs chiefly as weeds in this part of the country. This communication gives an account on the cytology of *Lantana crenulata* L. and *L. trifolia* L.

The flower buds and root tips, pre-treated with 8-hydroxyquinoline were fixed in Carnoy's fluid (1:1:3 chlor - acetic - ethanol). Smears of anthers and squashes of root tips were made in 2% acetocarmine. Photomicrographs of the required stages were taken from fresh preparations. Pollen fertility is determined by the stainability of pollen in 1:1 acetocarmine-glycerine mixture. The systems proposed by Stebbins (1958), Levan et al. (1964) and Walker (1985) were followed for karyomorphological analysis.

In *L. trifolia* pollen mother cells of this species showed 11 bivalents at diakinesis (Fig.1). Squashes of root tips showed 2n=22 chromosomes (Fig.2). The karyotype is heterogeneous. The length of the chromosomes varies from 2.83µm to 4.67µm. Pollen sterility is estimated to be 17%. The third, eighth and eleventh pairs of the chromosomes are of st types while others are of sm types. Pollen sterility is estimated to be 17%. In *L. crenulata,* at diakinesis, 11 bivalents were noticed in each PMC (Fig 3). Somatic chromosome number is found to be 2n=22 (Fig 4). The karyotype is heterogeneous. Chromosome length varies from 2.4µm to 4.17µm. The fourth, seventh and eighth pairs of chromosomes are of sm, third pair of m and others are of st types. Pollen sterility is 17.68%.

From the existing cytological data, it is seen that the proposal for the basic chromosome numbers of many genera are primarily based on the lowest known gametic number (Darlington & Wylie 1955). As such, based on the previous reports (Paterman 1938, Tijo 1948, Arora 1960, Cherubini 1982, Choudhury & Roy 1982, Spies & Stirton 1982) and the present study on chromo-

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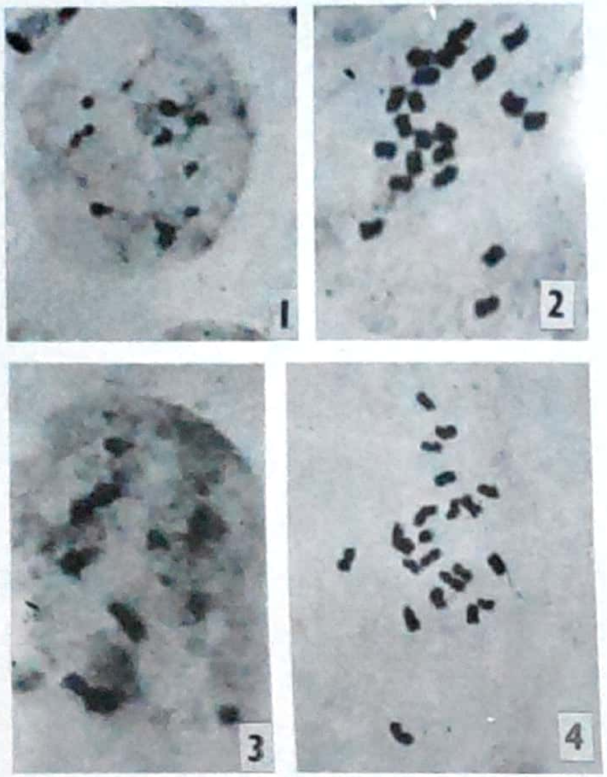

Figs. 1-4: Meiotic and somatic chromosomes of *Lantana*. 1-2. *L. trifolia*. 1, PMC at metaphase I (n=11). 2, Somatic chromosomes (2n=22). 3-4. *L. crenulata*. 3, PMC at diakinesis (n=11). 4, Somatic chromosomes (2n=22). (all X 1500).

some counts of *L. trifolia* (n=11 2n=22) and *L. crenulata* (n=11, 2n=22) the basic chromosome number of the genus *Lantana* is 11. The existence of an hexaploid taxa of *L. trifolia* with 2n=48 with basic chromosome number x=8 is in record (Paterman 1938). However, the diploid species of *L. trifolia* and *L. crenulata* each with n=11 constitute the first report.

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SOCGI CHROMOSOME NUMBER REPORTS XII

Presented by
B. H. M. NIJALINGAPPA

These reports are intended to publish/stray chromosome counts of plants and animals which otherwise might remain unpublished. The taxa from unexplored regions/areas particularly of tropical Asia, Africa and America studied for the first time would be given preference. Each chromosome number report should be based on a critical taxonomic identification of the material, exact counting of the chromosome number, documentation of an authentic material by depositing in a recognised herbarium. Two copies of the reports prepared in accordance with the format of the reports appeared in the latest issue of the journal should reach **Prof. B.H.M. Nijalingappa, Depratment of Botany, Bangalore University, Bangalore 560 056, India.** All contributors shall become the members of the Society of Cytologists and Geneticists, India before the reports are submitted for publication. Contributors receive the reprints on payment of nominal reprint charges *in advance* which will be intimated at the time of acceptance.

Reports by S.S. KUMAR, MANISELVAN and MANJU ARORA Department of Botany, Panjab University, Chandigarh 160 014, India. Vouchers in PAN.

BARTRAMIACEAE

Philonotis fontana (Hedw.) Mitt. n=12. India : Nilgiris, Naduvattum, *4372.*

BRACHYTHECIACEAE

Homalothecium sericeum (Hedw.) B.S.G. n=11. India : Nilgiris, Naduvattum, *4389.*

BRYACEAE

Bryum billardieri Schwaegr. n=10. India : Nilgiris, Naduvattum, *4377.*

B. capillare Hedw. n=10. India : Nilgiris, Naduvattum. *4378.*

B. medianum Mitt. n=22. India : Nilgiris, Naduvattum, *4379.*

B. uliginosum (Brid.) B.S.G. n=11. India : Nilgiris, Naduvattum, *4381.*

B. Wightii Mitt. n=22. India : Nilgiris, Udhagamandalam, *4382.*

DICRANACEAE

Dicranella divaricata (Mitt.) Jaeg. n=11. India : Nilgiris, Naduvattum, *4383.*

FUNARIACEAE

Entosthodon piliferum Mitt. n=26. India : Nilgiris, Naduvattum, *4384.*

GRIMMIACEAE

Grimmia apophysata Hamp. n=12. India : Nilgiris, Udhagamandalam, *4393.*

POLYTRICHACEAE

Pogonatum aloides (Hedw.) Beauv. forma *aloides*. n=7. India : Nilgiris, Naduvattum, *4389*.

P. aloides (Hedw.) Beauv. forma *Neesii* (C. Muell.) Gangulee. n=7. India : Nilgiris, Naduvattum, *4390*.

P. himalayanum Mitt. n=7, n=14. India : Nilgiris, Doddabetta, *4391, 4392*.

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COVER : Somatic chromosomes of *Hippeastrum reginae* (See p. 71)