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EFFECTS OF CESIUM CHLORIDE ON CHROMOSOMES AND CELL DIVISION IN *TRIGONELLA FOENUM-GRAECUM* L.

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SUMMARY

Cesium chloride was found to be cytotoxic on seeds of *Trigonella foenum-graecum* following acute exposure in vivo. The end points screened were mitotic index and frequencies of aberrant cells. The number of aberrant cells was directly proportional to the dosage used and the period of exposure. The frequency was reduced in seeds exposed to lower concentrations on being transferred to cesium-free medium for recovery. The effects on mitotic index was variable, lower concentrations being weakly mitogenic and higher ones being mitostatic.

Key Words : Cesium toxicity, chromosomal aberrations, cell division, metal toxicity.

INTRODUCTION

The most important reason for interest in cesium (Cs) toxicity is that Cs is an industrially important element and is being increasingly used in different industries. In addition, it closely resembles the biologically active alkali earth metals, sodium (Na), potassium (K) and lithium (Li). Cs is present in air (Dittrich & Cothern 1975), soil (Bertrand & Bertrand 1952, Wallace et al. 1982) and water (Ehmann 1986, Folsom et al. 1964) in various concentrations permitting an efficient transfer through the soil-plant-food chain to the human system.

Physiological effects and toxicity, following exposure to Cs has been observed in lower organisms and plants (Chang 1986, Kordan 1987a,b, Nagata 1988; Tarasov et al. 1986, Vlasyuk et al. 1970). The effect of Cs is pronounced on chlorophyll synthesis and activity of certain enzymes (Marschner 1965, Rotfarb et al. 1970). No information is, however, available on the effects of Cs on plant cell division.

The present investigation was undertaken to study the effects of acute exposure to cesium chloride (CsCl) on chromosomes and cell division on seeds of *Trigonella foenum-graecum*.

MATERIAL AND METHODS

CsCl (Molecular weight 168.36, Sisco laboratory, India) was dissolved in glass distilled water to give concentrations of 10, 100 and 1000 ppm. Seeds of *Trigonella foenum-graecum* were procured from the local market in bulk quantity. The seeds were soaked in different concentrations of the salt for 24 h. As no

data are available on the cytotoxicity of the metal on plant systems, several trials have been carried out for determining the concentrations and the duration used. Control sets were maintained for each concentration of the chemical used. After 24 h, all seeds from each set was thoroughly washed in distilled water and transferred to Knop's nutrient medium for recovery. Subsequently, from each set, 10 root tips in each, were excised after 24, 48, 72, 96 and 120 h of recovery. Root tips were fixed for an hour and a half in 1:2 aceto-ethanol, kept for 2-3 min in 45% acetic acid, stained in 2% aceto-orcein:1N HCl mixture (9:1) and squashed in 45% acetic acid (Sharma & Sharma 1980).

About 5000 cells were scored for treatment and each recovery period per concentration. The number of dividing cells were recorded and the mitotic index (MI) was calculated.

The abnormalities observed in dividing cells were of 3 categories and were expressed as percentage of total cells scored (5000) : Group I : effect due to spindle disturbances, such as laggards, multipolarity, sticky bridge, early separation, group II : effects on chromosomes, such as breaks and fragments and group III : lethality as shown by pycnosis and gross pulverisation (Table 1).

RESULTS AND DISCUSSION

Effects of acute treatment

On frequency of dividing cells (M I) : The effect of CsCl on MI was mitostatic at the higher concentrations and mitogenic at the lower concentration and directly related to the concentration used. The higher two doses reduced MI significantly after treatment for 24 h. The lowest dose increased the mitotic index, though not to a significant level. The enhancement of mitotic index at lower concentrations has been recorded earlier for other metals as well (Sharma & Talukder 1987).

On frequency of aberrant cells : The frequency of aberrant cells was directly proportional to the concentration used. The principal aberrations induced were spindle disturbances and very few breaks. The higher two doses increased aberrations to a signiiicant level. The frequency induced by the lower dose was very low, even after 24 h of treatment.

Effects of recovery in Cs–free medium

The seeds were transferred to Knop's nutrient medium after being washed thoroughly to remove traces of CsCl. The observations were made after 24, 48, 72, 96 and 120 h of recovery till the mitotic index and aberrant cells reached the normal control level.

On frequency of mitotic index : After treatment with the highest dose, MI remained significanty low even on being allowed to recover. The effect was lethal as shown by cell death at 72 h. The mitotic index increased with duration of recovery in plants treated with the two lower doses.

TABLE 1 : Effect of CsCl on seeds of *Trigonella foenum-graecum* after single acute exposure (5000 cells scored per set)

Seeds soaked in water

Duration (in hours)	MI (%)	Total abnormal cells (%)	Abnormalities (%)		
			I	II	III
Direct					
24	5.86	1.0	1.0	—	—
Recovery after					
24	5.84	1.0	1.0	—	—
48	5.76	1.4	1.2	—	0.2
72	5.06	1.6	1.0	—	0.6
96	4.88	1.4	1.0	—	0.4
120	5.02	1.6	1.0	—	0.6

Seeds soaked in 10 ppm CsCl

Duration (in hours)	MI (%)	Total abnormal cells (%)	Abnormalities (%)		
			I	II	III
Direct					
24	6.9	8.2*	5.8	0.4	1.8
Recovery after					
24	6.0	7.0*	5.0	0.2	1.8
48	5.6	5.8*	4.0	0.2	1.6
72	5.4	4.0*	3.2	—	0.8
96	5.2	3.0*	2.6	—	0.4
120	5.0	1.6	1.4	—	0.2

Table 1 : Contd.

Seeds soaked in 100 ppm CsCl

Duration (in hours)	MI (%)	Total abnormal cells (%)	Abnormalities (%) I	II	III
Direct 24	3.5*	15.2*	13.4	0.4	1.4
Recovery after 24	3.6*	12.6*	11.2	0.2	1.2
48	3.5*	11.8*	10.8	—	1.0
72	3.7	11.2*	10.4	—	0.8
96	3.7	10.0*	9.4	—	0.6
120	4.0	9.6*	8.6	—	1.0

Seeds soaked in 1000 ppm CsCl

Duration (in hours)	MI (%)	Total abnormal cells (%)	Abnormalities (%) I	II	III
Direct 24	2.8*	21.0*	18.0	0.6	2.4
Recovery after 24	2.2*	16.2	14.6	0.2	1.2
8	1.9*	14.8	13.8	—	1.0
72	—	—	—	—	—
96	—	—	—	—	—
120	—	—	—	—	—

*Significant a $P \leq 0.05$ compared to control at that particular duration.

On frequency of aberrant cells : The time required for regaining normalcy depended on the concentrations used and on the period of recovery for the two lower concentrations. The cells treated with 10 ppm reached normal level after 120 h of recovery. With the middle dose (100 ppm), the extent of recovery was less as compared to the lowest dose. The cells did not recover after treatment with the highest dose (1000 ppm) and lethality occurred.

Cs has been recorded in different organs of plants including algae (Ishikawa et al. 1986), fungi (Seeger & Schwein-Shaut 1981) and other higher plants (Teherani 1987).

The present data indicate that CsCl affects cell division adversely in the seeds, the degree of effects being directly proportional to the concentration used. Return to the normal state occurs only after exposure to lower concentrations.

The high frequency of spindle disturbances indicates that the action of Cs is on spindle formation and physiological activity of dividing cells rather than directly on chromatin in the plant systems. This report is of importance as stable cesium has been located in large amounts in plant parts after a nuclear fallout.

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EFFECTS OF GAMMA RAYS AND EMS ON MEIOSIS IN SOME CEREALS

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SUMMARY

The effect of gamma rays and/or EMS on meiosis in rye (*2x*), wheat (*4x*, *6x*) and triticale (*4x*, *6x*) were studied. Cytological abnormalities like quadrivalents, rod bivalents, univalents, laggards, bridges, fragments and micronuclei were increased with the elevation of dose and duration of a mutagen. The irregularities were decreased with increase in ploidy level of the taxa. In substituted triticales, the cytological effects of mutagens were less pronounced than complete rye triticales.

Key Words : Cytological aberrations, induced mutation, cereals.

INTRODUCTION

The effects of various physical and chemical mutagens on cytological studies in different genotypes were made to understand and to compare the mutagenic effects of various mutagens and also to elucidate the response of various genotypes to a particular mutagen (Reddy et al. 1991a). The effects of gamma rays, EMS and sodium azide were studied in diploid barley, *4x* and *6x* wheats (Reddy et al. 1991b). The present communication reports the cytological effects of gamma rays and EMS in different cereals which differ in ploidy but having common genomes.

MATERIALS AND METHODS

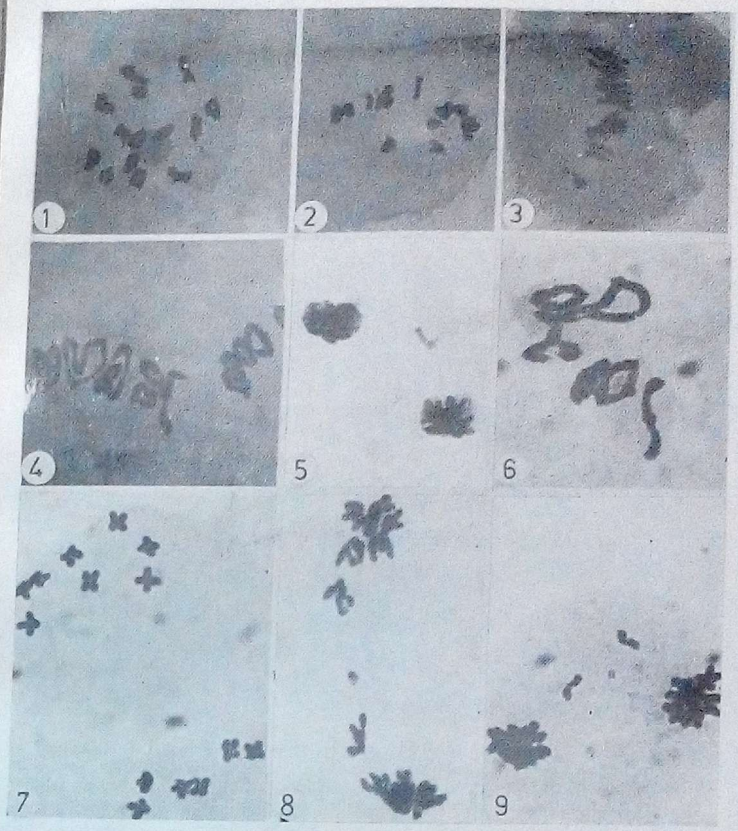
Seeds of one variety each of diploid rye (King II), *4x* wheat (Jairaj), *6x* wheat (WH147), *4x* triticale (T 4 x 1) and 3 varieties of *6x* triticales (Carman, Beagle, Towan) were used in the present study. Seeds of rye, wheat and triticale (*6x*) were procured from IARI, Regional Research Station, Wellington, while *4x* triticales were obtained from INRA (Institut national de la recherche agronomique), France.

Seeds of all the genotypes were treated with 3 doses of gamma rays (15, 20, 25 kR), 3 durations of 0.5% aqueous solutions of EMS (8, 10, 12 h) and combination of GR+EMS for 3 treatments (15 kR+12 h, 20 kR+10 h, 25 kR+8 h). For each treatment, 100 seeds were used. After EMS treatments, seeds were thoroughly washed in running water and planted immediately in the field along with respective controls.

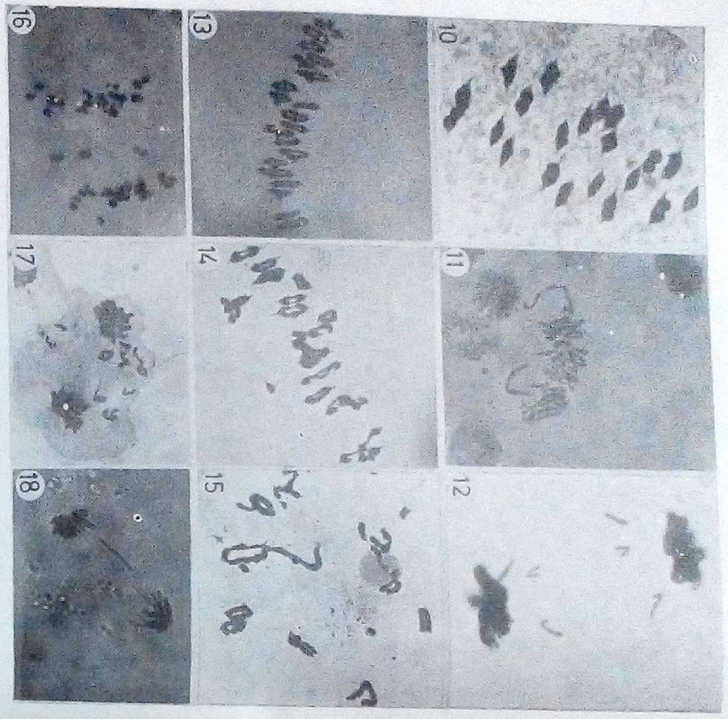
Meiotic studies were made on 25 randomly selected M_1 plants from each treatment. Spikes were fixed in Carnoy's fluid. Squashes were made in 2% aceto-carmine. Data on various cytological parameters such as quadrivalents, bivalents, univalents, laggards, bridges, fragments and micronuclei were recorded at appropriate meiotic stages. 'T' test was applied to test the mean cytological values of treated plants with that of control.

RESULTS AND DISCUSSION

The data on chromosomal behaviour during meiosis in gamma ray and EMS induced M_1 populations of rye ($2x$), wheat ($4x$, $6x$) and triticale ($4x$, $6x$) are presented in Table 1 and the representative cytological configurations in various genotypes were shown in Figs. 1-18. The results presented in the Table suggest that irrespective of the type of mutagen either individually or in combination, the mean values of quadrivalents, rod bivalents, univalents, lagging chromosomes, bridges, fragments and micronuclei were increased significantly over control, while mean values of ring bivalents and chiasma were decreased significantly Cytological abnormalities like quadrivalents, univalents, laggards, bridges, fragments and micronuclei were completely absent in controlled materials of rye and wheat while in triticales very small frequency of univalents, and micronuclei could be noticed in untreated control. However, within the individual mutagenic treatments, the meiotic irregularities were increased with increase in dose/duration of mutagen. The combination treatments produced more cytological irregularities relative to individual treatments. Within the combination treatment, in terms of cytological irregularities genotypic variation was noticed. The mean values of quadrivalents were high in combination treatments of 20 kR×10 h in $4x$ and $6x$ wheats while in rye and triticales, the mean values of quadrivalents were more in 25 kR×8 h treatment followed by 20 kR+10 h and 15 kR+12 h treatments. However, in all the genotypes, other meiotic irregularities like univalents, laggards, bridges, fragments and micronuclei had a linear relationship with the dose of gamma rays. Such a linear relationship between mutagenic dose/duration and the frequency of various cytological aberrations including translocations were noticed earlier in a number of cereal crops like barley (O'Mara 1987, Reddy et al. 1991a,b, Vazquez & Sanchez-Monge 1987, Swaminathan et al. 1962, Prina et al. 1986), rice (Reddi and Reddi 1985, Ramakrishna et al. 1989), rye (Singh & Khanna 1988, Rogalska & Darbrowski 1981), and wheat and triticales (Singh & Singh 1989, Sham Rao & Sears 1964, Singh & Khanna 1988, Swaminathan et al. 1962, Reddy et al. 1991b, Pushpalatha et al. 1992). The mean values of various chromosomal irregularities noticed in the combined treatments were less than that of total frequency of chromosomal aberrations produced individually by gamma rays and EMS, thereby suggesting a 'less than additive' effect. However, chemicals like dES was shown to produce synergistic effect on chromosomal fragments in barley, when coupled with X-rays (Nilan et al. 1962). Among individual treatments, cytological abnormalities were more in gamma ray treatments as compared to EMS treatments. These results, therefore, support the view that gamma rays either alone or in combination with EMS produce more chromosomal rearrangements and chemical mutagens cause the other types of mutations. However, in the present study, the various cytological abnormalities were considerably higher in combined treatments as compared to individual treatments of gamma rays, indicating that EMS also is capable of causing chromosomal aberrations.



Figs. 1-9 : 1-2. Tetraploid wheat ($2n=28$), 1, Diakinesis, 2, MI with 2 rod and 12 ring bivalents, 3-5. Tetraploid triticale ($2n=28$), 3, MI with 4 rod and 10 ring bivalents, 4, MI with a quadrivalent, 5, AI with a laggard, 6-9. Rye ($2n=14$), 6, MI with a quadrivalent, one rod and 4 ring bivalents, 7, AI with perfect disjunction, 8, AI with univalents in dividing, 9, AI with laggards.



Figs. 10–18 : 10–12, Hexaploid wheat ($2n=42$). 10, MI with 21 ring bivalents. 11, AI with a quadrivalent, 3 rod and 16 ring bivalents. 12, AI with univalents in dividing. 13-18, Hexaploid triticale ($2n=42$). 13, MI with 4 rod and 21 ring bivalents. 14, MI with 4 rod 17 ring bivalents. 15, Diakinesis with a quadrivalent. 16, AI with late disjunction. 17, AI showing univalents in division. 18, AI with a bridge.

TABLE 1 : Cytological effects of gamma rays and EMS in rye (2x), wheat (4x, 6x) and triticale (4x, 6x). (First line is the mean and second line is range).

Variety/ Treatment	IV	II		u	Laggards/ Dividing univalents	Fragments/ Bridges	Micro-nuclei/ Microspore	Chiasmata per cell
		Rod	Ring					
1	2	3	4	5	6	7	8	9
4x Wheat								
Jairaj								
Control	—	0.64±0.11	13.36±0.02	—	—	—	—	27.13±0.08
		0-2	12-14					26-28
Gamma ays								
15 kR	0.25	**1.46±0.08	*11.98±0.04	0.31±0.06	0.38	0.41	0.24	*26.38±0.11
	0-1	0-2	11-17	0-2	0-2	0-1	0-2	25-27
20 kR	0.35	**1.69±0.14	**11.59±0.04	0.39±0.04	0.43	0.46	0.31	*26.19±0.09
	0-1	0-3	10-13	0-4	0-2	0-1	0-2	25-28
25 kR	0.50	**1.94±0.06	**11.14±0.06	0.42±0.06	0.51	0.50	0.36	*26.22±0.21
	0-1	0-3	10-13	0-4	0-2	0-1	0-2	25-28
EMS								
8 h	—	*1.06±0.10	*12.72±0.04	0.22±0.02	0.19	0.18	0.04	26.40±0.14
		0-2	12-14	0-2	0-2	0-1	0-2	25-28
10 h	0.22	*1.14±0.04	*12.28±0.06	0.26±0.04	0.26	0.21	0.08	26.78±0.06
	0-1	0-2	11-14	0-2	0-2	0-1	0-2	25-28
12 h	0.28	**1.28±0.06	*12.12±0.08	0.32±0.04	0.29	0.24	0.12	26.64±0.80
	0-1	0-2	11-14	0-2	0-2	0-1	0-2	25-28
GR+EMS								
15 kR+12 h	0.58	**1.76±0.05	**11.28±0.10	0.38±0.04	0.56	0.52	0.39	26.64±0.11
	0-1	0-2	10-13	0-4	0-2	0-1	0-2	25-28
20 kR+10 h	0.75	**2.32±0.08	**10.37±0.04	0.56±0.02	0.58	0.58	0.46	*26.06±0.10
	0-1	0-3	10-12	0-4	0-2	0-1	0-2	25-28

Contd..

TABLE 1 : (Contd.)

1	2	3	4	5	6	7	8	9
25 kR+8 h	0.66	**2.19±0.11	**10.71±0.06	*0.44±0.02	0.64	0.63	0.52	26.45±0.13
	0-1	0-3	9-12	0-4	0-2	0-1	0-3	25-28
4x Triticale T 4X1								
Control	—	0.92±0.04	12.98±0.06	0.10±0.02	0.05	0.05	—	26.88±0.08
		0-2	12-14	0-2	0-2	0-1		25-28
Gamma rays								
15 kR	0.46	**2.65±0.08	**11.47±0.06	*0.42±0.04	0.39	0.43	0.68	26.43±0.06
	0-1	0-3	11-13	0-4	0-2	0-1	0-3	25-28
20 kR	0.53	**1.83±0.06	**11.16±0.06	*0.48±0.06	0.46	0.50	0.73	26.27±0.04
	0-1	0-3	10-13	0-4	0-2	0-1	0-3	25-28
25 kR	0.61	**2.04±0.04	**10.79±0.04	*0.56±0.04	0.50	0.54	0.79	*26.06±0.09
	0-1	0-3	10-12	0-4		0-20-1	0-3	25-28
EMS								
8 h	0.23	*1.26±0.02	12.23±0.02	*0.28±0.02	0.26	0.29	0.53	26.64±0.08
	0-1	0-2	12-14	0-2	0-2	0-1	0-3	25-28
10 h	0.30	**1.38±0.08	11.99±0.02	0.33±0.02	0.31	0.37	0.59	26.56±0.11
	0-1	0-2	11-14	0-2	0-2	0-1	0-3	25-28
12 h	0.38	**1.52±0.08	*11.72±0.94	*0.38±0.04	0.38	0.44	0.64	26.48±0.10
	0-1	0-2	11-13	0-4	0-2	0-1	0-3	25-28
GR+EMS								
15 kR+12 h	0.69	**1.95±0.06	**10.88±0.02	*0.48±0.02	0.57	0.53	0.77	26.47±0.06
	0-1	0-3	10-12	0-4	0-2	0-1	0-3	25-28
20 kR+10 h	0.84	**2.06±0.08	**10.56±0.04	*0.54±0.04	0.64	0.57	0.81	26.54±0.11
	0-1	0-3	10-12	0-4	0-2	0-1	0-3	25-28
15 kR+8 h	0.92	**2.17±0.04	**10.30±0.04	*0.61±0.02	0.68	0.61	0.98	26.45±0.07
	0-1	0-3	0-12	0-4	0-2	0-1	0-3	25-28

TABLE 1. : (Contd.)

1	2	3	4	5	6	7	8	9
2x Rye King II								
Control	—	0.44±0.02	6.56±0.02	—	—	—	—	13.56±0.06
		0-2						12-14
Gamma rays								
15 kR	0.46	**1.34±0.04	**4.80±0.02	0.40±0.04	0.41	0.44	0.31	12.78±0.04
	0-1	0-2	3-6	0-2	0-2	0-2	0-2	12-14
20 kR	0.53	**1.57±0.02	**4.43±0.04	0.47±0.06	0.52	0.49	0.45	*12.55±0.06
	0-1	0.3	3-6	0-2	0-2	0-2	0-2	12-14
25 kR	0.66	**1.76±0.04	**4.04±0.06	0.54±0.02	0.60	0.56	0.49	*12.48±0.07
	0-1	0-3	3-6	0-2	0-2	0-2	0-2	12-14
EMS								
8 h	0.24	**1.10±0.01	**5.38±0.04	0.28±0.04	0.23	0.22	0.11	12.82±9.011
	0-1	0-2	4-7	0-2	0-2	0-1	0-1	12-14
10 h	0.32	**1.26±0.02	**5.10±0.02	0.32±0.04	0.28	0.25	0.15	12.74±0.013
	0-1	0-3	4-7	0-2	0-2	0-1	0-1	12-14
12 h	0.40	**1.50±0.04	**4 69±0.06	0.41±0.06	0.34	0.31	0.21	*12.48±0.11
	0-1	0-3	3-6	0-2	0-2	0-2	0-1	12-14
GR+EMS								
15 kR+12 h	0.73	**1.91±0.02	**3.75±0.06	0.61±0.06	0.59	0.54	0.43	*12.33±0.12
	0-1	0-3	3-6	0-2	0-2	0-2	0-2	12-14
20 kR+10 h	0.80	**2.35±0.04	**3.13±0.04	0.72±0.04	0.61	0.61	0.51	**11.81±0.06
	0-1	0-3	2-5	0-2	0-2	0-2	0-2	11-13
25 kR+8 h	0.86	**2.12±0.04	**3.34±0.04	0.68±0.04	0.68	0.69	0.56	*12.24±0.07
	0-1	0-3	2-5	0-2	0-2	0-2	0-2	11-13
6x Wheat WH 147								
Control	—	0.55±0.04	20.45±0.06	—	—	—	—	41.45±0.11
		0-2	18-2					39-42
Gamma rays								
15 kR	0.48	**1.03±0.02	**19.13±0.06	0.36±0.02	0.33	0.34	0.18	41.21±0.09
	0-1	0-2	18-21	0-2	0-2	0-1	0.2	39-42

TABLE 1 : (Contd.)

1	2	3	4	5	6	7	8	9
20 kR	0.52	**1.23±0.04	**18.81±0.04	0.44±06	0.41	0.40	0.23	*40.93±0.06
	0-1	0-3	17-21	0-2	0-2	0-1	0-2	39-42
25 kR	0.64	**1.42±0.04	**18.43±0.06	0.51±0.06	0.49	0.47	0.32	*40.84±0.04
	0-1	0-3	17-20	0-2	0-2	0-1	0-2	39-42
EMS								
8 h	0.24	*0.71±0.02	*19.86±0.05	0.19±0.04	0.11	0.14	0.11	41.39±0.04
	0-1	0-3	18-21	0-2	0-2	0-1	0-2	39-42
10 h	0.32	*0.78±0.04	*19.64±0.06	0.26±0.02	0.17	0.22	0.16	41.34±0.06
	0-1	0-3	18-21	0-2	0-2	0-1	0-2	39-42
12 h	0.36	*09.4±0.02	*19.36±0.04	0.34±0.04	0.21	0.28	0.19	41.10±0.11
	0-1	0-3	18-21	0-2	0-2	0-1	0-2	39-42
GR + EMS								
15 kR + 12 h	0.68	**1.25±0.02	**18.60±0.10	*0.47±0.04	0.49	0.42	0.25	41.17±0.08
	0-1	0-3	17-20	0-2	0-2	0-1	0-2	39-52
20 kR × 10 h	0.84	**1.58±0.04	**17.97±0.08	*0.61±0.02	0.54	0.47	0.36	*40.88±0.11
	0-1	0-3	16-19	0-2	0-2	0-1	0-2	38-42
25 kR × 8 h	0.76	**1.52±0.04	**18.14±0.06	*0.58±0.02	0.62	0.53	0.44	**40.84±0.10
	0-1	0-3	16-19	0-4	0-2	0-1	0-2	38-42
6x Triticale Carman								
Control	—	1.98±0.04	18.71±0.04	0.31±0.02	—	—	0.07	39.58±0.09
		0-3	18-21	0-2			0-2	37-41
Gamma rays								
15 kR	0.56	**3.45±0.02	**15.85±0.06	**1.21±0.04	0.32	0.19	0.61	**37.37±0.11
	0-1	1-4	14-19	0-2	0-2	0-1	0-2	36-40
20 kR	0.06	**3.82±0.01	**15.32±0.04	**1.28±0.08	0.41	0.29	0.66	**36.86±0.12
	0-1	1-5	14-18	0-4	0-2	0-1	0-2	35-40
25 kR	3.68	**4.01±0.02	**14.97±0.05	**1.36±0.06	0.48	0.33	0.74	**36.67±0.04
	0-1	0-6	13-17	0-4	0-2	0-1	0-2	35-39

TABLE 1 : (Contd.)

1	2	3	4	5	6	7	8	9
EMS								
8 h	0.28	**3.60±0.02	**16.03±0.04	**1.10±0.05	0.19	0.14	0.44	**36.76±0.06
	0-1	1-4	11-49	0-2	0-2	0-1	0-2	35-39
10 h	0.36	**3.77±0.02	**15.71±0.06	**1.16±0.04	0.24	0.19	0.52	**36.63±0.08
	0-1	1-5	14-18	0-4	0-2	0-1	0-2	35-39
12 h	0.48	**3.89±0.04	**15.39±0.08	**1.24±0.06	00.32	0.24	0.61	**36.59±0.04
	0-1	1-5	14-18	0-4	0-2	0-1	0-2	35-39
GR + EMS								
15 kR + 12 h	0.76	**3.86±0.04	**14.96±0.02	**1.42±0.04	0.49	0.31	0.71	**36.82±0.06
	0-1	1-5	13-17	0-4	0-2	0-1	0-2	35-39
20 kR + 10 h	0.88	**3.98±0.04	**14.64±0.04	**1.54±0.07	0.56	0.36	0.76	**36.78±0.06
	0-2	1-5	13-17	0-4	0-4	0-2	0-2	35-39
25 kR + 8 h	0.96	**4.22±0.05	**14.17±0.06	**1.66±0.02	0.63	0.44	0.89	**36.40±0.04
	0-2	1-6	13-17	0-4	0-4	0-2	0-2	35-39
6x Triticale Beagle								
Control	—	1.82±0.06	19.00±0.06	0.18±0.04	—	—	0.05	39.82±0.05
		0-3	18-21	0-2			0-2	38-42
Gamma rays								
15 kR	0.45	**3.40±0.06	**15.98±0.07	**1.17 0.04	0.27	0.14	0.54	**37.16±0.06
	0-1	1-5	14-18	0-2	0-2	0-1	0-2	36-40
20 kR	0.49	**3.71±0.05	**15.56±0.08	**1.24±0.02	0.36	0.20	0.62	**36.79±0.04
	0-1	1-5	14-18	0-2	0-2	0-1	0-2	35-40
25 kR	0.57	**3.92±0.04	**15.20±0.08	**1.31±0.06	0.44	0.27	0.68	**36.60±0.07
	0-1	1-6	14-17	0-4	0-4	0-1	0-2	35-40
EMS								
8 h	0.25	**3.29±0.04	**16.42±0.04	**1.04±0.05	0.13	0.12	0.36	**37.13±0.07
	0-1	1-5	14-19	0-2	0-2	0-1	0-2	36-40
10 h	0.34	**3.54±0.02	**15.98±0.06	**14.0±0.06	0.16	0.18	0.44	**36.86±0.06
	0-1	1-6	14-18	0-2	0-2	0-1	0-2	35-40
12 h	0.42	**3.69±0.03	**15.68±0.06	**1.21±0.04	0.26	0.22	0.55	**36.73±0.04
	0-1	1-5	14-18	0-4	0-2	0-1	0-2	35-40

TABLE : 1 (Concluded)

1	2	3	4	5	6	7	8	9
GR × EMS								
15 kR + 12 h	0.68	**3.71±0.02	**15.29±0.04	**1.32±0.04	0.45	0.26	0.67	**37.01±0.06
	0–2	0–5	14–19	0–2	0–2	0–1	0–2	35–41
20 kR + 10 h	0.72	**3.86±0.03	**14.93±0.06	**1.49±0.02	0.52	0.34	0.71	**36.60±0.07
	0–2	1–6	14–18	0–4	0–4	0–2	0–2	35–40
25 kR + 8 h	0.86	**4.11±0.04	**14.46±0.06	**1.57±0.02	0.59	0.41	0.84	**36.47±0.04
	0–2	0–6	14–18	0–4	0–4	0–2	0–2	35–40
6x Triticale Towan								
Control	—	1.64±0.04	19.22±0.06	0.14±0.06	—	—	0.03	40.08±0.05
		0–3	18–21	0–2			0–2	39–42
Gamma rays								
15 kR	0.41	**3.31±0.05	**16.17±0.04	**1.11±0.04	0.21	0.14	0.49	**37.29±0.40
	0–1	0–4	15–18	0–2	0–2	0–1	0–2	36–41
20 kR	0.44	**3.69±0.06	**15.69±0.06	**1.18±0.02	0.33	0.19	0.55	**36.83±0.03
	0–1	1–4	14–18	0–2	0–2	0–1	0–2	35–41
25 kR	0.52	**3.84±0.04	**15.37±0.04	**1.27±0.04	0.41	0.26	0.67	**36.66±0.02
	0–1	1–5	14–18	0–4	0–4	0–2	0–2	35–40
EMS								
8 h	0.19	**3.18±0.02	**16.63±0.04	**1.00±0.06	0.10	0.11	0.32	**37.20±0.06
	0–1	1–4	15–19	0–2	0–2	0–1	0–2	36–40
10 h	0.27	**3.41±0.03	**16.21±0.06	**1.11±0.04	0.14	0.16	0.41	**36.91±0.11
	0–1	1–4	14–18	0–2	0–2	0–1	0–2	35–40
12 h	0.36	**3.57±0.04	* *15.91±0.07	**1.16±0.2	0.24	0.23	0.56	**36.83±0.09
	0–1	1–4	14–18	0–4	0–2	0–1	0–2	35–40
GR + EMS								
15 kR + 12 h	0.55	**3.77±0.05	**15.40±0.04	**1.28±0.02	0.40	0.24	0.62	**36.77±0.07
	0–1	1–6	14–18	0–4	0–2	0–1	0–2	35–40
20 kR + 10 h	0.68	**3.90±0.04	**14.98±0.04	**1.44±0.02	0.48	0.29	0.70	**36.58±0.06
	0–1	1–6	63–17	0–4	0–4	0–1	0–2	35–40
25 kR + 8 h	0.81	**4.01±0.02	**14.66±0.02	**1.52±0.02	0.56	0.38	0.80	**36.57±0.07
	0–2	1–6	13–16	0–4	0–4	0–2	0–2	35–40

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

The chromosomal abnormalities were more in rye (2*x*) as compared to wheat (4*x*, 6*x*) and triticales (4*x*, 6*x*). In wheat and triticales, tetraploid forms produced slightly more abnormalities than hexaploid ones. These results suggest that diploids are more sensitive than tetraploids, and the latter ones are more mutable than hexaploids. Similar observations for various mutations including cytological aberrations were made in different ploidy levels in wheat and barley (Valeva 1976, Edwards & Williams 1966, Bhatia & Swaminathan 1963). Bhaskaran & Swaminathan (1961) noticed high frequency of quadrivalents in neutron-irradiated populations of 4*x* wheat and observed that their frequency was increased with increase in the dose and such a trend has not been noticed at the hexaploid level. Within hexaploid triticales, triticale Carman (having full rye complement) had higher meiotic irregularities in mutagenic treatments as compared to 'Beagle' (one rye pair substituted for one pair of D-genome chromosomes of wheat) and 'Town' (two pairs of rye chromosomes were substituted), thereby suggesting that substituted triticales are slightly resistant to mutagens relative to triticales with full chromosome complement. This was explained by the presence of genes for meiotic stability on D-genome chromosomes (Queiroz et al. 1991).

Increase in mean number of quadrivalents with dose and duration of mutagens indicating structural alterations leading to the rearrangement of chromosomes. Both translocations and inversions may be involved in this process. Earlier, Caldecott et al. (1954) noted that in barley, the frequency of translocations were dependent on the dose of ionizing radiations. The presence of laggards, fragments, anaphase bridges and micronuclei in high proportion in all the mutagenic treatments suggest that fragmentation and rejoining of the broken ends of the chromosomes are the result of mutagens. In wheat and triticales, the mean frequency and number of univalents were increased with the elevation of mutagen dose, however, in rye only the frequency was increased. An increase in univalent formation suggests that mutagenesis did result in non-pairing of homologous chromosomes which might have resulted from point mutations or deletions. In the present study, despite increase in quadrivalent frequency, the chiasma frequency decreased significantly in most of the treatments indicating the effect of increased rod bivalents on decreasing the chiasmata. The reduction in chiasma frequency also attributed to failure of coiling or reduction of pairing at pachytene. The absence of fragments along with anaphase bridges noticed in the present study might be due to subchromatid exchanges. Reduction in chromosome pairing has also been attributed to mutations in the genes governing homologous chromosome pairing and/or chromosomal structural changes (Reddy & Annadurai 1992, Reddy et al. 1992).

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INDUCED DESYNAPSIS IN *CLITORIA BIFLORA* DALZ.

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SUMMARY

Three desynaptic plants (Ds_1, Ds_2, Ds_3) in *Clitoria biflora* Dalz. were isolated in the M_1 of either combined (post-irradiation) treatment of 25 kR+0.15% NMU (Ds_3) or 40 kR+0.15% NMU (Ds_3) or from the seeds irradiated with 40 kR gamma rays (Ds_2). The average number of univalents per cell ranged from 9.6 in Ds_1 to 12.8 in Ds_2. The variation in the mean number of univalents in Ds_1, Ds_2 and Ds_3 is indicative of possibly polygenic control of synapsis. The univalent frequency per cell in M_2 plants of Ds_1 and Ds_3 was relatively less, possibly due to changed environment.

Key Words : Desynapsis, asynapsis, *Clitoria biflora*.

INTRODUCTION

The study of homologous chromosome pairing at zygotene followed by crossing over at late pachytene had been a popular subject of study. Mutations in the genes governing coordinated meiotic systems or drastic change in the external factors like temperature, water and nutrition may lead the course of meiosis to deviate in various ways. Therefore, either mutations in such genes or drastic fluctuations in the external factors have been brought about artificially with a view to study their consequent impact on chromosome pairing. The homologous chromosomes may fail to pair at zygotene (asynapsis) or else, fall apart after pairing due to failure of subsequent chiasma formation (desynapsis). Since resultant observation in asynapsis as well as desynapsis is varying number of univalents at late prophase I and metaphase I, the clear demarcation between both the phenomena can only be carried out by studying pachytene which is not amenable in most of the plants due to technical difficulties (Gottschalk & Kaul 1980 a, b).

The following study is about 3 desynaptic lines (Ds_1, Ds_2, Ds_3) isolated in the mutant population of *C. biflora* (Leguminosae).

MATERIAL AND METHODS

A pure lot of air dried seeds (4.5% moisture) of *C. biflora* was exposed to 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, and 70 kR gamma rays (15 seeds in each dose) at National Botanical Research Institute, Lucknow. After irradiation,

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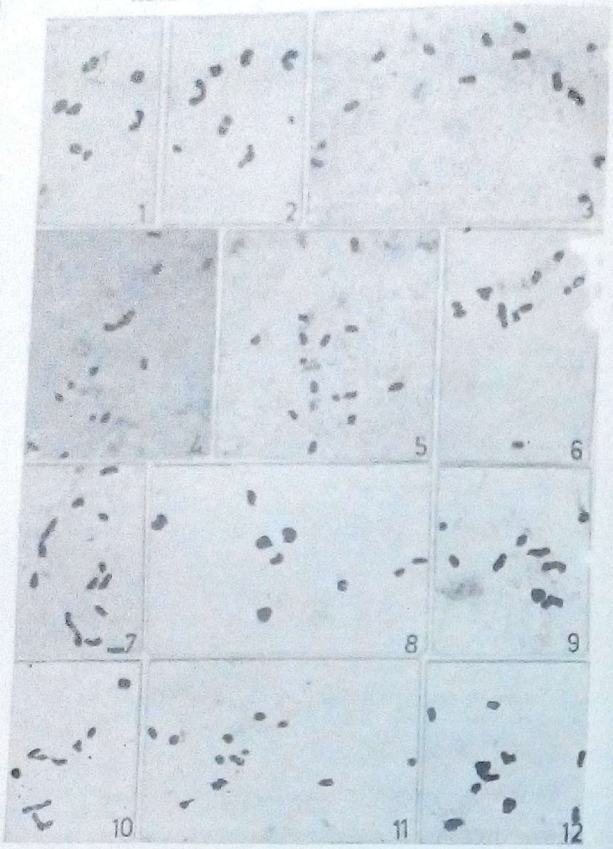

the seeds were treated with conc. H_2SO_4 and then washed in running water for 24 h. Ten seeds each, exposed to 10, 20, 25, 30, 35, 40, 50, 60 and 70 kR were immersed (post-irradiation) in 0.15% aqueous solutions of sodium azide, Methyle methane sulphonate, Ethyle methane sulphonate and Nitroso methyleurea separately with intermittent shaking for 9 h. The seeds were subsequently washed in running tap water for 24 h and sown in pots. The remaining 5 seeds of each dose and all the 15 seeds of 5 kR dose were directly sown in pots. For meiotic analysis, flower buds fixed overnight in 1:3 acetic-ethanol were squashed in 1% acetocarmine.

OBSERVATIONS

Post-irradiation treatment with Naz, MMS or EMS was met with 100% lethality as no seed germination was recorded in these treatments. Likewise, beyond 40 kR dose of gamma rays also the germination as well as survival percentage was nil. Three desynaptic lines were isolated in the mutation population. Observations recorded on type, average and range of associations have been given in Tables 1 and 2.

Ds_1 : This mutant was detected in the combined treatment of 25 kR gamma rays + 0.15% NMU. Five of 8 plants obtained from the same treatment were normal diploids.

The Ds_1 plant was characterized by the presence of univalents in all cells. The average number of associations per cell was 2.18 bivalents + 9.6 univalents, range being 0—5 and 4—14, respectively (Fig. 3). The bivalents, on the average resolved into 0.08 ring and 2.1 rod bivalents, range being 0—2 and 0—5, respectively. In comparison, the control plants had on the average 6.08 bivalents + 1.84 univalents, range being 5—7 and 0—4, respectively (Figs. 1,2). The bivalents in control plants resolved into 3.48 ring and 2.6 rod bivalents, range being 0—7 and 0—6, respectively. Evidently, ring bivalents in Ds_1 were more prone to univalent formation. Number of chiasmata in Ds_1 ranged from 0—5, mean being 2.3 (terminalized), giving terminalization coefficient of 1.0. In comparison, the control plants had on the average 9.6 chiasmata, range being 6—14, out of which 8.8 were terminalized giving terminalization coefficient of 0.91.

Sixty-five per cent of the cells at anaphase I had equal (7:7) distribution of chromosomes followed by 8:6 and 9:5 distribution in 20.0 and 5.0% cells, respectively. The remaining 2 cells (10.0%) had 2 chromosomes lagging behind. The

←

Figs. 1-12 : 1,2. 7_{II} and $6_{II}+2_I$ respectively at metaphase I in control. 3,4. 14 I at metaphase I in M_1 and M_2 of Ds_1 respectively. 5. 14_I at metaphase I of M_1 in Ds_2. 6,7. $3_{III}+8_I$ and $2_{II}+10_I$ respectively in M^1 of Ds_3. 8-12. $6_{II}+2_I$, $5_{II}+4_I$, $2_{III}+3_{II}+2_I$, $2_{II}+10_I$ and $4_{II}+6_I$ respectively in M_3 of Ds_3 (X 1330).

TABLE 1 : Average number and range of associations.

Material	Gene-ration	No. of Cells anal-ysed	Association								Polien staina-bility (%)	Mean seed set/pod
			Ring bivalents		Rod bivalents		Total bivalents		Univalents			
			Range	Mean	Range	Mean	Range	Mean	Range	Mean		
Control		25	0-7	3.48 ±1.93	0-6	2.6 ±1.68	5-7	6.08	0-4	1.84	61.0	3.0(20)*
Ds_1	M_1	20	0-2	0.08 ±0.4	0-5	2.1 ±1.23	0-5	2.18	4-14	9.6	28.0	1.0(4)
	M_2	25	0-7	3.18 ±2.9	0-4	1.8 ±1.96	0-7	4.98	0.14	4.02	50.0	1.50(14)
Ds	M_1	20			0-4	1.9 ±1.25	0.4	1.9	6-14	10.2	24.0	0.8(5)
	M_2	31	1-6	2.55 ±2.12	2-5	2.58 ±1.81	1-7	5.13	0-12	3.61	40.0	1.5(8)
Ds_2	M_1	20	0-2	0.22 ±0.5	0-3	0.38 ±0.69	0-3	0.6	8-14	12.8	20.0	—

* Number of pods collected.

TABLE 2 : Associations at metaphase I.

Material	Gener-ation	No.of cells anal-ysed	Cells with chromosome associations %								Univalents/cell	
			7_{II}	$6_{II}+2_I$	$5_{II}+4_I$	$4_{II}+6_I$	$3_{II}+8_I$	$2_{II}+10_I$	$1_{II}+12_I$	14_I	Range	Mean
Control		25	36.0	36.0	28.0	—	—	—	—	—	0-4	1.84
Ds_1	M_1	20	—	—	5.0	—	25.0	35.0	25.0	10.0	4-14	9.6
	M_2	25	20.0	36.0	16.0	8.0		16.0		4.0	0-14	4.02
Ds_2	M_1	20	—	—	—	—	5.0	15.0	15.0	65.0	8-14	12.8
Ds	M_1	20	—	—	—	15.0	5.0	55.0	5.0	20.0	6-14	10.2
	M_2	31	25.8	28.98	12.88	22.54	3.22	3.22	3.22	—	0-12	3.61

distribution in the control was equal (7:7) in 71.42% cells and in 28.56% cells the distribution was either unequal (8:6) or 1—2 univalents/bivalents were seen to lag behind. The pollen stainability in Ds_1 was 28% as compared to 61% in the control plants. The seed set never exceeded 1/pod as against 4—6 seeds/pod in the control and in all, 4 seeds were collected from Ds_1 mutant. All the four seeds collected from Ds_1 were sown for M_2 generation and of these, 2 were meiotically analysed. The average number of associations per cell in M_2 plants was $4.98_{II} + 4.02_I$ and the bivalents resolved into 3.18 ring and 1.8 rod bivalents (Fig. 4). A few PMCs had very small bivalents for the reasons unknown. The stainable pollen showed marked improvement from 28% in M_1 to 50% in M_2. The average number of seeds/pod and pods/plant also showed upward trend from 1 and 4 in M_1 to 1.5 and 14 in M_2, respectively.

Ds_2 : This plant was isolated in M_1 of the seeds irradiated with 40 kR gamma rays.

The average number of associations per cell was $0.6_{II} + 12.8_I$. The bivalents resolved into 0.22 ring and 0.38 rod bivalents, range being 0–2 and 0–3 respectively (Fig. 5). Number of chiasmata ranged from 0—4, mean being 0.75 (terminalized) giving terminalization coefficient of 1.0.

The cells at anaphase I had equal (7:7) distribution in 50% of cells and unequal (8:6, 9:5) or lagging univalents/bivalents in another 50% of cells. The pollen stainability was only 20% and the plant was total seed sterile (Table 1).

Ds : The plant was detected in M_1 of post-irradiated (40kR+0.15% NMU) treatment.

The average number of associations per cell was $0.9_{II} + 10.2_I$ and all bivalents were rod bivalents (Figs. 6,7). The range of chiasmata was 0—4, mean being 1.9 (terminalized), giving terminalization coefficent of 1.0.

At anaphase I, 60% of cells had equal disjunction. Remaining 40.0% cells had either unequal distribution (9:5, 8:6) of chromosomes or 2 chromosomes were lagging behind. The pollen stainability was 24%. The total number of seeds collected from the 5 pods was 4 at an average of 0.8 seed/pod. The 4 seeds collected from Ds_3 were sown for M_2 and out of these, only 2 plants could be meiotically investigated.

The mean frequency of univalent bearing cells was relatively less than those in M_1. The average number of associations was $5.13_{II} + 3.61_I$ The mean number of ring (2.55) and rod (2.58) bivalents per cell was almost the same (Figs. 8-12). The number of chiasmata ranged from 2-12, mean being 8.06, out of which 7.26, were terminalized giving terminalization coefficient of 0.9.

All the ten cells analysed at anaphase I had equal (7:7) disjunction. Pollen stainability increased from 24% in M_1 to 40% in M_2. In all, 12 seeds were collected from M_2.

DISCUSSION

The desynaptic or asynaptic nature of a synaptic mutant could be ascertained only after pachytene analysis. However, wherever partial failure of pairing is observed, even pachytene analysis is not helpful in deciding either asynaptic or desynaptic nature of these mutants (Riley & Law 1985). In the absence of pachytene studies the present mutant lines have been tentatively designated as desynaptic lines (Ds_1, Ds_2, Ds_3) although prevalence of a high frequency of rod bivalents would tempt to conclude that mutated genes in these 3 mutants do not govern chiasma formation but chromosome pairing. The pairing would be interrupted as soon as it starts at the end of the chromosomes. With the result, rod bivalents with mostly terminal chiasmata were seen even at early stages or as univalents when chiasmata were not formed.

Besides being found spontaneously, synaptic mutations have also been induced by gamma or X-rays (Martini & Bozzini 1966, Bozzini & Martini 1971, Gottschalk & Baquar 1971, Singh et al. 1977), chemical mutagens (Sharma & Reinbergs 1974, Tyagi & Das 1975, Singh et al. 1977) and by combined treatments of physical and chemical mutagens (Singh et al. 1977). Although the mutants studied presently also appeared after the mutagenic treatments, it is not possible to conclude whether the mutations were induced or spontaneous solely because the mutants appeared as whole plant mutants in M_1. There is reason to believe that it might not have been induced by the treatment but occurred in the material as a spontaneous mutation arisen in an earlier generation.

In the absence of detailed segregational studies it was not possible to ascertain the number of gene(s) controlling desynapsis in Ds_1, Ds_2 and Ds_3. At the same time it is interesting to note that all the 3 mutants, under the same climatic and edaphic conditions, differed markedly in the degree of desynapsis. How far such positive evidence favours polygenic control of synapsis is a matter to be investigated in detail.

ACKNOWLEDGMENTS

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INDUCED TETRAPLOIDY IN *CLITORIA*

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SUMMARY

Induction of tetraploidy in *Clitoria ternatea* Linn. cv. white, *C. ternatea* cv. violet, *C. ternatea* cv. sutton, *C. ternatea* var. *pleniflora* f. *pleniflora* Fantz and *C. biflora* Datz. was achieved by cotton swab and cotyledonary immersion methods. All morphological features of induced polypoidy such as slow growth, late flowering, increase in cell size and gigantism were expressed in the *ternatea* lines. The tetraploids of all the cultivars of *C. ternatea* were much better ornamentals than their corresponding diploids. The double flowers of *C. ternatea* var. *pleniflora* f. *pleniflora* were most expressive and beautiful to look at. The tetraploids had a low quadrivalent frequency and a high bivalent average. The smaller chromosomes formed more quadrivalents than larger chromosomes in cultivars of *C. ternatea*. Nevertheless, all but *C. ternatea* cv. white were total seed sterile. The 2C DNA amount and chromatin area in the tetraploid *C. ternatea* cv. white were approximately twice the value observed in the diploid progenitor.

Key Words : *Clitoria*, colchicine, polyploidy, chromosome associations, DNA.

INTRODUCTION

Clitoria ternatea Linn. (Leguminosae), commonly known as butterfly pea is widely grown in warmer parts of the world for its multiple uses in horticulture, medicine and agriculture (Fantz 1980). Four of 10 available cultivars of *C. ternatea* viz., *C. ternatea* cv. white, *C. ternatea* cv. violet, *C. ternatea* cv. sutton and *C. ternatea* var *plenifora* f. *pleniflora* were selected for inducing tetraploidy mainly because of their relatively better ornamental value. In *C. ternatea* cv. sutton the number of flowers is always more than one per axil and the size of the petals is relatively smaller. *C. ternatea* var. *pleniflora* f. *pleniflora* has beautiful showy "double" flowers with all the petals of almost equal size. *C. biflora* was also subjected to colchicinization, chiefly because it differed from *C. ternatea* in chromosome number.

Successful induction of autotetraploidy in *C. ternatea* cv. white (C_0) had been already reported (Srivastava & Raina 1982 b). Present communication deals with a further generation (C_1) of above colchitetraploids and also the C_0 generations of *C. ternatea* cv. violet, *C. ternatea* cv. sutton, *C. ternatea* var. *pleniflora* f. *pleniflora* and *C. biflora*. For comparison, the data of induced tetraploidy in

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C. biflora cv. white (C_{10}) have also been incorporated in the present communication. Methods followed for colchicination and meiotic analysis have been detailed elsewhere (Srivastava & Raina 1982 a, b).

OBSERVATIONS

Efficiency of colchicination

The induction of tetraploidy was only by cotton swab and cotyledonary leaves immersion methods. The seed treatment method was met with complete failure. Not a single soaked germinated after the seed treatment. In *C. ternatea*, out of 125 seedlings treated (0.15% colchicine) for 12 h (cotton swab method) equally spread over 2 days, 9 were cytologically confirmed as tetraploids. The highest number of tetraploid plants was recovered in *C. ternatea* cv. white (5) followed by *C. ternatea* cv. violet (2), *C. ternatea* cv. sutton (1) and *C. ternatea* var. *pleniflora* f. *pleniflora* (1). In *C. biflora*, 2 out of the 20 seedlings treated (cotton swab method) were found to be tetraploids. The highest (16.66%) and the lowest (5.0%) percentage success was observed in *C. ternatea* cv. white and *C. ternatea* var. *pleniflora* f. *pleniflora*, respectively. The cotyledonary leaves immersion method was successful in only *C. ternatea* cv. sutton. Out of the 20 seedlings treated, only one plant (10.0%) turned out to be a tetraploid.

Growth rate

The immediate effect of colchicinization was reflected in the seedling of apical region of the seedlings. As compared to the diploids, the first leaf emerged after 10-15 days in expected colchiploids. They exhibited slower growth rate and the flowering was delayed by 10-15 days in *C. biflora* and about 20-30 days in the 4 cultivars of *C. ternatea*.

Cell size

The doubling of chromosome number was associated with increase in cell size and such increase was reflected in pronounced increase in the size of stomata and pollen grains (Table 1). The size of pollen grains increased by 10.29, 11.42, 11.42, 15.38 and 40.44 per cent in *C. ternatea* cv. sutton, *C. ternatea* cv. white, *C. ternatea* var. *pleniflora* f. *pleniflora*, *C. ternatea* cv. violet and *C. biflora*, respectively. The stomatal frequency per unit area showed 37.5, 37.5, 46.2, 46.2 and 38.55 per cent decrease in *C. ternatea* cv. violet, *C. ternatea* var. *pleniflora* f. *pleniflora*, *C. ternatea* cv. white, *C. ternatea* cv. sutton and *C. biflora* colchitetraploids, respectively.

Morphology

Plant height, measured only in *C. biflora* as it has erect herbaceous habit was reduced by 42.5 per cent. All the tetraploids had dark green leathery leaves. Other parts such as, rachis, internodes leaflets, calyx, corolla, stamens and gynoecium also had pronounced effect due to colchicinization (Table 1). The flowers in

TABLE 1 : Morphological features of diploids and corresponding tetraploids

Characters	*C. biflora*		*C. ternatea* cv. white		*C. ternatea* cv. violet		*C. ternatea* cv. sutton		*C. ternatea* var. *pleniflora* f. *pleniflora*	
	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x
Leaf texture Leaf colour	Thin Green	Thick Dark green	Thin Green	Thick Dark green	Thin Green	Thick Dark green	Thin Green	Thick Dark green	Thin Green	Thick Dark green
Length of rachis (cm)	3.5	5.5	5.5	6.5	4.5	5.5	6.0	7.5	7.5	9.0
Length of odd leaflet (cm)	6.0	6.6	2.5	3.0	2.5	3.0	3.5	4.0	3.5	4.2
Breadth of odd leaflet (cm)	2.7	3.1	2.2	2.7	1.2	1.5	2.0	2.2	2.5	2.7
Length of stomata (μm)	22.1	28.2	20.0	25.0	20.0	25.0	20.0	25.0	20.0	25.0
Breadth of stomata (μm)	14.3	13.8	15.0	20.0	15.0	20.0	15.0	20.0	15.0	20.0
No. of stomata per unit area	16.6	10.2	39.0	21.0	40.0	25.0	39.0	21.0	40.0	25.0
Internodal legth (cm)	3.0	3.2	8.0	9.5	7.5	9.0	4.5	5.0	7.5	9.0
No. of flowers per axil	2-3	2-4	1-2	1-4	1	1	1-3	1-5	1	1
Length of standard petal (cm)	0.7	0.9	3.5	4.2	3.0	3.5	2.5	2.8	4.0	4.3
Breadth of standard petal (cm)	0.4	0.7	3.5	4.0	2.5	3.0	2.0	2.2	3.0	3.2
Length of wing petal (cm)	0.4	0.6	2.0	2.5	2.0	2.5	1.8	2.0	4.0	4.4
Breadth of wing petal (cm)	0.1	0.3	1.0	1.2	1.8	1.0	1.0	0.9	3.0	3.0
Length of calyx tube (cm)	0.6	0.7	1.0	1.2	1.8	2.0	1.5	1.8	1.3	1.7
Length of stamen (cm)	0.1	0.2	0.5	0.7	0.3	0.4	0.3	0.4	1.7	2.0
Length of gynoecium (cm)	1.0	1.2	1.2	1.4	0.9	1.0	0.7	0.8	2.5	2.7
Pollen size (μm)	50.9	72.5	70.0	78.0	65.0	>80	68.0	75.0	70.0	78.0
Pollen stainability (%)	31.0	16.0	86.0	71.0	90.0	48.0	85.0	52.0	65.0	53.0
Length of pod (cm)	4.0	—	8.5	7.0	8.5	—	8.5	—	6.5	—
Seeds per pod (range)	2-4	—	2-8	0-1	2-8	—	2-8	—	4-7	—
Height of the plant (cm)	80-0	46.0	—	—	—	—	—	—	—	—

autotetraploids were of bigger size and their lustre period was prolonged by 5 days. In *C. ternatea* cv. white, the number of flowers per axil increased from normal 1-2 to 1-4 in synthetic tetraploids. The colchitetraploids of all the 4 cultivars of *C. ternatea* were clearly much better ornamentals than their corresponding diploids.

Cytology

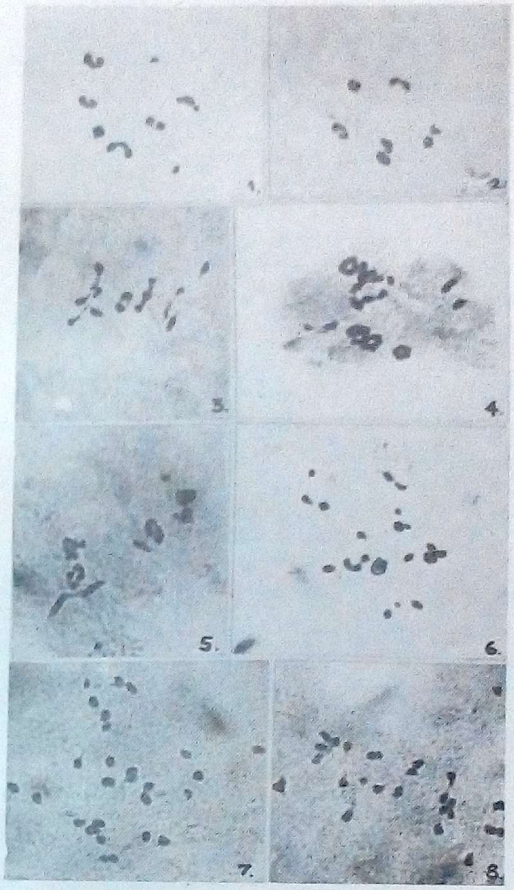
C. biflora : In the diploid, the most common associations encountered were 7_{II} (36%) and $6_{II}+2_{I}$ (36%), followed by $5_{II}+4_{I}$ in 28% of cells (Figs. 1-3). On the average there were 8.08_{II} and 1.84_{I} per cell. The bivalents resolved into 3.48 ring and 2.6 rod bivalents. The chiasma frequency was 9.6 per cell.

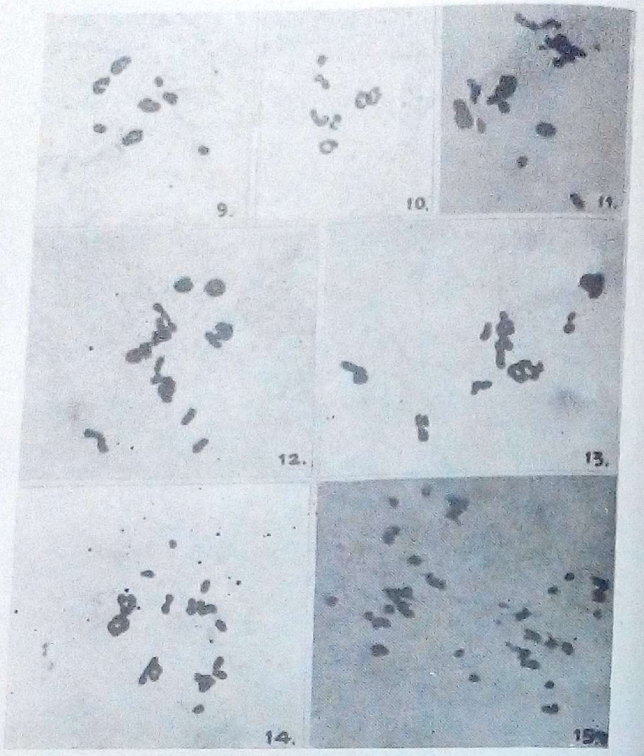
In the 2 autotetraploids (Figs. 4-6), the mean number of bivalents per cell (5.48) was appreciably higher than those of quadrivalents (2.96) and trivalents (0.16), range being 1-10, 2-5 and 0-1 respectively. The mean number of univalents (4.72) was also quite high. Most of the multivalents were either of ring or chain type. The average number of associations per cell was $2.96_{IV}+0.16_{III}+5.48_{II}+4.72_{I}$. The range of chiasmata was 10-27, mean being 19.72, out of which 19.28 were terminalized, giving terminalization coefficient of 0.98. Only 40% of cells at anaphase I exhibited equal (14:14) distribution of chromosomes. This was followed by unequal (15:13, 16:12) distribution in 36.0 per cent of cells. The remaining 24% of cells had either 2-3 univalents or a bivalent lagging behind. Anaphase II and telophase II were highly irregular and all the cells analysed had more than 4 poles.

C. ternatea cv. white : The diploid had 8_{II} in 90% of cells followed by $I_{IV}+6_{II}$ and $6_{II}+4_{I}$ in 10% of cells. The average number of associations per cell was $0.03_{IV}+7.86_{II}+0.2_{I}$. The bivalents resolved into 5.04 ring and 2.82 rod bivalents. The mean number of chiasma per cell was 13.26.

The 5 colchitetraploid plants were characterized by the presence of multivalents, bivalents and univalents and the range of hexavalents, quadrivalents, trivalents, bivalents and univalents was 0-1, 0-6, 0-3, 2-14 and 0-8, mean being 0.01, 3.32, 0.45, 8.0 and 1.3 respectively. The ring and chain quadrivalents outnumbered other types of configurations. The number of chiasmata ranged from 18-32, mean being 25.0, out of which 22.75 were terminalized giving terminalization coefficient of 0.91. Out of 25 cells analysed at anaphase I, 52% had equal (16:16) distribution of chromosomes followed by unequal (15:17) distribution in 16% ofcells. The remaining 32% of cells had 1-2 univalents/bivalents lagging behind.

Figs. 1-8 *C. biflora* 1-3. Diploid. MI ($6_{II}+2_{I}$) 2, 3. MI (7_{II}). 4-8. Antotetraploid. 4-6. MI (4, $4_{IV}+5_{I}+2_{II}$, 5, $5_{IV}+3_{II}+2_{I}$, 6, $3_{IV}+5_{II}+6_{I}$). 7,8-Autotetraploid, early AI. (all X 1330).





Figs. 9-15 : 9-12, 15. *C. ternatea* cv. violet. 9,10. Diploid, MI (8_{II}). 11,12. Autotetraploid, 11, MI ($4_{IV}+8_{II}$) 12, MI ($3_{IV}+9_{II}+2_{I}$) 15.AI(16:16). 13, 14. *C. ternatea* cv. white. 13, C_1, MI($4_{IV}+1_{III}+6_{II}+1_{I}$) 14, ($3_{IV}+9_{II}+2_{I}$) (all X 1330).

In comparison to C_0 plants the 2 C_1 plants showed a slight increase in the mean number of quadrivalents and trivalents. Further, due to high frequency of univalents per cell there was appreciable decrease in mean number of bivalents. The average number of associations per cell was $3.5_{IV} + 0.71_{III} + 5.91_{II} + 4.03_{I}$ range being 0-5, 0-3, 0-12 and 0-11 respectively (Figs. 13, 14). The ring and chain quadrivalents predominated over other configurations. While the quadrivalents (3.5) resolved into 1.5 larger and 2.0 smaller quadrivalents, the trivalents (0.71) resolved into 0.4 big and 0.31 small trivalents indicating thereby that smaller chromosomes in the complement are slightly more favoured in the multivalent formation. The number of chiasmata ranged from 16-29, mean being 23.08 out of which 22.35 were terminalized giving terminalization coefficient of 0.97. Majority of cells (55%) at anaphase I had equal (16:16) distribution of chromosomes.

The root tip cells of one of 10 seeds collected from C_0 plants had 26 instead of 32 chromosomes. The 26 chromosomes resolved into 8 groups of 2 or 4 chromosomes each. The other 9 seeds had 32 chromosomes which resolved into 8 groups of 4 chromosomes each.

C. ternatea cv. violet : The diploid had 8_{II} in all the cells analysed (Figs. 9, 10). The bivalents on the average resolved into 5.46 ring and 2.54 rod bivalents. The mean chiasma frequency per cell was 13.6.

The average number of associations per cell in the 2 tetraploids was $0.16_{IV} + 0.03_{V} + 3.2_{IV} + 0.39_{III} + 7.87_{II} + 1.1_{I}$, range being 0-2, 0-1, 0-6, 0-4, 3-12 and 0-6, respectively (Figs. 11,12). The ring and chain quadrivalents outnmbered other types of configurations. Number of chiasmata ranged from 18-31, mean being 25.1, out of which 23.97 were terminalized giving terminalization coeffient of 0.96. Majority (55%) of the cells at anapahase I were characterized by the presence of unequal (14:18, 15:17, 13:19) distribution of chromosomes, followed by equal (16:16, Fig. 15) distribution of chromosomes in 45% of cells.

C. ternatea cv. sutton : In the diploid, majority (76.66%) of the cells were characterized by 8_{II} (Fig. 16) followed by $7_{II}+2_{I}$ in 23.34% of cells. The bivalents on the average resolved into 5.47 ring and 2.3 rod bivalents per cell. The mean chiasma frequency per cell was 14.32.

In the 2 colchitetraploids, bivalents (8.0) outnumbered other associations. The average number of associations per cell was $3.04_{IV}+0.29_{III}+8.0_{II}+2.94_{I}$, range being 0-6, 0-2, 4-14 and 0-9 respectively (Figs. 17, 18). The ring and chain quadrivalents predominated over other types of configurations. The number of chiasmata ranged from 12-29, mean being 21.17, out of which 20.21 were terminalized giving terminalization coefficient of 0.94. At anaphase I as many as 60 per cent of cells had either unequal (18:14, 17:15, 19:13) distribution of chromosomes or the abnormalty was due to lagging of 4 chromosomes.

C. ternatea var. *pleniflora* f. *pleniflora*. The diploid had 8_{II} in majority (94.23%) of the cells (Fig. 19). The remaining 5.77% of cells had 9 bivalents instead of normal 8. The 8 bivalents resolved on the average into 6.44 ring and 1.56 rod bivalents. The mean chiasma frequency per cell was 14.32.

The colchitetraploid plant had on the average $4.35_{IV}+0.42_{III}+5.32_{II}+2.68_{I}$ per cell, range being 2-6, 0-2, 2-10 and 0-8 respectively. The quadrivalents (4.35) resolved into 1.98 larger and 2.37 smaller sized quadrivalents. The average frequency of smaller (1.21) and larger (1.21) trivalents was, however, equal. Thus the average frequency of small multivalents, as in *C. ternatea* cv. white, was higher than that of bigger multivalents (Figs. 21-11). The ring and chain quadrivalents outnumbered other types of configurations. The number of chiasmata ranged from 19-31, mean being 16.65, out of which 15.41 were terminalized giving terminalization coefficient of 1.95. As in *C. ternatea* cv. violet and *C. ternatea* cv. sutton, majority (61%) of the cells at anaphase I had abnormalities either due to unequal (17:15) distribution or lagging bivalents/univalents.

Pollen stainability

The change in the ploidy level from diploid to autotetraploid was accompanied by decrease in pollen stainability from 31.0 to 16.3, 86.1 to 71.0, 91.1 to 48.1, 85.1 to 52.1 and 65.1 to 55.1 in *C. biflora*, *C. ternatea* cv. white, *C. ternatea* cv. violet, *C. ternatea* cv. sutton and *C. ternatea* var. *pleniflora* f. *pleniflora* respectively (Table 1). The C_1 plants in *C. ternatea* cv. white did not show any appreciable change in the pollen stainability.

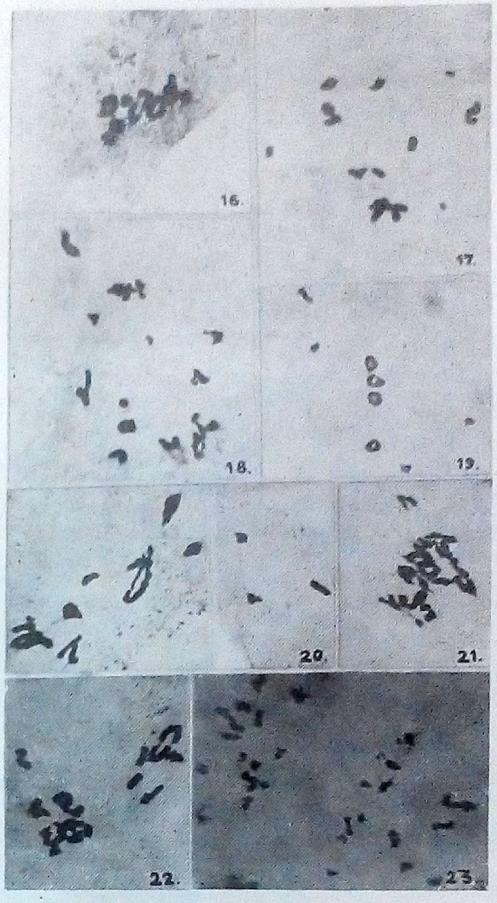
Seed set

Partial seed set was noticed only in *C. ternatea* cv. white. The number of seeds per pod in C_0 and C_1 plants of *C. ternatea* cv. white ranged from 0-1. In all, 21 seeds were collected from from C_0 and C_1 plants. The autotetraploids of *C. biflora*, *C. ternatea* cv. violet, *C. ternatea* cv. sutton and *C. ternatea* var. *pleniflora* f. *pleniflora* were total seed sterile (Table 1).

Nuclear DNA and chromatin area

As expected, the 2C DNA (9.83 pg) and chromatin area (81.8 arb. units) values in C_0 generation of *C. ternatea* cv. white were almost double to their corresponding values in the diploids (4.84 pg., 44.12 arb. units) with no appreciable change in DNA density.

Figs. 16-23: 16-18. *C. ternatea* cv. sutton. 16. Diploid, MI (8_{II}). 17,18. Autotetraploid. 17. MI ($3_{1IV}+2_{III}+6_{II}+2_I$) 18. MI($2_{IV}+7_{II}+10_I$) 19-23. *C. ternatea* var. *pleniflora* f. *pleniflora*. 19. Diploid MI (8_{II}). 20-23, Autoteraploid. 20, MI($3_{IV}+10_{II}$). 21, MI($3_{IV}+9_{II}+2_I$) 22. MI($4_{IV}+11_8$) 23. AI(16:16). (all X1330.)



DISCUSSION

The cotton swab method, as in *Tephrosia* (Srivastava & Raina 1982a), was found to be most efficient in inducing tetraploidy in *Clitoria*. The colchitetraploids were no exception to the general morphological and cytological features met with in induced polyploids. The increase in cell size evidenced by the size of pollen grains and stomata and consequent decrease in number of cells per unit area was reflected by the stomatal frequency. The dark green leaves were broader and thicker. The determinate organs like floral parts and seeds also showed a considerable increase in size and the violet petals and deeper pigmentation. All these features are towards better ornamental value of *C. ternatea* tetraploids as in *Impatiens balsamina* (Jalil et al. 1978), *Verbena tenuisecta*, *V. incisa*, *V. platensis* (Arora 1975), *Tabernaemontana divaricata* (Chauhan & Raghuvanshi 1978), *Bougainvillea* (Zadoo et al. 1975), *Zinnia elegans* (Gupta & Koak 1976) *Gerbera jamesonii* (Jalil 1978) and *Phlox drummondii* Rama Rao et al. 1981). The double flowers in the colchitetraploid of *C. ternatea* var. *plenifloro* f. *pleniflora* were most impressive and beautiful to look at. Total seed sterility in all, but *C. ternatea* cv. white is circumvented by the perennial nature of *C. ternatea*. The fooder value of *C. ternatea* tetraploids could be ascertained only after all the data analysis is made on the total yield per plant and also the nutrient content of the more important parts of the plant(s).

The synthetic colchitetraploids were characterized by low quadrivalent and high bivalent average. The average number of quadrivalents ranged from 1-96 in *C. biflora* to 4.35 in *C. ternatea* var. *plenifloro* f. *pleniflora*. Interestingly, the cultivars of *C. ternatea* exhibited considerable differences in mean number of associations inspite of having overall symmetry (4 larger and 4 smaller chromosome pairs) of the chromosome complement in the diploids. Such differences in the mean frequency of associations clearly irdicate the inherent genotypic differences among the 4 cultivars.

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SYNAPTIC MUTANTS IN POLYPLOID *TEPHROSIA PURPUREA* (LINN.) PERS.

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SUMMARY

Induction of colchitetraploidy in *T. purpurea* was achieved in 2 seedlings. Of these, 2 types of cytocolchitetraploids were recognized, one showing meiotic behaviour typical of autotetraploids throughout the year and the other behaving as a desynaptic mutant in different seasons of the year. The desynaptic mutant (C_0Ds_1) and the normal autotetraploid plants had on the average only 1.67 I per cell, which in the former increased to 32.15 I during cold weather. Another desynaptic mutant (Ds_1), recovered from the reverted seed of colchioctoploid, had mean univalent frequency of 33.6 per cell during cold weather. This plant (Ds_1) behaved like a normal tetraploid (0.39 I /cell) on the onset of favourable conditions.

It is concluded that the differential behaviour of colchiploids at meiosis might be attributed to genetic dissimilarity of seeds used for colchicinization and desynaptic cytocolchitetraploid might have been desynaptic even at lower ploidy level. In the case of Ds_1, the seed from which the octoploid was derived might have been heterozygous for desynaptic gene (S) and segregation resulted in a mutant plant homozygous for mutant genes.

Key Words : *Tephrosia purpurea*, autotetraploids, desynapsis.

INTRODUCTION

Contrary to the long list of desynapsis and asynapsis in diploid plants, the spontaneous occurrence of asynapsis and desynapsis in both induced and natural polyploids is confined to hardly a few taxa, *Primula kewensis* (Newton & Pellew 1929), *Allium cepa* (Gohil & Kaul 1971), *Lolium perenne* (Ahloowalia 1969 a), *Secale cereale* (Kolobaeva 1974), *Artemisia doughlasiana* (Estes 1971), *Paspalum longifolium* and *P.commersonii* (Pi & Chao 1974), *Pennisetum typhoides* (Subba Rao 1978) and *Tephrosia wallichii* (Srivastava & Raina 1981).

During the course of present investigation two desynaptic mutants, Ds_1 and C_0Ds_1 were isolated in natural tetraploids and colchitetraploid populations of *Tephrosia purpurea* respectively.

MATERIAL AND METHODS

The seeds of diploid and tetraploid *T. purpurea* growing in western Rajasthan, India were treated with conc. H_2So_4 for 10-30 min and sub-subsequently washed thoroughly in tap water for 24 h. The seeds were then sown both

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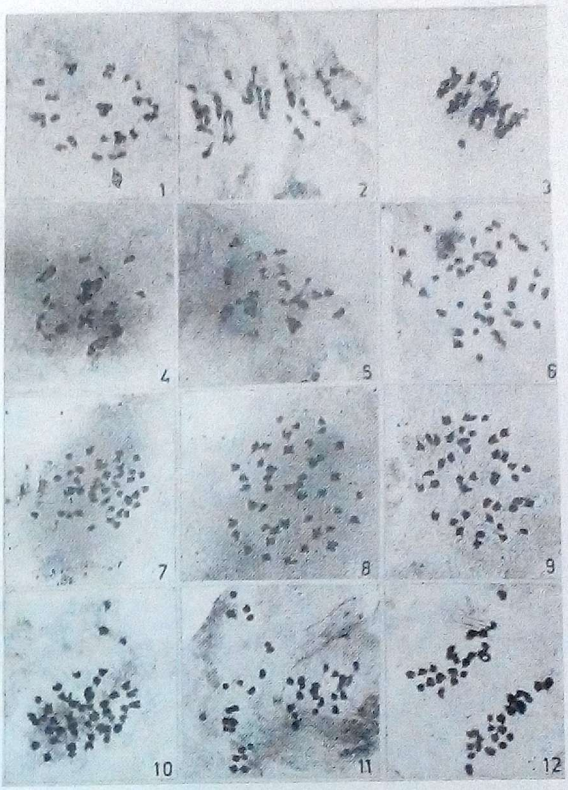
in pots and on moist filter paper. For colchicinization seed treatment, cotton swab and cotyledonary leaves immersion methods were employed as detailed elsewhere (Srivastava & Raina 1981). For meiotic analysis, flower buds fixed overnight in acetic-ethanol (1:3) were squashed in 1% acetocarmine.

OBSERVATIONS

Colchicinization was successful in only 2 out of 20 seedlings treated. Of them, one (C_0) was normal behaving as colchitetraploid and the other one (C_0Ds_1) was desynaptic in behaviour. The Ds_1 plant was raised from a seed collected from a colchioctoploid plant due to reversion. All other plants (4) raised from the seeds collected from the same octoploid plant, were normal tetraploids. All morphological features except the plant height were similar in C_0 and C_0Ds_1 plants. The C_0 plant (60 cms.) was taller by 25 cm. On the contrary, the Ds_1 and other 4 'revert back' tetraploids were similar in all morphological features.

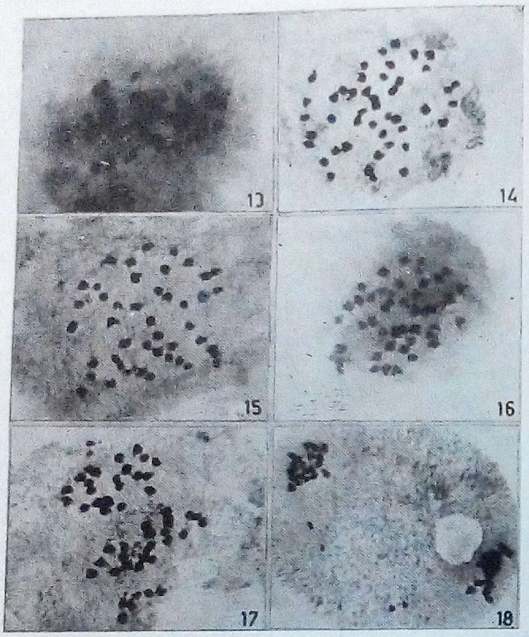
Cytology

The chromosome number in *Tephrosia purpurea* is 2x=22 and 4x=44. The diploid plants exhibited 11_{II} at metaphase I. Meiotic behaviour in both C_0 and C_0Ds_1 was exactly the same. The average number of associations per cell was $3.13_{IV}+0.67_{III}+13.9_{II}+1.67$ their range being 1-5, 0-2, 8-20 and 0-4, respectively. Thus C_0Ds_1 behaved like C_0 when suitable environmental conditions of 35-40°C temperature and 70-80% humidity were available (Figs. 1-5). The differential chromosome synapsis was expressed when temperature (10-15°C) and humidity (40-50%) were relatively much low. The mean number of associations in C_0 plant matched entirely with the analysis during suitable climatic conditions. In C_0Ds_1, however, the average number of univalents per cell rose from 1.67 to 32.16, their range being 0-44 (Figs. 6-11). Nine out of 30 cells analysed had complete failure (44_I) of pairing. The average number of associations was not $0.47_{IV}+0.1_{III}+4.83_{II}+32.16_I$, their range being 0-5, 0-1, 0-15 and 0-44, respectively. Under favourable climate, the mean number of chiasmata in C_0 and C_0Ds_1 was 31.76, their range being 25–42, out of which 31.6 were terminalized, giving terminalization coefficient of 0.99. The corresponding values for C_0Ds_1 under low temperature and humidity were 10.76, 0.38, 10.64 and 0.99. The C_0 plant however, exhibited the same chiasmatic behaviour. Under favourable climate out of the 20 cells analysed in C_0Ds_1 11 had equal (22 : 22) distribution of chromosomes followed by unequal 23 : 21, 20 : 24 distribution in 4 cells. In the remaining 5 cells 1 or 2 chromosomes were seen to lag behind. Almost the same kind of frequency of abnormality was noticed in C_0 plant also. When C_0Ds_1 was again analysed under low temperature and humidity, it did not show much deviation from the above data (Fig. 12).



Figs. 1-12 : Male meiosis in normal and desynaptic (C_0Ds_1) colchiploid of *T. purpurea*. (all × 1330). 1-3. MI in normal colchiploid. 1. $3_{IV}+14_{II}+4_I$. 2. $2_{IV}+16_{II}+4_I$. 3. $5_{IV}+10_{II}+4_I$. 4-5. M I in C_0Ds_1, 4. $6_{IV}+9_{II}+2_I$, 5. $2_{IV}+14_{II}+4_I$, 6. Diakinesis in C_0Ds_1 showing 44_I, 7-10. M I in C_0Ds_1 showing 44_I, 11. M I in C_0Ds_1 with $5_{II}+34_I$. 12. A I in C_0Ds_1 with 22 : 22 distribution.

During cold weather, the Ds_1 plant exhibited total failure of pairing (44_I) in 10 out of 20 cells (Figs. 13-17). The remaining 10 cells had $14_{II} + 16_I$, $10_{II} + 24_I$ and $9_{II} + 26_I$ in 2, 4 and 4 cells, respectively. Quadrivalents were altogether absent. The mean number of associations per cell was $5.2_{II} + 33.6_I$, range being 0-14 and 16-44, respectively. For 2 other normal tetraploids the mean number of associations was $0.29_{IV} + 21.22_{II} + 0.39_I$, range being 0–2, 16-22 and 0-4, respectively. Interestingly, when Ds_1 was analysed on the onset of favourable weather, it behaved like a normal tetraploid. During cold weather,



Figs. 13-18 : 13-18. Male meiosis in desynaptic tetraploid (Ds_1) *T. purpurea*, 13-16. M I with 44_I, 17. A I with 25 : 19 distribution, 18. A I showing 4 lagging univalents.

number of chiasmata in Ds_1 ranged from 0-23, their mean being 8 out of which 7.9 were terminalized giving terminalization coefficient of 0.98. The corresponding values for other tetraploids were 30-72, 49.06, 25-54 and 0.52, respectively. Nine (45%) out of 20 cells in Ds_1 had equal distribution at anaphase I. The remaining 11 (55%) cells had either unequal (23 : 21) distribution or 1-4 univalents were found to lag behind (Fig. 18). Some cells, where chromosome number could not be counted at the poles, had 1 or 2 precociously dividing univalents. The normal tetraploids had equal distribution in all the cells analysed.

Pollen stainability

The percentage of pollen stainability was same (70%) in both C_0 and C_0Ds_1, but during cold weather, the latter showed a decrease of 10% stainability.

Compared to normal tetraploids (95%) the pollen stainability in Ds_1 was 70% during cold weather.

Seed set

Both C_0 and C_0Ds_1 were partial seed fertile (1-5 seed/pod). During cold weather, although partial seed fertility was maintained in C_0 plant, the C_0Ds_1 plant was total seed sterile.

The average number of seeds set per pod in Ds_1 was 3 during cold weather which increased to normal 5 under favourable weather.

DISCUSSION

The spontaneous occurrence of asynapsis and desynapsis as mentioned earlier has been reported in a large number of plants. Besides being found spontaneously it has also been induced by physical and chemical mutagens (Gottschalk & Kaul 1980 a, b). In the present investigation synaptic mutants were detected among colchitetraploids (C_0) and 'revert back' tetraploids raised as C_1 plants from the seeds collected from a colchioctoploid (C_0) plant of *T. purpurea*. Since both Ds_1 and C_0Ds_1 mutants were recovered following colchicine treatment and not in natural populations, one could infer that they are colchicine induced as stray reports indicate that alkaloid colchicine besides being a classic mitotic inhibitor may act as mutagenic agent also (Eigsti & Dustin 1955). It is reported to induce chromosome/chromatid breaks (Soriano 1957) and also inhibit DNA synthesis (Ilan & Quastel 1966). A perusal of literature reveals that colchicine might reduce pairing to some degree (Sybenga 1972) but not to the extent found in the present material. As the C_0Ds_1 mutant appeared as the only whole plant mutant in C_0 generation there is also reason to believe that it might not have been induced by the colchicine treatment but occurred in the material as the spontaneous mutation arisen in an earlier generation at diploid level. To ascertain whether the present mutants are asynaptic or partially so, is rather difficult. Complete

univalent formation in all or majority of the PMCs at post pachytene stages in the present material does not necessarily mean that the plant is asynaptic or partially so in nature. The only reliable evidence should have come from the pachytene which on technical grounds is very difficult to analyse in *Tephrosia*. In the absence of such studies the present mutants are tentatively designated as desynaptic as some cases of supposed asynaptics have in fact turned out to be desynaptics after pachytene studies (Celarier 1955).

Various causes such as drastic temperature fluctuations, ageing, water content, humidity, soil conditions, apomixis, loss of chromosome pair, structural and numerical changes of chromosomes, interspecific and intergeneric hybrids and above all gene mutation (Gottschalk & Kaul a, b, Sybenga 1972) have been suggested for causing desynapsis/asynapsis.

Under the environmental stress all the plants raised following colchicinization in the present case should have behaved in a similar way, but that was not so. Some plants exhibited normal synapsis of chromosomes while others (Ds_1 and C_0Ds_1) did not. Among the various causes listed above, therefore, genetic control seems to be responsible for the abnormal behaviour. In fact, recent investigations, have clearly demonstrated that like several other events in meiosis, chromosome pairing and subsequent chiasma formation also are controlled by genes at different stages of meiosis (Celarier 1955, Ahloowalia 1969a, Sajodin 1970). Barring *Crepis* (Hollingshead 1930) where monofactorial dominant inheritance has been observed, most of the mutants have been found to exhibit monogenic recessive inheritance. The digenic recessive inheritance has also been reported in some plants (Weaver 1971, Lakshmi et al. 1979). Some plants are known to have desynapsis/asynapsis controlled by more than 2 non-allelic recessive genes (Gottschalk 1973). In the absence of data regarding segregation pattern in the present mutants nothing can be said about the number of gene/genes controlling desynapsis. In the autotetraploid C_0Ds_1 mutant the desynapsis was expressed by reduced multivalent bivalent frequency and increased univalent frequency. In comparison, other autotetraploid (C_0) from the same lot exhibited normal meiosis and were not desynaptic This significant difference between the tetraploids could be attributed to genic differences among the diploids from which tetraploids were derived because autopolyploids arising from desynaptic diploids are expected to be desynaptic as well. The above assumptions regarding causes of desynapsis in *Tephrosia* are in conformity with earlier presumptions derived by present authors (1981) for desynaptic colchitetraploids of *T. wallichii*

The parent of Ds_1 was a hybrid colchioctaploid ($A_1A_1A_1A_1A_2A_2A_2A_2$, Srivastava & Raina 1992) and Ds_1 mutant was recovered from one of the 5 reverted seeds. The remaining 4 did not show desynapsis. It is probable that

the seed from which octaploid was produced was heterozygous for desynaptic mutant and segregation resulted in a mutant plant (Ds_1) homozygous for mutant genes. As the ratio 4:1 or 7:1 (octoploid plants included) does not fall in monofactorial segregation it could be presumed that the desynapsis is governed by more than a pair of allelic genes.

The highest mean frequency of univalents observed in the desynaptic mutants (Ds_1, C_0Ds_1) was found at 10-15°C temperature and 40-50% relative humidity and the lowest mean frequency was observed at 35-40°C temperature and 70-80% relative humidity. In fact, mean number of associations per cell in Ds_1 and C_0Ds_1 tallied more or less with their respective non-desynaptic plants. During cold weather, the mean number of univalents was 33.6 and 32.16 in Ds_1 and C_0Ds_1 respecticely. In comparison, the corresponding values in non desynaptic plants were 0.38 and 1.67, respectively. Such cytological variation of the mutants in different weather is not uncommon (Prakken 1943, Ahloowalia 1969 b). Like Ds_1 anb C_0Ds_1 of *T. purpurea* and earlier reported desynaptics of colchitetraploid *T. wallichii* (Srivastava and Raina 1981), some of the synaptic mutants have normal chromosome pairing and chiasma formation at a particular temperature and humidity (Ahloowalia 1969 b) but show desynapsis under altered temperature and humidity. The mutant genes are considered to be less buffered against environmental fluctuations, than normal genotypes and, therefore, a slight change in the environment might have pronounced effect on meiotic behaviour (Darlington 1958).

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EFFECT OF RESERPINE ON NUCLEAR MORPHOLOGY AND MITOSIS IN *CYCLOTELLA MENEGHINIANA* KUTZ

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SUMMARY

Rawolphia alkaloid, reserpine induced distortion in nuclear morphology and the migration of nucleus to peripheral layer of cytoplasm. Nucleus in 6-80 % of the cells was affected at various concentrations of the alkaloid ranging from 1 to 10 mm. Nutrient medium with additional phosphorus could revert the effect of reserpine up to 3 mm while the effects of 5 and 10 mm alkaloid remained irreversible.

Key Words : Reserpine, mitotoxic, phosphorus

INTRODUCTION

Effect of alkaloids on plants as well as animals and on their cellular mechanisms revealed that most of the alkaloids regulate cell cycle through various inhibitory mechanisms such as accumulation of prophase and metaphase (Sopova et al. 1983), depolymerization of mitotic spindles (Borisy et al. 1975), inhibition of cytokinesis (Encina & Becerra 1986) and protein synthesis (Felipo et al. 1986). However, such inhibitory mechanisms in algae are poorly understood in comparison to higher plants (Sarma & Tripathi 1973, Sarma & Chaudhary 1977). Present paper deals with effects of *Rawolphia* alkaloid, reserpine on the division process of a freshwater centric diatom.

MATERIAL AND METHODS

Cyclotella meneghiniana f. *unipunctata* was grown in Chu-10 medium (Chu 1942) supplemented with D7-micronutrient solution (Arnon et al. 1974) and maintained in a culture chamber with 2.5 klux light intensity, 25±1°C temperature and 16:8 h light and dark periods. Material from 15 day-old fully grown cultures were concentrated, reinoculated in freshly prepared sterilized Chu-10 medium and allowed to grow under standard culture conditions referred above for 48 h before the execution of following experiments.

i) Exponentially growing 48 h-old cells of *C. meneghiniana* f. *unipunctata* were treated with (1. 0, 2. 0, 3. 0, 5. 0 and 10.0 mM reserpine for various time periods ranging from 2-12 h. Cells were washed thoroughly with sterile Chu-10 medium and resuspended in fresh medium devoid of alkaloid. The alga was allowed

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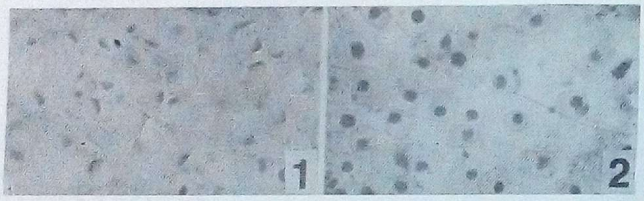
to grow further under standard culture conditions. Subsequently, cells were fixed in acetic-alcohol mixture (1:3) at 1 h interval till 60 h from the time of alkaloid injection and squashed in acetocarmine.

ii) Actively growing cells as referred above were treated simultaneously with different concentrations of reserpine in the presence of 1-10 mM phophorus for 6 h and then squashed as described above.

RESULTS AND DISCUSSION

In reserpine-treated cells the nucleus was observed as shrunken crescent-shaped structure occupying peripheral layers of cytoplasm (Fig. 1) Nuclear morphology was affected in 6-8% of cells when subjected to 1-10 mM reserpine treatments respectively. This resulted in mitotic delay of 0.5 to 60 h in alkaloid affected cells (Table 1). Such a type of excentric nucleus was characteristic of undividing centric (Reimann 1960, Crawford 1973) as well as pennate diatoms (Giri 1989). Retention of nucleus to peripheral layers of cytoplasm and distortion in its morphology as induced by reserpine indicate mitotoxic nature of this alkaloid. Nuclear retention could be due to affected nucleic acid synthesis prior to cell division (Giri 1989) and/or damage of rediating tubules (Wordeman et al. 1986). Either of the 2 mechanisms could have led to mitotic delay ranging from 30 min to 60 h in different reserpine concentrations. Mitotic suppression was also pronounced by other plant alkaloids like caffeine and theobromine in *Oedogonium acmandrium* (Sarma & Tripathi 1973) and *Sphaeroplea annulina* (Sarma & Chaudhary 1977). According to Giri (1989) the inhibition of nuclear migration to central region in *C. meneghiniana* f. *unipunctata* was due to affected nucleic acid synthesis while distortion in nuclear morphology and peripheral suppression could be due to damage of radiating microtubules. Simultaneous addition of reserpine and phosphorus in the medium reduced the number of cells with crescent shaped nuclei (Fig.2) and also significantly reduced mitotic delay (Table 2). Effects of 1, 2, and



Figs. 1 & 2 : *Cyclotella meneghiniana*. 1. Cells with peripherally disposed crescent shaped nuclei. 2. Cells with spherical nuclei showing normal migration to central region prior to mitosis.

TABLE 1 : Effect of reserpine on division process of *Cyclotella meneghiniana* f. *unipunctata*.

Concentration (mM)	Mitotic delay (h) after chemical treatment (9 h)				
	2	4	6	8	12
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1	0.5 (2)	1.2 (4)	2.0 (6)	2.0 (6)	2.0 (6)
2	1.5 (4)	3.3 (10)	5.4 (15)	5.4 (15)	5.4 (15)
3	2.0 (6)	4.5 (13)	8.0 (20)	8.0 (20)	8.0 (20)
5	3.0 (9)	9.0 (21)	22.0 (36)	22.0 (36)	22.0 (36)
10	7.0 (17)	30.0 (40)	60.0 (75)	60.0 (75)	60.0 (80)

Numbers in paranthesces indicate % of cells with crescent nuclei

TABLE 2 : Effect of simultaneous addition of reserpine and phosphorus on the division process of *C. meneghiniana* f. *unipunctata*

Reserpine concentration (mM)	Mitotic delay (h) after 6 h treatment of reserpine				
	0.1 mM Phosphorus (Control)	1 mM Phosphorus	2.5 mM Phosphorus	5 mM Phosphorus	10 mM Phosphorus
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1	2 (6)	1 (3)	0 (0)	0 (0)	0 (0)
2	5.5 (15)	3 (8)	1 (3)	0 (0)	0 (0)
3	8 (20)	6 (16)	2.5 (7)	1 (3)	0 (0)
5	22 (36)	22 (36)	8 (20)	3.3 (10)	2 (6)
10	60 (80)	60 (80)	60 (55)	30 (40)	30 (40)

Numbers in parantheses indicate % of cells with shrunken nuclei

3 mM reserpine was successfully reverted by 2, 5, 5 and 10 mM phosphorus respectively. However, effects of 5 and 10 mM alkaloid were reduced partially. Nuclear morphology in phosphorus protected cells was found to be spherical as in controls with normal migration to the central region prior to cell division. This could be due to complete synthesis of nucleic acids during interphase and normal functioning of radiating tubules operating in equatorial region. Supplementation of additional phosphorus in the medium might have resulted in competetive inhibition of reserpine from binding sites and subsequently in the preservation of ATP-dependent prerequisite cellular processes for mitosis.

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MUTATION AFFECTING SEED COAT AND SEED PROTEIN IN *PHASEOLUS VULGARIS* L.

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SUMMARY

Dry and dormant seeds of *Phaseolus vulgaris* L. var.Top crop has light brown mottled seed coat colour and 23.7% of average seed protein. Mutants having dark brown, deep reddish brown, shining green and pink mottled seed coat colour were isolated in M_2 generation. These mutants bred true in M generation. Data on plant height, number of pods/plant, seed weight/plant, total seed protein, protein fractions and protein profile were recorded both in M_2 and M_3 generations. Plant height showed decreasing trends whereas no marked effects on number of seeds/plant, seed weight/plant, has been observed in different mutants. Marked alteration in seed protein content and protein fractions were recorded. Alteration in seed protein composition is evident from the electrophoretograms. It is thus presumed that these traits may function as morphological markers in breeding for higher protien quantity and quality.

INTRODUCTION

The use of induced mutation for improving seed protein quantity and quality is well documented in literature and have been reviewed by Gottschalk & Wolff (1983). But in the absence of any morphological marker which may be correlated with the alteration in protein quantity or quality, it is a rigorous task to isolate mutants with altered protein characters, because it will need analysis of a large number of samples. Although morphological characters are closely correlated with changes in protein quantity and/or quality in some cases (Prasad et al. 1984) it should not be emphasized that this is a general phenomenon. However, it is emphasized that correlation be sought between easily measured phenotypic characters such as seed size, seed shape, leaf shape etc (Annonymous 1973). Mutants with changed phenotypic characters together with altered protein quantity or quality have been isolated in legumes (Gottschalk & Muller 1970, Bhamburkar and Bhalla 1983, Prasad et al. 1984). In view of the importance of the grain legumes as principal protein source, an attempt to induce and isolate mutants with morphological markers was made and some seed coat colour mutants were isolated. The utilization of these being markers for higher protein quantity and better quality is discussed in the present report.

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MATERIAL AND METHODS

Seeds of *Phaseolus vulgaris* var. Top crop having light brown mottled seeds were obtained from Pocha Seeds, Pune, India. Healthy seeds (13% moisture) were subjected to 5, 10, 15, 20 and 25 kR of ^{60}Co gamma rays. Salient features of the variety, method of irradiation and handling of irradiated materials have been described earlier (Prasad et al. 1984, 1986). Four types of mutants viz. dark brown mottled, deep reddish brown, shining green and pink mottled seed coat colour were isolated from treated population at M_2 generation. These mutants bred true in M_3 generation. Data on some important agronomic traits were recorded in field condition at maturity for both M_2 and M_3 generations. Seed protein content was determined by multiplying microkjeldahl determined nitrogen of 100 mg seed powder with the factor 6.25 (McCkenzie & Heather 1953). The protein content is expressed in terms of mg protein/100 mg seed powder. The albumin and globulin fractions of the protein were assayed after Lowry et al. (1951) and expressed as mg /100 mg seed powder. The protein profile of the mutants and the control seeds were evaluated by extracting protein from the seed powder in 0.2 M phosphate buffer. The polyacrylamide gel electrophoresis was carried out by using 7.5% acrylamide gel (Davies 1964). Protein was separated in anodic system using tris-glycine buffer pH 8.3. After electrophoresis, the gels were stained in 0.25% aqueous solution of comassic brilliant blue and destained in 7% acetic acid.

OBSERVATIONS

Morphological characters, protein content, albumin and globulin content and albumin/globulin ratio of the seed coat colour mutants and the control seeds in M_2 and M_3 generations are presented in Table 1. The results show reduction in height of the plants in mutants in both M_2 and M_3 generations. The maximum reduction occurred in pink mottled seed coat colour mutant which has been isolated at 25 kR. No significant difference in the number of pods/plant, seed weight/plant was observed in the mutants as compared to the control.

Marked increase in seed protein, albumin and globulin content and alteration in albumin and globulin ratio as compared to control has been recorded in the mutants (Table 1). Mutant with pink mottled seed coat colour has highest seed protein, albumin and globulin content followed by mutant having dark brown mottled seeds. Protein content, expressed as per cent that of control, in the mutant seeds is presented in Fig. 1. Magnitude of increase in the protein has been found to be associated with the nature of the mutants. For example, mutant with shining green seed coat which has been isolated at 5 kR showed a minimum increase of 5.58% as compared to pink mottled mutant isolated at 25 kR having 28% higher protein than the control. Nevertheless, all the 4 mutants have higher seed protein content as compared to the control. Albumin and globulin contents

of the mutants have simultaneously been altered. All the mutants showed higher amount of these 2 protein fractions. Alteration in the albumin and globulin content of the mutants is also evident from the change in A/G ratio (Table 1).

TABLE 1 : Agronomic characteristics and protein characters of seed coat colour mutants of *Phaseolus vulgaris* var. Top crop.

Mutants	Generation	Plant height	Pods/ Plant	Seed wt./ Plant (g)	Protien (mg/ 100 mg seed meal)	Albumins (mg/100 mg seed meal)	Globulins (mg/100 mg seed meal)	A/G ratio
	M_2	25.05	5	10.56	24.35	8.65	8.73	1.28:1
SG	M_3	25.15	5	10.80	24.60	8.73	6.86	1.27:1
	M_2	25.18	5	11.05	25.18	8.95	7.10	1.26:1
DRB	M_3	24.97	6	11.56	28.26	8.99	7.15	1.25:1
	M_2	22.30	6	11.64	28.14	9.35	8.15	1.15:1
DBM	M_3	23.05	7	12.01	28.91	9.50	8.30	1.14:1
	M_2	22.08	6	11.54	30.35	10.18	8.75	1.16:1
PM	M_3	22.17	5	11.65	30.71	10.65	8.45	1.26:1
	M_2	26.53	8	12.98	23.75	8.21	6.10	1.34:1
IL	M_3	26.25	7	11.87	23.86	8.30	6.21	1.33:1

SG, Shiny green ; DRB, Dark reddish brown mottled ; DBM, Dark brown mottled ; PM, Pink mottled ; IL, Initial line.

PAGE-protein profile of the normal and mutant seeds in M_3 generation is presented in Fig. 2. The number of protein bands in the zymogram varies from 11-14 in the normal and the mutated seeds. Clear differences were found in the banding pattern of the soluble seed protein of the normal and the mutated seed coat colour seeds. In the zymogram of the normal seeds the number of visible bands were 11 at different REM. In the coat colour mutant seeds the number of visible bands on the gel column was higher (13-14). The mutated seeds also showed a different banding pattern both in the presence and absence of some bands at specific REM. Presence of dark broad band at REM 4.3 in dark brown mottled seed coat mutant, a dark narrow band at REM 8.6 and 9.1 in shining green seed coat and pink seed coat colour mutant respectively and dark broad band at REM 3.1 in all the mutants indicate synthesis of new proteins due to mutation.

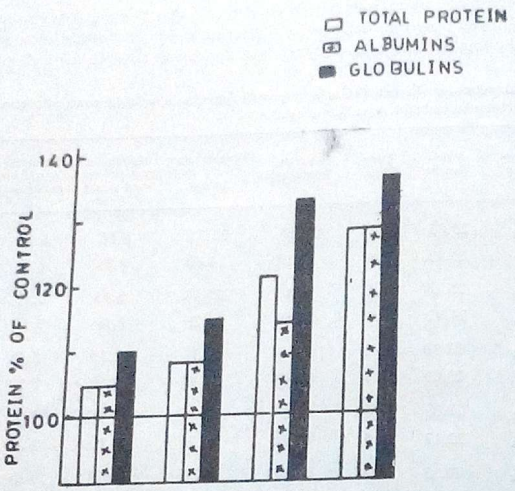

Fig. 1 : Composition of seed protein, albumin and globulin of normal and coat colour mutant seeds in M_3 generation of *Phaseolus vulgaris* var. Top crop

DISCUSSION

The potentialities of physical and chemical mutagens for inducing various types of morphological mutants are well documented (Prasad & Jha 1989). Micke (1970) seggested that if induction of higher protein mutants is associated with morphological changes, screening of protein mutants may be done with ease without performing biochemical analysis of a large number of samples. Seed coat colour mutants with higher protein has been reported earlier in black gram (Bhamburkar & Bhalla 1983) and in *P. vulgaris* (Prasad 1979, Prasad et al. 1984). Prasad et al. (1984) emphasized their role as markers for higher protein quantity and quality. Increase in total seed protein associated with increase in albumin and globulin content and change in albumin/globulin ratio in the seed coat colour mutants as observed in the present investigation suggest that quantitative and qualitative improvement of seed protein has been achieved. In addition, changes in the protein profile of the mutants also confirm, the alteration in the polypeptide composition of the seed protein.

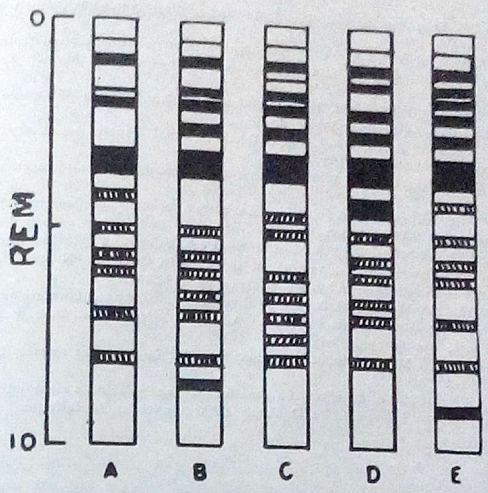

Fig. 2 : Electrophoretograms of seed protein of normal and coat colour mutants in M_3 generation. A. Control; B. Shiny green; C. Dark reddish brown mottled; D. Dark mottled; E. Pink mottled.

A comparison of the total seed protein, albumin and globulin contents albumin/globulin ratio and protein profile of the sead coat colour mutants. in M_2 generation and their true breeding nature in M_3 generation clearly indicates that genes responsible for controlling these traits are mutated simultaneously. The findings suggest that seed coat colour mutations may be considered as 'morphological markers' in the breeding experiments aimed to isolate protein mutants, if not in all the cases but at least in legumes.

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CHROMOSOMAL INVERSIONS IN *ANOPHELES STEPHENSI* LISTON-A MALARIA MOSQUITO

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SUMMARY

A preliminary survey of the natural populations of *Anopheles stephensi* Liston from India has revealed the presence of 6 autosomal paracentric inversions. Three inversions were observed on chromosome 2R, 2 on 2L and 1 on 3L. Each of these inversions is described and an account of their distribution in the strains examined is given.

Key Words : *Anopheles stephensi*, chromosomes, inversions.

INTRODUCTION

Chromosomal polymorphism originating from paracentric inversions are widespread among anopheline mosquitoes, and almost all species, even those closely related, appear to be differentiated by one or more fixed inversions which frequently overlap (Coluzzi 1970).

The study of inversion polymorphism, both in the field and in the laboratory, should provide a better understanding of the adaptive and evolutionary significance of such genetic variability and could help in clarifying certain patterns of biological and morphological variability in malaria vectors. (Coluzzi et al. 1973).

Anopheles stephensi is one of the important vectors of malaria in Indian subcontinent. It belongs to the order 'Diptera' and subgenus 'Cellia'. Very little work has been done on chromosomal polymorphism from Indian populations of *An. stephensi*. Hence, a survey was initiated to determine the occurrence of inversions and their frequencies in different populations of *An. stephensi*. It was felt that if the populations were found to be chromosomally polymorphic and differed in the kinds and/or frequencies of inversions, it may be possible to differentiate vector from non-vector populations on the basis of these inversions.

This paper reports naturally occurring inversion polymorphism by using polytene chromosomes from *An. stephensi*.

MATERIALS AND METHODS

Six strains of *An. stephensi* from different geographical areas of India and 6 strains from around Bangalore city were successfully colonized. The material was collected in the field usually in the form of larvae and gravid females.

Colonies of *An. stephensi* were maintained in the laboratory. The adults were reared in cages of iron frame covered by mosquito net. The adults were fed on glucose or honey. Females were provided blood meal on mice. Enamel water bowls lined with a strip of filter paper were placed in the population cages for oviposition. Rearing procedures for larvae and adults were followed by the procedure of Shetty (1983).

The cytological techniques were used according to the method of French et al. (1962) and Coluzzi et al. (1973).

All the inversions included in the present study were observed from the polytene chromosomes of the ovarian nurse cells. The polytene chromosomes from the ovarian nurse cells were prepared from half gravid females, 28 to 30 h after administering the blood meal. A female was randomly chosen and separated into a test tube and killed by striking the lower end of the tube against the palm. The female was then placed on a slide in a drop of dilute Carnoy' fixative. A quick and successful method to remove the ovaries was to hold the anterior part of the abdomen with one needle (left hand) and the penultimate segment of the abdomen with another and then give a sharp pull posteriorly to cut the last two segments. The abdomen was then gently pressed, spilling the ovaries posteriorly. The internal organs usually come out in a group.

For routine staining of the polytene chromosomes, synthetic orcein was the standard stain. The stain was mixed with glacial acetic acid and lactic acid, in the following proportion: 2 g of orcein in 50 ml of 85% lactic acid and 50 ml of 100% glacial acetic acid. The stock solution was stored in a cool and dry place. Refrigeration was not necessary. The stock solution was further diluted in lactic acid and acetic acid (1:1) prior to use to avoid overstaining.

After fixation, the drop of fixative along with the material on the slide was fixed with a drop of stain of similar size. After staining, a clean coverslip was placed on the top of the material and a gentle cover pressure was applied. The amount of pressure required to spread the chromosomes evenly is best gained by experience. The temporary mounts were ringed with nail polish around the coverslip.

The inversion nomenclature and their frequency was followed according to the method of Coluzzi et al. (1973).

RESULTS AND DISCUSSION

Altogether, 6 paracentric, heterozygous inversions were observed by using polytene chromosomes from the ovarian nurse cells of *An. stephensi* (Table 1).

TABLE 1 : Chromosomal inversions in the laboratory populations of *Anopheles stephensi*.

Sl No.	Strain	X	2R		2L		3R	3L
1	Bangalore							
	a) Chamarajpet	—	b/+	—	—	—	—	—
	b) Gandhinagar	—	—	—	—	—	—	—
	c) Jayanagar	—	b/+	—	—	—	—	—
	d) Yeshwanthpur	—	b/+	—	—	—	—	—
2	Coonoor	—	b/+	g/+	h/+	e/+	—	—	k/+
3.	Delhi	—	b/+	—	—	—	—	—	—
4.	Mandya	—	b/+	—	—	—	f/+	—	—
5.	Poona	—	b/+	—	—	—	—	—	—
6.	Salem	—	—	—	—	—	—	—	—

Tentative break points in 2R	Tentative break points in 2L	Tentative break points in 3L
b/+ = 11D–16C	e/+ = 23C–27D	k/+ = 39A–40B
g/+ = 8C–13A	f/+ = 24C–28B	
h/+ = 9C–16C		

These include 3 inversions on 2R, 2 on 2L and 1 on 3L. The frequencyof these inversions in different populations were also recorded (Table 2). No per centric, heterozygous or homozygous inversion were observed from the laboratory populations of the same species. No inversions were observed on the x-chromosome. The paracentric inversions observed on the arm 2R, designated as *b*, *g* and *h* respectively.

2R.*b*: The break points invoved in inversions *b* were 11 D-16 C (Fig. 1). This type was observed in Chamarajpet, Jayanagar, Yeshwanthpur, Coonoor, Delhi, Mandya and Poona strains. The frequencies of this inversion were 5%, 4.5%, 27.5%, 12.85%, 2·5%, 28% and 24% respectively (Table 2). This suggests that the inversion 2 R. *b* is fairly common in Indian populations.

2R. *g*: The break points involved in invesion *g* were 8C-13A (Fig. 2). This was observed in the Coonoor strain and the frequency of this inversion was, $g = 1.42$ %.

2R. *h*: The break points involved in inversion *h* was 9 C-16 C (Fig.3). The *h* inversion was observed in the Coonoor strain and frequency was, $h = 1.42\%$.

TABLE 2 : Percentage of inversions in the laboratory populations of *Anopheles stephensi*.

Sl. No.	Strain	Type of inversion	Chromosomal arm involved	Percentage of homozygosity
1.	Bangalore			
	a) Yeshwanthpur	Heterozygous	2R (b/+)	27.50
	b) Chamarajpet	Heterozygous	2R (b/+)	5.00
	c) Jayanagar	Heterozygous	2R (b/+)	4.50
2.	Coonoor	Heterozygous	2R (b/+)	12.85
		Heterozygous	2R (g/+)	1.42
		Heterozygous	2R (b/+)	1.42
		Heterozygous	2L (e/+)	1.42
		Heterozygous	3L (k/+)	1.42
3.	Delhi	Heterozygous	2R (b/+)	2.50
4.	Mandya	Heterozygous	2R (b/+)	28.00
		Heterozygous	2L (f/+)	4.00
5.	Poona	Heterozygous	2R (b+)	24.00

Two paracentric inversions designated as *e* and *f* were observed on the arm 2L.

2L. *e*: The break points in *e* inversion were 23 C-27 D (Fig. 4). This inversion was observed in the Coonoor strain and the frequency of inversion was $e=1.42\%$.

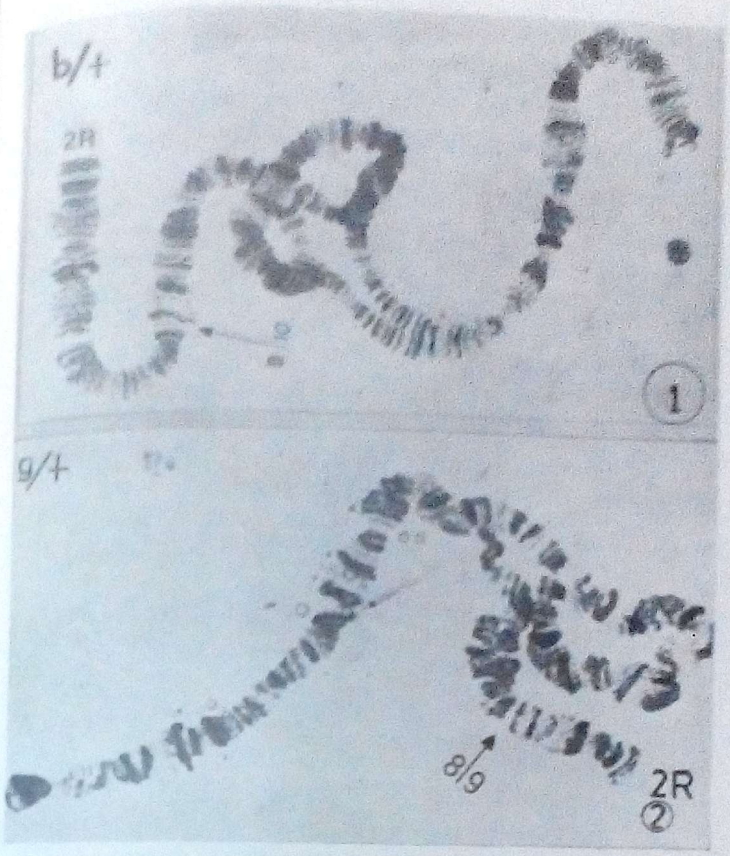
2L. *f*: The break points involved in *f* inversion were 24 C-28 B (Fig. 5). The *f* inversion was observed in the Mandya strain and the frequency was $f=4\%$.

One paracentric inversion was observed on the arm 3L, designated as *k*.

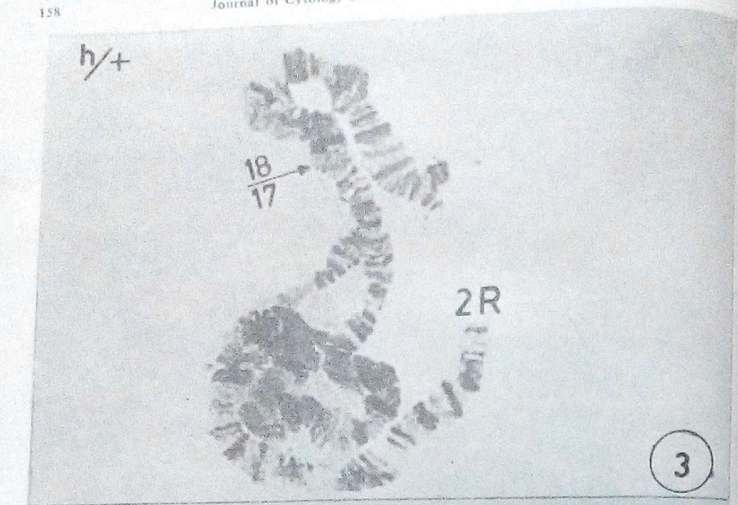
3L.*k*: The break points involved in this inversion were 39A-40B. (Fig. 6). The inversion was observed from the Coonoor strain and the frequency of this inversion was, $k=1.42\%$.

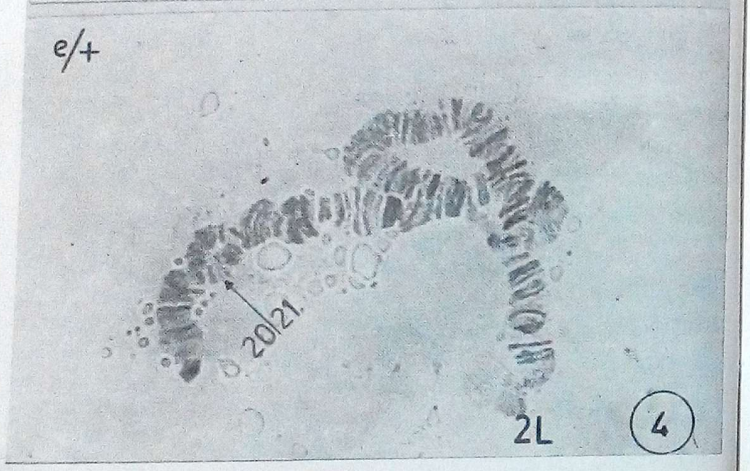
No inversions were observed on 3R-chromosome.

Among the 5 autosomal inversions included in the present study, 4 inversions, g/+, b/+ (2R), e/+, f/+ (2L) and k/+ (3L) are altogether new and reported for the first time from our laboratory. The differences in the frequencies and type of inversions in the natural populations of this species were very striking (Table 2.)



Figs. 1 & 2 : Chromosomal inversions in *An. stephensi*. 1. Arm 2R, Inversion b/+; 2. Arm 2R, inversion g/+.





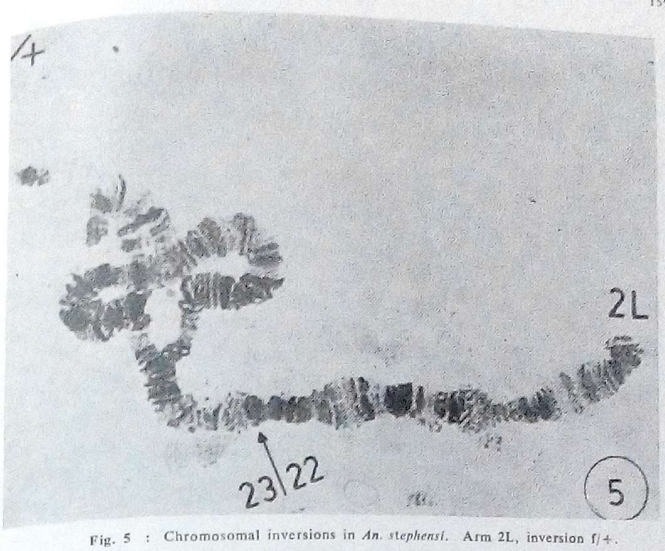


Fig. 5 : Chromosomal inversions in *An. stephensi*. Arm 2L, inversion f/+.

Coluzzi et al. (1970 , 1973) observed 6 heterozygous paracentric inversions in the laboratory maintained populations of *An. stephensi*. Mahmood & Sakai (1984) reported 16 paracentric autosomal inversions in the same species from Pakistan. Four of the paracentric inversions reported by Coluzzi et al. (1973) were also observed by Mahmood & Sakai (1984). Only one of the inversions reported here, the b/+ inversion was described by the above authors.

The relationship between behavioural and biological variations on the inversion 2R: b/+, has been demonstrated in the laboratory strains of *An. stephensi* by Suguna (1981) and Coluzzi et al. (1973). Coluzzi et al. (1973) showed a relationship between inversion polymorphism and egg size in an Iraq strain of *An stephensi*. Colonies with shorter eggs (*Mysorensis*) were characterised by a ×/+ arrangement, while inversion (2R: b/+) was present in colonies with longer eggs. It was, therefore, suggested that the presence or absence of this inversion was related to the morphological variants described as *mysorensis* and type from *An. stephensi*.

Figs. 3 & 4 : Chromosomal inversions in *An.stephensi*. 3. Arm 2R, inversion h/+ ; 4. Arm 2L, inversion e/+.

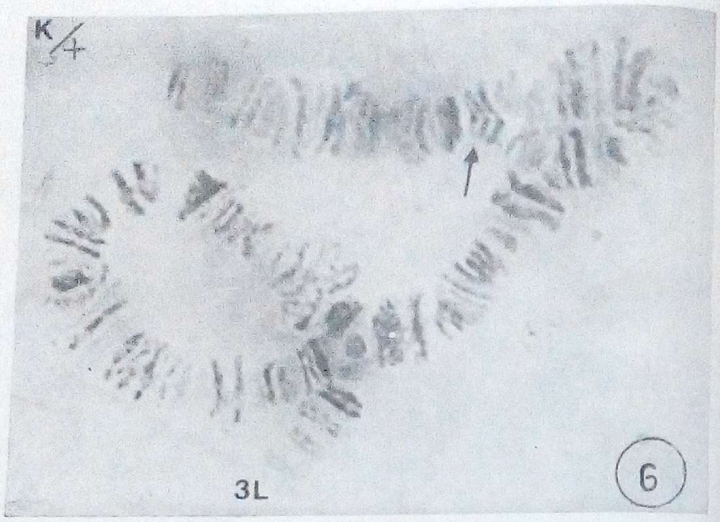


Fig. 6 : Chromosomal inversions in *An. stephensi*. Arm 3L, inversion k/+.

Apart from the evolutionary interest of chromosomal rearrangements, the investigation of inversion polymorphism in relation to the biology and behaviour of malaria vectors may improve our understanding of mosquito behaviour and of the epidemiology of mosquito borne diseases. The possibility of correlating inversion rates with tolerance of insecticide selection pressure has received much attention (Holstein 1957, Mason & Brown 1963).

The differences in the frequency of inversion observed, may be due to the nature of breeding habitats which may facilitate or affect the survival of inversion heterozygotes. Dobzhansky (1951) stated that the inversion polymorphisms within one species *(Drosophila)* may vary in the same locality at different seasons and also over a period of years between geographically separated populations adapted to somewhat different environments. Fluctuating frequencies of inversions has been linked to the seasonal changes in overall biting cycle and with vectorial capacity (Kreutzer et al. 1972).

ACKNOWLEDGMENTS

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The **title** of the paper must be brief and contain words useful for indexing. Serial titles are to be avoided. The authors are also expected to suggest a short running title (of not more than 50 characters, including author's name and spaces). The full names of the author(s) and address (es), the institute(s) at which the work was carried out (the present address of the author(s), if different from the above, should appear in a footnote) must be given.

Each paper should be preceded by a short SUMMARY, and by KEY WORDS. Summary should be a lucid digest of the whole paper, complete in one paragraph with no numbered parts and not to exceed 80 words. Three to 5 key words should suffice to denote the more important taxa and problems treated in the paper in a form that can be fed into a data bank.

(continued on page 174)

J. Cytol. Genet. 27 : 163-173 (1992)

KARYOMORPHOLOGY OF *FIMBRISTYLIS* FROM PUNJAB

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SUMMARY

Karyomorphological studies on 8 species of sedge genus *Fimbristylis* have been made. Karyomorphological differences have been noticed in *F. bisumbellata, F. dichotoma, F. ferruginea* and *F. quinquangularis* on population basis and in *F. bisumbellata* even on individual basis within the same population. This indicates the evolution of species at micro level and the usefulness of chromosomal analysis on population basis. Based on present analysis it is surmised that the perennial species of *Fimbristylis* (*F. bisumbellata, F. dichotoma, F falcata* and *F. ferruginea*) have more evolved karyotypes as compared to annual species (*F. miliacea, F. ovata, F. quinquangularis* and *F. tenera*). Acrocentric chromosomes and secondary constrictions are reflected in complements of perennial species only.

Key Words : *Fimbristylis*, karyomorphology, population, species, variability.

INTRODUCTION

Fimbristylis (Cyperaceae) is well represented in Indian flora with 50 species (Santapau & Henry 1973), out of which 14 are recorded from Punjab (Nair 1978). Chromosomal analysis of 9 species presently available in Punjab has earlier been made by Bir et al. (1992). Since the chromosome numbers recorded are quite different from the previous reports (Kumar & Subramaniam 1987), these studies have revealed the existence of 'biological species'.

In earlier studies on *F. dichotoma*, Bir et al. (1986) noted variations in karyomorphology of individuals of 2 populations of diploid (n=5) and 5 populations of tetraploid (n=10), the individuals exhibiting the same chromosome number within a population.

In order to find out whether the karyomorphological variations exist in other species or not, further investigations have been extended to *F. bisumbellata, F. dichotoma, F. falcata, F. ferruginea, F. miliacea, F. ovata, F. quinquangularis* and *F. tenera* on population basis. Although, reinvestigations have been made on the previously studied species (Bir et al. 1986) yet populations from different localities from Punjab were studied with a view to confirm whether or not the karyotypic variations in populations really exist.

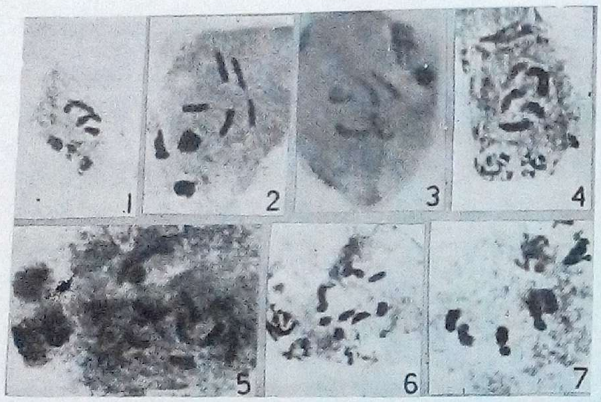
MATERIALS AND METHODS

Flower buds were fixed in Carnoy's fluid for 10-12 h and then stored in 95% ethanol. Acetocarmine squashes were made and preparations were mounted in

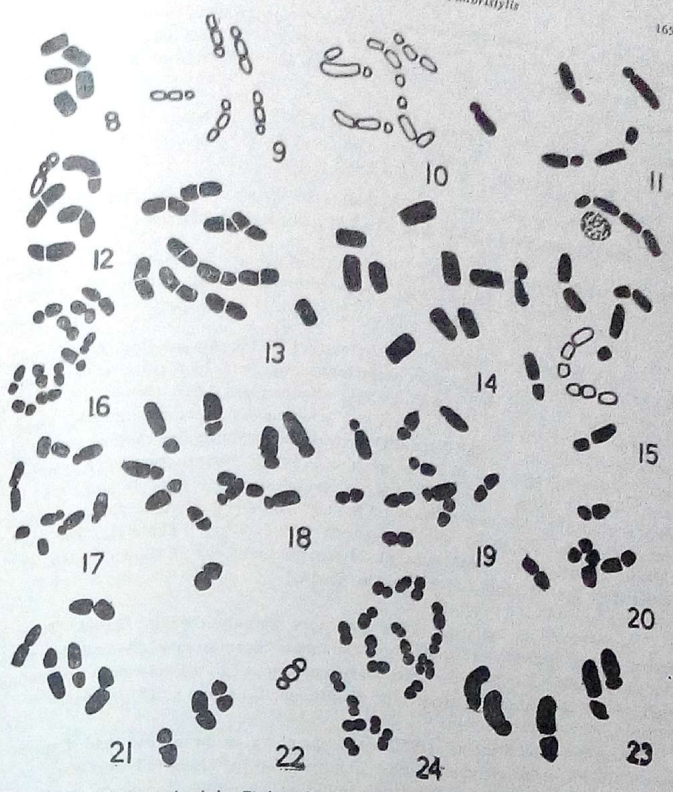
euparal. In order to record karyotypic variabilities at inter- and intraspecific levels, mitosis was studied in pollen grains from different inflorescences of the same species. Major guidelines for karyotypic analysis such as, position of centromere, presence/absence of secondary constriction regions, total haploid chromatin length, absolute and average chromosome size and type of symmetry are primarily taken from Stebbins (1971) and adopted for sedges by Bir et al. (1986). Voucher specimens are preserved in PUN.

OBSERVATIONS

Haploid sets of chromosomes as appeared at first pollen mitosis have been examined in 16 populations belonging to 8 species, namely, *F bisumbellata* (n=5; 4 pop.), *F. dichoroma* (n=10; 3 pop), *F. falcata* (n=11; 1 pop.), *F. ferruginea* (n=10; 3 pop.), *F. miliacea* (n=5; 1 pop.), *F. ovata* (n=5; 1 pop.), *F. quinquangularis* (n=5; 2 pop.) and *F. tenera* (n=20; 1 pop.) (Figs. 1-24). In all these species, the nucleus of the functional microspore undergoes division as the other three microspores degenerate, the result being a pseudomonad characteristic of sedges. Karyotypes have been studied from metaphase chromosomes at first pollen mitosis with an advantage of the presence of a single set of chromosomes making karyotypic analysis easy.



Figs. 1-7 : Photomicrographs showing pollen mitosis in various species of *Fimbristylis*. 1-4, *F. bisumbellata* (n=5) Pop. i, iva, ivb and x respectively ; 5, *F. dichotoma* (n=10) Pop. v; *F ferruginea* (n=10) Pop. i; 7, *F. quinquangularis* (n=5) Pop. v.



Figs. 8-24: Pollen mitosis in *Fimbristylis* 8-12, *F. bisumbellata* (n=5) Pop. i, iva, ivb, viii and v respectively; 13-15, *F. dichotoma* (n=10) Pop. i, iv and v respectively ; 16, *F. falcata* (n=11) ; 17-19, *F. ferruginea* (n=10) Pop. ii and iii respectively ; 20, *F. miliacea* (n=5) Pop. iii ; 21, *F. ovata* (n=5) ; 22-23, *F. quinquan- gularis* (n=5) Pop. i and v respectively ; 24, *F. tenera* (n=20).

Data on various karyotyotypic parameters as karyotype formulae, analysis, range of absolute chromosome size, categorisation on the basis of Stebbins (1958) are presented in Table 1. A glance on the karyotypic details reveals wide differences

in chromosome morphology between the complements of different as well as of the same population (Figs. 25-41). Within the genome of each taxon the chromosome size exhibits gradual gradation; the size range of the presently studied chromosomes is as follows :

Long (Double or more than double the length of the shortest chromosome within total species of the genus worked out here): A=2·55–4.5 μm, B=2.26-4.74 μm, C=2.37-5. 56 μm, D=2.41-2.7 μm.

Medium (From one and a half times to double the length of the shortest chromosome): E=2.18 μm, F=1.82-2.18 μm, G=2.18 μm, H=1.8-2.18 μm.

Short (Less than one and a half times the length of the shortest chromosome): I=1.45 μm, J=1.09-1.45 μm. K=no chromosome falls under this category, L=1.09-1.45 μm.

Average chromosome size ranges between 1.49 and 4.6 μm (Fig. 43). Following Bir et al. (1986) the taxa based on chromosome size can be divided into following three categories : (a) Taxa with average chromosome size above 3.0 μm : *F. bisumbellata* pop. iv (a), iv (b), viii, x; *F. dichotoma* pop. v; *F. ovata* pop. i and *F. quinquangularis* pop. v, (b) Taxa with average chromosome size ranging from 1 5-3.0 μm: *F. bisumbellata* pop i; *F. dichotoma* pop. ii, iv; *F. falcata* pop. i; *F. ferruginea* pop. i-iii; *F. miliacea* pop. iii and *F. quinquangularis* pop. ii and (c) Taxa with average chromosome size below 1.5 μm : *F tenera* pop. i. The absolute chromosome size within the genus ranges between 1.09-5.56 μm (Table 1). The haploid chromatin length of the complements of different populations is shown in Fig. 42. It ranges from 8·91-38.64μm in the species studied.

It is seen that submetacentrics are more prevalent (Figs. 25–41, Table 1). Secondary constrictions are always on the larger chromosomes (B– and C–types) of the complement. Further, the secondary constrictions are common to the long arms of the chr omosomes except for *F. bisumbellata* pop.x (Fig. 29).

According to Stebbins (1971) some members of Juncaceae and Cyperaceae have diffuse centromeric regions and thus cannot be classified according to his categorisation. Presently, out of 16 populations studied, localized centromeres could not be identified in two taxa, namely, *F. bisumbellata* pop. i (Figs. 1,8,25) and *F. dichotoma* pop. iv (Figs. 14,31) while the rest of the populations are with localized centromeres.

Comparative values for GI and SI for various populations are represented in Fig. 44. Evaluating GI values independently of SI, it is clear that the karyotypes are slightly to highly symmetrical for all the species. SI values

TABLE 1 : Data on karyotypes in *Fimbristylis*.

Species++	Chrom. No.	Karyotype fromulae++	Karyotypic analysis	Chrom. size (μm)	Category
F. bisumbellata (Forsk.) Bub.					
Pop. i	n=5	NI_5	H_5	1.82-2 18	—
Pop. iva*+	n=5	J_5^{sc}	$C_4 1 + G_1 1$	3.10-3.28	4A
Pop. ivb*+	n=5	J_5^{sc}	$C_5 1$	4.01-5.56	4A
Pop. viii	n=5	L_4+J_1	B_4+G_1	2.0-2.73	2A
Pop. x	n=5	$V_2+L_2+L_1^{sc}$	$A_2+B_2+B_1^s$	3.28-3.74	1A
F.dichotoma (L.) Vahl					
Pop. ii	n=10	$V_5+L_3+J_1$	$A_5+B_3+K_1$	2.37-3.35	2A
Pop. iv	n=10	NI_{10}	D_5+H_5	1.80-2.70	—
Pop. v	n=10	$V_1+L_7+L_1^{sc}+J_1^{sc}$	$F_1+B_7+B_1 1+C_1 1$	3.10-4.74	3A
F. falcata (Vahl) Kunth					
Pop. i	n=11	V_5+L_6	$A_2+E_1+I_2+F_5+J_1$	1.45-2.91	1B
F. ferruginea (L.) Vahl					
Pop. i	n=10	$L_2+J_2+I_6$	$F_2+G_2+H_2+L_4$	1.09-2.18	4B
Pop. ii	n=10	$V^1+L_6+J_3$	$E_1+B_5+F_1+C_2+G_1$	2.18-4.01	2A
Pop. iii	n=10	$V_2+L_6+J_2$	$E_2+B_2+F_4+C_5$	2.18-3.10	2A
F. miliacea (L.) Vahl					
Pop. iii	n=5	L_5	B_5	2.26-3.10	1A
F. ovata (Burm.f.) Kern					
Pop. i	n=5	L_5	B_5	3.28-3.83	1A
F. quinquangularis (Vahl) Kunth					
Pop. ii	n=5	$V_1+L_3+L_1^{sc}$	$E_1+B_2+F_1+B_1 1^s$	2.09-2.95	1A
Pop. v	n=5	L_3+J_2	B_3+C_2	2.91-4.37	2A
F. tenera Schult.					
Pop. i	n=20	$V_{10}+L_{10}$	$I_{10}+F_4+J_6$	1.09-1.82	1A

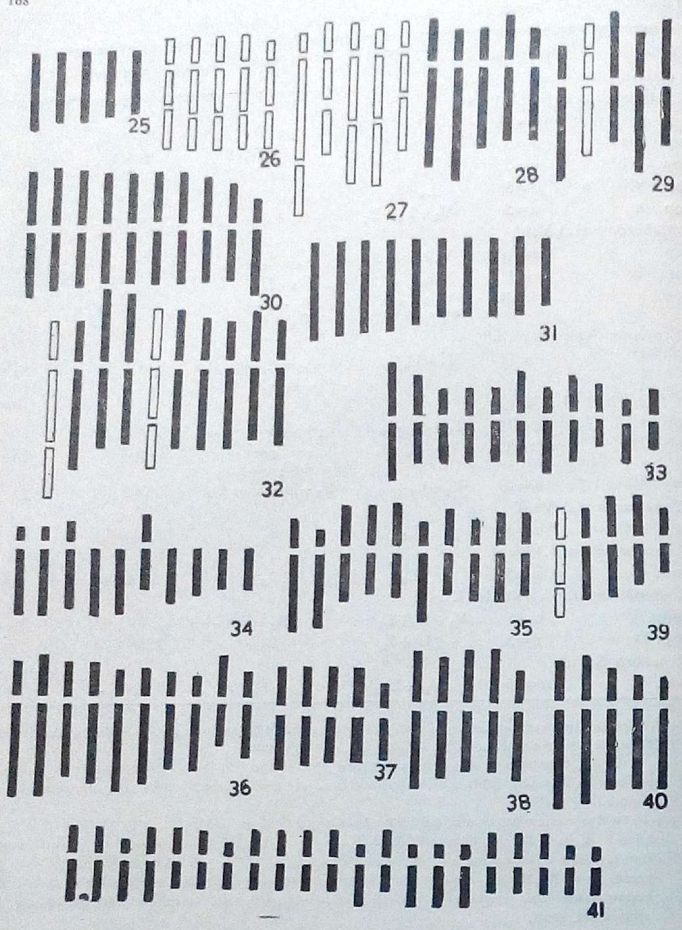
+ The population numbers coincide with those earlier cited for meiotic studies (cf. Bir et al. 1992).

** Different "Karyotypic units" within the same population.

++ V, L, J, I chromosome categories are according to White (1945) whereas 'NI' stand for chromosomes with nonidentifiable centromeres and hence no arms concept. Secondary constrictions are denoted by 'sc'

+++ Twelve basic types on size basis have been recognised as under :

Long : A, B, C, D ; Medium : E, F, G, H ; Short : I, J, K, L. The position of centromere in relation to chromosome is A, E, I : Metacentric ; B, F, J : submetacentric ; C, G, K : acrocentric, D, H, L: telocentric in complements with normal chromosomes with localised centromeres. The symbols '1's relate to chromosomes with secondary constrictionf on long arms/short arms.



indicate something contrary to it. *F. bisumbellata* pop. iva, b; *F. ferruginea* pop.i and *F. quinquangularis* pop.v have highly asymmetrical karyotypes. On the basis of Stebbins' (1958) categorisation, all the taxa are scored as with symmetrical karyotypes falling in 1A, 2A, 4A, 1B and 4B categories (Table i).

Following variations at infraspecific level have been noted :

(1) *F. bisumbellata* (n=5) : In pop. i (Fig. 25) the centromere is not identifiable whereas rest of the populations exhibit localized centromeres. Interestingly, various populations with localized centromeres exhibit different components of meta–, submeta– and acrocentric chromosomes. Further, pop. iv and x have secondary constrictions but former on long arms of all the 5 acrocentric chromosomes (Figs. 2, 3, 26, 27) and latter on the short arm of one submetacentric chromosome (Figs. 4, 29). Even within the same population different pollen grains exhibit different karyomorphology as is clear from data in Table I and by comparison of Figs. 26 and 27.

(2) *F. dichotoma* (n=10) : Pop. iv of this species is without identifiable centromere (Figs. 14, 31) whereas the rest of the populations, have localized centromeres. The ratio of V, L and J chromosomes is different in the 2 populations. (Figs. 13–15, 30, 32). Pop. v has 2 chromosomes with secondary constrictions (Figs. 15, 32).

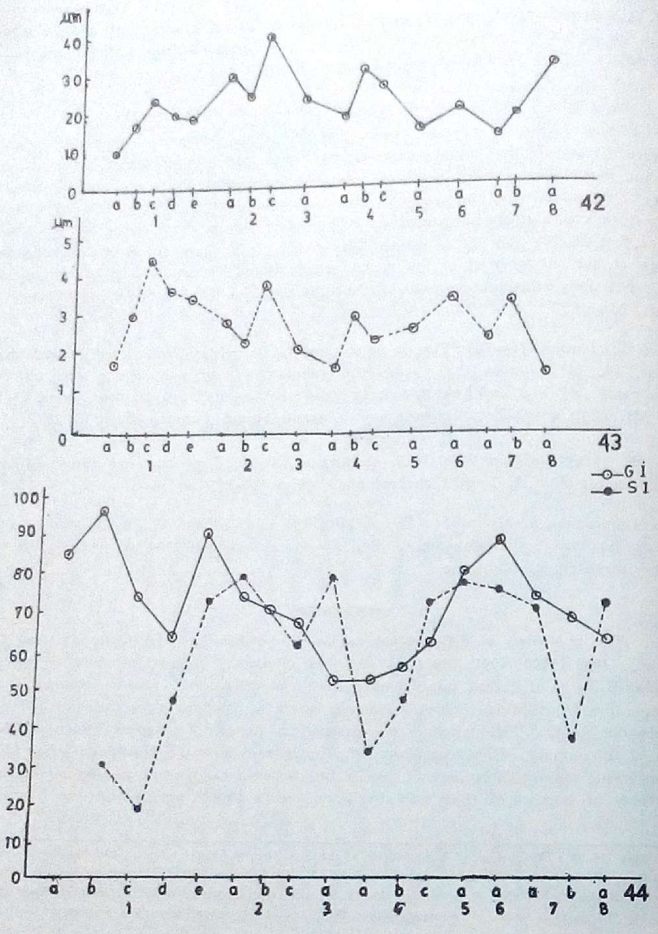
(3) *F. ferruginea* (n=10) : It is seen that all the 3 populations show different components of V, L, J and I chromosomes (Figs. 17-19, 34-36).

(4) *F. quinquangularis* (n=5) : Pop. ii (Fig. 39) and v (Figs. 7, 23, 40) differ in ratio of various types of chromosomes. Pop. ii also has secondary constriction on one submetacentric chromosome.

DISCUSSION

The recording of different chromosome numbers in different populations (Bir et al. 1988, 1990, 1991, 1992) of the same species accompanied by karyotypic variabilities in different populations (Bir et al. 1986) and present communication points out the usefulness of chromosomal analysis on population basis or even on individual basis. This indicates the evolution of 'species' at micro level as reflected by *F. dichotoma*, *F. bisumbellata*, *F. ferruginea* and *F quinquangularis*. These karyotypic variabilities cannot easily be correlated with morphological features. It may be mentioned that individuals reflecting these variabilities are growing

Figs. 25–41 : Idiograms of haploid sets of chromosomes in *Fimbristylis* 25-29, *F. bisumbellata* Pop. i, iva, ivb, viii and x respectively ; 30-32, *F. dichotoma* Pop. i, iv and v respectively ; 33, *F. falcata* ; 34-36, *F. ferruginea* Pop. i, ii, and iii respectively ; 37, *F. miliacea* Pop. iii ; 38, *F. ovata* ; 39-40, *F. quinquangularis* Pop. i and v respectively; 41, *F tenera*.



under identical growth conditions. Possibly in due course of time the infraspecific karyotypic character variabilities are reflected at phenotypic level and may be helpful in segregating the species into subspecific or varietal or formal categories. This is how the evolution seems to progress further at infraspecific level amongst the plants with same chromosome numbers.

The absence of SAT-chromosomes in *F. dichotoma*, *F. bisumbellata*, and *F. falcata* is in contrast to the observations of Sanyal & Sharma (1972) and Nijalingappa (1973, 1975, 1977) whose findings are based on somatic chromosomes from the root tips. Presently, no secondary constrictions on metacentric chromosomes have been seen which is contrary to earlier reports on *Fimbristylis dichotoma* (Bir et al. 1986), *F. quinquangularis*, (Nijalingappa 1975), *F. ferruginea*, *F. miliacea*, *F. quinquangularis*, *F. ovata (F. monostachya)* (Sanyal and Sharma 1972) which show their presence on these chromosomes. Further, presently, secondary constrictions are not observed in complements of *F. falcata* and *F. miliacea* although earlier reports indicate their presence (Bir et al. 1986). For *F. bisumbellata* we clearly demarcated secondary constrictions for 2 populations (Table 1,Figs. 26, 27,29) in contrast to their absence being earlier shown by Nijalingappa (1975). By comparing the present data with the observations of earlier workers, it is seen that the ratio of metacentric and submetacentric chromosomes may vary considerably in *F. dichotoma*, n=10 (Sanyal & Sharma 1972, Nijalingappa 1973, Bir et al 1986); *F. bisumbellata*, n=5 (Nijalingappa 1975); *F falcata*, n=11 (Sanyal & Sharma 1972, Bir et al. 1986); *F. quinquangularis*, n=5 (Nijalingappa 1975, Bir et al. 1986) and *F. tenera*, n=20 (Bir et al. 1986). Telocentrics were hitherto unrecorded in the complements of sedges but we have noticed their presence in *F. ferruginea* pop. i. In the light of earlier and present observations, *Fimbristylis* is now fully known to possess localized centromeres (Sharma & Bal 1956, Sanyal & Sharma 1972, Nijalingappa 1975, 1977, Patnaik & Rath 1983, Bir et al. 1986). In contrast, Cronquist (1968) considered the chromosomes of various taxa of Cyperaceae including *Fimbristylis* as diffuse-centric.

Shrama & Sharma (1959) are of the view that karyotypic changes are due to breakage, reunion in reverse postion and translocation of chromosome segments. According to Stebbins (1971) and Sharma (1976), gradual evolution from symmetrical to asymmetrical karyotype takes place either through the shift of centromeric position from metacentric to submetacentric or acrocentric or accumulation of differences in relative size between chromosomes of the same complement.

Figs. 42-44 : Total chromatin length of haploid complement (42), average chromosome size (43) and chromosome SI and GI (44) for species of *Fimbristylis* 1a-e, *F. bisumbellata* Pop. i, iva, ivb, viii and x respectively ; 2a-c, *F. dichotoma* Pop. i, iv and v respectively ; 3a-c, *F. falcata* ; 4a-c, *F. ferruginea* ; Pop. i, ii and iii respectively 5a, *F. miliacea* Pop. iii, 6a, *F. ovata* ; 7a-b, *F. quinquangularis* Pop. i and v respectively ; 8a, *F. tenera*.

A perusal of karyotypic data reveals that there is no definite correlation between haploid chromatin length and level of ploidy.

As far as annual and perennial habits are concerned, it is seen that telocentric and diffuse-centric chromosomes are totally absent in annuals (*F. miliacea, F. ovata F. quinquangularis* and *F. tenera*) but these are present in perennial species (*F. bisumbellata, F. dichotoma, F. falcata* and *F. ferruginea*). Interestingly, acrocentric chromosomes and secondary constrictions are primarily present in karyotypes of perennials. This indicates that perennial species of *Fimbristylis* have more evolved karyotypes.

Chromosomes of species of *Fimbristylis* show considerable size differences among different species or even different populations of the same species. This is in confirmation of earlier postulation of Stebbins (1971) for the herbaceous angiosperms on the whole.

ACKNOWLEDGMENTS

Grateful thanks are expressed to UGC, New Delhi [project grant no. F-3-58/89 (SR-II)] and Punjabi University, Patiala for financial assistance for chromosomal analysis of sedge flora of North India.

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SUGGESTIONS TO CONTRIBUTORS *(continued from page 162)*

THE TEXT : The paper must be set out under the following headings : INTRODUCTION, MATERIALS AND METHODS, OBSERVATIONS and DISCUSSION. Main headings are in full capitals and **bold face**. Subheadings are in lower case and **bold face**. **Bold prints** indicated by double underlining. ACKNOWLEDGMENTS should be placed between the text and the literature REFERENCES.

Scientific names should conform to the International Rules of Nomenclature. Complete scientific names should be given when organisms are first mentioned. The generic name may subsequently be abbreviated to the initial. Authors of names of taxa should be cited both in the SUMMARY and at the first mention of a taxon in the text, but not elsewhere. The scientific names of organism will be printed in *italics* and should be underlined.

Citations in the text of works listed in the REFERENCES should be set out as follows : Nagl & Ehrendorfer (1974), more than two authors : Evans et al. (1972), or : (Nagl & Ehrendorfer 1974, Evans et al. 1972), indication of page or figure Teppner (1974 : 63, Fig. 1). The units of measurement should conform to the SI notation and should be abbreviated in standard form (e.g., μm, mg, l, M) and not followed by full points.

TABLES AND FIGURES : Tables and figures should be used sparingly. The appropriate place of insertion of them in the text should be indicated in the margin of the manuscript. Tables must be typed on separate sheets, numbered and cited in arabic numerals with caption as concise as possible.

When choosing illustrations, please note that the print area of the Journal is 13×19 cm. Line drawings should be neatly executed in black ink and with clear, well-spaced lines that allow for reduction (if possible, by half). Photographs should be high constrast, glossy prints in sharp focus. Photographs may be grouped to make up a plate in such a manner that no space is wasted in between them. Line drawings should be mounted close to each other for only page width (13 cm) reproduction. In both cases, *enough space should be left for the legend* at the bottom of the page. Number all figures (photographs, line drawings or graphs) consecutively from one. Both in the text and legends, the figures should be referred to as Figs. 1, 2, 3 and so on. Superimposed numbers and explanatory letters should be dark on light ground, or light on dark ground and should be bold enough to withstand reduction during reproduction and must be done with stencils of appropriate size or with Letraset (instant letters). Legends should be concise and typewritten on a separate sheet. Copies of original illustration may accompany the duplicate manuscript.

(continued on page 178)

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INDUCED VARIABILITY FOR DIFFERENT BIOLOGICAL PARAMETERS IN SOYBEAN

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SUMMARY

Induced variability for different biological parameters was studied in 2 cultivars of soybean (*Glycine max* (L.) Merr.) viz. KHSB -2 and MACS -124. The chemical mutagens (dES and MH) affected the germination percentage, seedling height and plant survival more drastically than the physical mutagen (gamma rays). The plants treated with the highest dose/concentrations of the mutagens took a longer time to attain maturity as compared to the controls and the plants treated with lower dose/concentration. Both the varieties responded identically to all the mutagenic treatments.

Key Words : Induced variability, soybean.

INTRODUCTION

Genetic diversity is one of the important factors for crop breeding. Substantial variability for different quantitative and qualitative traits can be brought about through mutations. Since spontaneous mutation frequency is very less, induced mutagenesis has gained importance. While Muller (1927) and Stadler (1928) were the first to show the mutagenic effects of X-rays in *Drosophila* and barley respectively, Aurbach & Robson (1946) were the first to introduce chemical mutagenesis. Several varieties of crop plants produced through mutation breeding were reported (Swaminathan et al. 1962, Boreiko 1970, Chowdhary 1978).

Soybean, a high energy legume, was selected for the present report to study the differential effects of gamma rays, dES and MH on 2 varieties, KHSB-2 and MACS-124.

MATERIALS AND METHODS

Seeds of both KHSB-2 and MACS-124 obtained from LAM RARS, Guntur, were exposed to 20 kR, 40 kR and 60 kR doses of gamma rays and were soaked for 6-8 h prior to sowing. other sets of pre-soaked seeds were treated with 0.1%, 0.2% and 0.3% concentrations of dES and MH respectively for each variety. This was followed by a thorough washing with tap water and later by distilled water. One hundred and fifty seeds per each dose/concentration were sown in randomized block design (RBD) in 3 replications along with the respective controls. Data for some parameters like germination percentage, seedling height, period of maturity and percentage plant survival for each dose/concentration were recorded. The results are presented in Table 1.

RESULTS AND DISCUSSION

All the 3 mutagens affected the plant system as evidenced by the decreasing values of percentage germination, seedling height and plant survival and increasing durations of period of maturity in both the varieties of soybean (Table 1).

The germination percentage was drastically reduced at the highest concentration of the chemical mutagens viz, dES in KHSB-2 and dES and MH in MACS-124. The highest concentration of 0.3% MH however, produced a higher value of germination percentage. The value was appoximately similar to those obtained (in both the varieties) due to the highest dose of gamma rays. Hence, the chemical mutagens have produced a more drastic effect of germination than the physical mutagen in MACS-124, whereas the chemical mutagen MH produced a more drastic effect than the other chemical mutagen, dES and the physical mutagen, gamma rays in KHSB-2.

Almost all the values of seedling height were moderately affected by the mutagens and they decreased with increasing dose/concentration of the mutagens with the exception of the lowest dose of gamma rays which recorded a slight increase over the controls in both the varieties.

TABLE 1 : Differential effects of the mutagens on certain biological parameters of KHSB-2 and MACS-124 varieties of soybean.

Treatment	Dose/Conc.	Germination (%)		Seedling height (cm)		Period of maturity (days)		Plant survival (%)	
		KHSB 2	MACS 124	KHSB 2	MACS 124	KHSB 2	MACS 124	KHSB 2	MACS 124
Control	—	98.3	96.3	3.5	3.1	115	110	96.6	94.00
Gamma rays	20 kR	89.3	86.0	3.7	3.2	108	106	82.66	77.30
	40 kR	80.6	78.6	3.5	3.0	112	109	72.0	69.30
	60 kR	65.3	62.0	3.1	2.8	117	114	54.0	48.66
dES	0.1%	63.3	51.0	2.9	2.6	110	103	60.0	46.00
	0.2%	44.0	39.0	2.8	2.3	112	108	40.0	33.66
	0.3%	26.0	32.0	2.4	2.0	121	119	20.0	27.30
MH	0.1%	76.0	52.66	3.1	2.3	114	112	72.66	47.30
	0.2%	68.0	43.30	3.0	2.0	115	116	60.06	38.60
	0.3%	61.3	29.30	2.8	1.8	120	120	56.00	26.30

The period of maturity decreased with the lower doses/concentrations of gamma rays and dES, but increased at the highest doses of gamma rays and dES. The period of maturity increased at all the concentrations of MH treatment.

Plant survival was drastically affected at all the highest concentrations of gamma rays, dES and MH. The lowest dose of gamma rays, however, had only a mild effect. All the doses of the chemical mutagens, however, affected the plant survival moderately.

The early maturity in the lower doses of gamma rays and dES observed presently is in conformity with the reports of Boreiko (1970), Witherspoon & Kathleen (1970) and Baradjanegara (1980) working with induced mutagenesis in different varieties of soybean. Hence, comparatively, chemical mutagens (dES and MH) affected the germination percentage, seedling height and plant survival more drastically than the physical mutagen (gamma rays) supporting the view of Brock (1976). The plants treated with the highest dose/concentration of the mutagens took a longer time to attain maturity as compared to the controls and plants treated with the lower dose/concentration of the mutagens. Both varieties respond identically to all the mutagenic treatments in the production of variants in the present study. However, the applicability of the physical and chemical mutagenesis in the plant improvement programmes involves considerable uncertainty as the process of mutagenesis is not directional and the success depends on choosing the right doses and also the parameters apart from several other factors like standardization of various techniques and protocols.

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(b) JAYAKAR S D & ZONTA L 1990 Coevolution at two trophic levels In Weehrmann K & Jain S (eds) *Population biology* Springer-Verlag Berlin pp349-365

(c) CAVALIER-SMITH T 1985 *The evolution of genome size* John Wiley New York

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IMPACT OF TEXTILE FACTORY EFFLUENT ON MEIOSIS IN *CAPSICUM ANNUUM* L.

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SUMMARY

The impact of textile factory effluent on meiotic cells was studied by using *Capsicum annuum* as the test material. The effluents were alkaline and contained a high proportion of sodium and sulphates. Young flower buds treated with effluents yield a large number of abnormal PMCs in comparison with the control. Abnormal cells were maximal at both anaphase I and II and telophase. Abnormalities such as sticky chromosomes, laggards, bridges, fragments and multinucleate cells were most common. Fairly high amount of pollen sterility was also observed in the treated series. In all the cases, the response remained dose and duration dependent.

Key Words : Genotoxicity, meiosis, industrial effluents, chilli.

INTRODUCTION

Chromosomal aberrations are induced in various test systems either due to variation in physical environment or chemical make up of the medium. They play a key role in producing barriers between different species. Darlington (1965) opined that structural changes in chromosomes during meiosis produce nonviable gametes, and thus induce sterility. Therefore, it becomes imperative to study the meiotic anomalies induced by effluents in plants in view of likelihood contamination following their discharge from factories. Amer (1973) suggested that many chemicals are identical in their mode of action on meiosis, yet they differ in their effects on pollen mother cells (PMCs). In view of the above, the present work was undertaken to study the effects of effluents emanating from a textile industry in Bangalore.

MATERIAL AND METHODS

Effluent samples were collected from the point of discharge from the Binny Mills at a weekly interval for 4 weeks and stored at 10°C. The effluents were analysed by using the standard methods (APHA 1980). The heavy metals were estimated by atomic absorption spectrophotometry following the methods of Van Loon (1980). The data on physico-chemical characteristics of waste water represent the range values.

About 6-8 plants were treated for different durations with different concentrations of effluents. Young flower buds of *Capsicum annuum* were sprayed at the flowering stage for 2, 4 and 6 successive days with different concentrations of effluents and allowed to recover for 24 h. Flower buds sprayed with distilled

water served as control. The buds were excised and fixed in Carnoy's fluid for 2 h and anthers were squashed in acetocarmine/propionocarmine. Pollen sterility was assessed from sixth to eighteenth day, after spraying waste water at the time of flowering. For this, 5 successive sprays were given at an interval of 24 h. A total of 4 plants and a minimum of 1000 pollen grains were observed to assess pollen sterility. Stainability of pollen with acetocarmine was used as an index for determining sterility (Reddy & Rao 1981).

RESULTS AND DISCUSSION

The effluents were alkaline and contained substantial amounts of dissolved solids. Their sodium concentration was 0.16 g/l and sulphate concentration was 0.13 g/l. The concentration of heavy metals was low (Table 1). The percentage of abnormal PMCs in the control series of all the cases relatively remained low, while between 3.36% and 16.19% of abnormal PMCs were observed in the treated series (Table 1).

Abnormal PMCs at different stages of cell division were also scored. More abnormal PMCs belonged to anaphase I and II. A lower percentage of abnormal PMCs were found at M II. In general, more abnormal cells were seen at anaphase than at telophase and metaphase. The effect was generally dose and duration dependent (Table 2).

Following effluent treamet, a large number of PMCs showed multinucleate condition. In addition, PMCs with univalents, bridges, laggards, sticky chromosomes and micronuclei were not uncommon (Table 2). Occasionlly, however, the PMCs showing grouping of chromosomes and multipolar segregation were observed. The percentage of such abnormal PMCs was more at first meiotic division as compared to those at meiosis II.

The stickiness of chromosomes is quite predominant at diakinesis, M I and A I with a high percentage in diakinesis. However, it decreased at subsequent stages of meiosis and was not seen at A II and onwards which is attributed to either recovery of cells or to the probability that PMCs have not completed meiotic cycle (Soliman & Al-Najjar 1980). The stickiness of chromosomes is caused due to agglutination of chromosomes (Resende & Rijo 1948). They give rise to many other abnormalities, such as, single and multiple bridges, sticky bridges, laggards and asynchronised chromosomes during A II.

In the present investigation, 3 types of sticky bridges were noticed: single, double and mulitiple, the first type being more common. The percentage of bridges at A II remained lower than at A I. Klekowsky & Berger (1976) also made similar observations in a fern growing in polluted environment.

TABLE 1 : Physico-chemical characteristics of the waste water from Binny Mills (chemical characteristics in mg/l).

Characteristics	Range
pH	7.80 — 8.30
Electrical conductance, μMhos	2270.00—2348.00
Total solids	2088.60—2138.40
Total dissolved solids	1940.80—1983.70
Total suspended solids	147.80 — 154.70
Turbidity, NTU	84.00 — 96.30
Total alkalinity	986.40—1012.80
Total hardness	203.10 — 216.40
Residual chlorine	0.30 — 3.40
Hydrogen sulphide	8.20 — 10.30
Dissolved oxygen	5.00 — 6.80
Biological oxygen damand at 20°C for 5 days	390.20 — 416.00
Chemical oxygen demand	1240.60—1530.20
Cl	272.50 — 291.00
F	6.30 — 6.80
Ca	20.00 — 28.10
Mg	88.60 — 93.70
Mn	0.80 — 1.70
Na	1552.60—1603.90
K	8.20 — 9.60
Fe	2.62 — 2.83
SO_4	1328.10—1331.50
PO_4	3.21 — 3.63
NO_3	0.21 — 0.27
NO_2	0.53 — 0.61
SiO_2	0.41 — 0.53
Ni^{2+}	0.49 — 0.77
Pb^{2+}	1.83 — 3.13
Zn^{2+}	0.37 — 1.01
Cu^{2+}	0.96 — 1.80
Co^{3+}	1.20 — 5.74
Cr^{3+}	0.21 — 0.61
Ammoniacal nitrogen	21.30 — 28.40

The lagging bivalents in M I are formed due to the hindrance of their movement towards the equatorial plate (Barthelmess 1957) and due to stickiness of chromosomes as reported in *Lycopersicon esculentum* treated with DES (Bose

TABLE 2 : Percentage of abnormalities in meiosis of *Capsicum annuum* treated with effluent of Binny Mills.

Type of treat.	Conc. (%)	No. of plants	No. of PMCs	Percentage of cells with							Total (%)
				Sticky chrom	Laggards	Bi-and multinucleate cells	Micronuclei	Fragments	Univalents	Bridges	
A	25	4	1879	1.59	0.18	0.00	0.00	0.00	1.59	0.00	3.36
	50	7	2014	1.83	0.33	0.06	0.12	0.00	1.68	0.00	4.02
	75	6	2414	1.97	0.49	0.14	0.24	0.06	1.90	0.10	4.90
	100	8	2415	2.31	0.51	0.19	0.38	0.19	2.60	0.21	6.39
	Control	5	2318	0.10	0.00	0.00	0.00	0.00	0.12	0.00	0.22
B	25	8	1904	1.51	0.21	0.95	0.12	0.31	1.68	0.00	4.78
	50	6	2406	1.99	0.39	2.13	0.29	0.56	1.97	0.07	7.40
	75	9	2531	2.19	0.53	2.60	0.37	0.70	2.36	0.27	9.02
	100	7	2647	2.26	0.68	3.13	0.45	0.91	2.83	0.33	10.59
	Control	5	2541	0.15	0.00	0.11	0.00	0.00	0.16	0.00	0.42
C	25	5	2189	0.98	0.86	1.21	0.43	0.63	1.97	0.77	6.85
	50	8	2453	1.55	1.45	2.40	0.59	1.42	2.30	1.24	10.95
	75	7	2451	1.68	1.78	2.83	0.98	1.64	2.83	2.13	13.87
	100	9	2465	2.05	2.11	3.02	1.35	2.17	3.00	2.48	16.19
	Control	6	2044	0.19	0.04	0.03	0.00	0.06	0.19	0.05	0.56

A – Plants sprayed for 2 successive days.
B – Plants sprayed for 4 successive days.
C – Plants sprayed for 6 successive days.

& Bose 1972). A higher frequency of univalents at M I and their random distribution on the spindle is reported to be the outcome of disturbance caused during pairing of chromosomes (Rilly & Law 1965). The presence of multivalents is attributed to the occurrence of many breaks leading to reciprocal translocations (Shastri & Ramaiah 1961).

Disturbance in spindle formation leads to the formation of multipolar spindles. Walters (1958) suggested the origin of multipolar spindle from splitting of spindle organisers during prophase. In the present case, multipolar spindles were observed both at first and second divisions.

The occurrence of micronuclei is attributable to nonorientation of chromosomes, laggards, breaks and fragments (Reddy 1978, Tarar & Dnyansagar 1980). Multinucleate cells result due to the failure of cell plate formation.

Pollen sterility was evident on the sixth day and it reached the peak on the twelfth day after spraying. Subsequently, there was a reduction in the percentage of sterile pollen. Pollen sterility is considered as an indicator of disruption of reproductive mechanism (Reddy 1978). The univalents, laggards and micronuclei formed during meiosis contribute to pollen sterility (Novak & Betlach 1970). A time lag of 8 or 9 days is required for the full development of pollen from actively dividing PMC. The occurrence of maximum sterility after twelfth day of spraying confirms the disturbance caused during the developmental period. A sharp decline after twelfth day indicates that, the spray given is either insufficient or the effect of pollutants is restricted to a short period. Besides chromosomal abnormalities disturbances in physiological reaction within the cytoplasm also contribute to pollen sterility.

It is concluded that the various elements present in the effluents acted on the PMCs in combination. To study the influence of individual factor a detailed investigation using different elements is recommended. Nonetheless, it is better to consider waste water as a composite unit while making this type of study, since the plant communities under natural conditions are exposed to effluents in a similar manner.

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