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CYTOLOGICAL STUDIES IN SOME SPECIES OF *CASSIA* FROM SOUTH INDIA

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(Received 16 November 1992, accepted 21 December 1992)

SUMMARY

Meiotic studies were conducted in 10 species of the genus *Cassia* L. (*C. alata* L., *C. biflora* L., *C. didymobotrya* Fresen, *C. fistula* L., *C. grandis* L., *C. hirsuta* L., *C. javanica* L., *C. nodosa* Buch-Ham ex Roxb., *C. senna* L. and *C. nigricans*)) from South India of which 9 are tetraploids and one diploid. It is suggested that x = 7 is the most common basic number in the genus.

Key Words: *Cassia*, cytology, basic number.

INTRODUCTION

Cassia L. is the largest genus of the family Caesalpiniaceae comprising 600 species (Willis 1973) distributed in the tropical and warm temperate regions. They are popularly known as 'shower trees' and include forms having high ornamental as well as medicinal value. A perusal of the cytological data has shown that the species which are cytologically investigated are from the North and Central India and other geographical regions of the world (Irwin & Turner 1960, Frahm-Leliveld 1960, Baquar et al. 1966, Bir & Sidhu 1966, Bir & Kumari 1981, 1982, Gill & Husaini 1982, 1985, Coleman & De Menezes 1980). It has been found that no serious cytological study has been attempted on this genus from South India. In this paper, the results of the cytological studies in 10 species of *Cassia* from South India are reported.

MATERIALS AND METHODS

The materials for the present study were collected from different localities of Kerala and Tamil Nadu. Young flower buds were fixed in a modified Carnoy's fluid (6 absolute alcohol: 3 chloroform : 1 glacial acetic acid) and were squashed in 2% acetocarmine. Photomicrographs were taken from temporary preparations.

RESULTS AND DISCUSSION

The results of chromosome analysis made on 10 species during the present investigation are shown in Table I and Figs. 1-10. All species showed normal meiotic divisions except a few abnormal divisions in *C. nodosa* and *C. javanica*.

Nine out of the 10 species of *Cassia* studied here are n = 14 except *C. nigricans* which is n = 8. *C. nigricans* is a diploid on x = 8 while the other 9 species are tetraploids on the basic chromosome number x = 7. Cumulative worldwide cytological data revealed that *Cassia* is a multibasic genus with x = 6,7,8,10,11,13 and 15. However, all the Indian species so far investigated are based on x = 6,7 and 8 (Kumari & Bir 1987). It has been found that the common basic chromosome numbers are x = 7 and 8 as far as the Indian species are concerned. It is suggested that species with n = 14, the most

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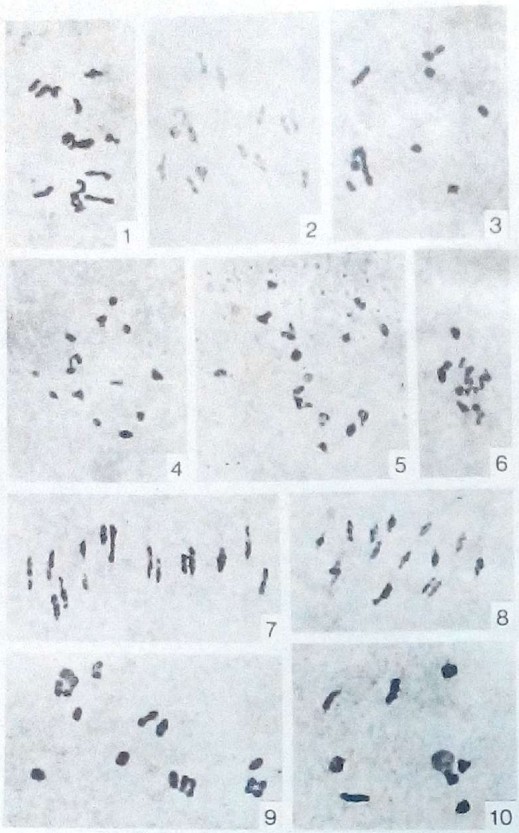

Figs.1-10 : Chromosome numbers of *Cassia* species (all x 2000). 1. *C. alata*, metaphase I (n = 14), 2. *C. biflora*, metaphase I (n = 14), 3. *C. didymobotrya*, metaphase I (n = 14), 4. *C. fistula*, metaphase I (n = 14), 5. *C. grandis*, metaphase I (n = 14), 6. *C. hirsuta*, metaphase I (n = 14), 7. *C. javanica*, metaphase I (n = 14), 8. *C. nodosa*, metaphase I (n = 14), 9. *C. senna*, metaphase I (n = 14), 10. *C. nigricans*, metaphase I (n = 8).

TABLE 1 : List of species studied, their localities and chromosome numbers.

Species	Chromosome number		Locality	Author/s & Year/s
	n	2n		
Cassia alata	14	-	Thiruvananthapuram	Present study
	12	-		Sanjappa &Gupta 1981
	12, 14	-		Gill & Husaini 1982, 1985
C. biflora	14	-	- do -	Present study
	-	28		Irwin & Turner 1960
C. didyamobotrya	14	-	Coonoor	Present study
	14	28		Bir & Kumari 1981, 1982, Berger et al. 1958 Sethi 1930
C. fistula	14	-	Thiruvananthapuram	Present study
				Bir & Sidhu 1967, Tandon & Bhat 1970, Mehra & Hans 1971, Mehra & Sareen 1973, Gill & Husaini 1982, 1985
	13	-		Bir & Sidhu 1966
	12	-		Nanda 1962
	-	28		Berger et al. 1958, Pantulu 1946, 1960
	14	28		Bir & Kumari 1977, 1981, 1982, Choudhary & Choudhary 1988
C. grandis	14	-	- do -	Present study
	-	28		Jacob 1940, Atchison 1951
C. hirsuta	14	-	Thiruvananthapuram, Courtallam	Present study
	-	28		Frahm-Leliveld 1953, 1957
	-	56		Sampath & Ramanathan 1949
	-	28, 56		Irwin & Turner 1960
	8, 8+1B	-		Gill & Husaini 1985
C. javanica	14	-	Thiruvananthapuram	Present study
				Bir & Sidhu 1966, 1967, Tandon & Bhat 1970
	14	28		Bir & Kumari 1981, 1982
	-	28		Ramanathan 1950
C. nodosa	14	-	- do -	Present study, Mehra & Hans 1969, Tandon & Bhat 1970
	-	24		Atchison 1951, Irwin & Turner 1960, Baquar et al. 1966
	14	28		Bir & Kumari 1981, 1982
C. senna	14	-	Thirunelveli	Present study

TABLE 1: (Contd.)

Species	Chromosome number		Locality	Author/s & Year/s
	n	2n		
(=*C. angustifolia*)	14	28		Bir & Kumari 1977, 1981, 1982, Frahm-Leliveld 1960, Irwin & Turner 1960, Baquar et al. 1966
	-	28, 56		Sampath & Ramanathan 1949
C. nigricans	8	-	Ambasamudram	Present study
	-	16		Miege 1960

References cited in Table 1 are drawn from Fedorov (1974), Bir & Kumari (1981, 1982), Kumari & Bir (1987) and IOPB chromosome number reports published in Taxon.

successful number in the genus, might have had a polyploid origin from x = 7. It is also suggested that x = 7 would be the primary basic number and species with n = 8 might be an aneuploid derivative from x = 7. Bir & Kumari (1981) have also suggested the aneuploid origin of x = 8 from x = 7.

Out of 26 species of the genus studied from North and Central India, all species are polyploids (tetraploids, hexaploids and octoploids) and aneuploids with the exception of a diploid *C. mimosoides* var. *demidiata* and var. *wallichiana* (Bir & Kumari 1981). On the basis of the high incidence and grade of polyploidy coupled with a highly variable karyotype, Bir & Kumari (1981) suggested that the genus *Cassia* is in an active state of evolution.

In contrast to this, the species studied from Kerala and Tamil Nadu in South India (present study) all exist at the tetraploid level except one species, *C. nigricans*. Polyploids above the tetraploid level or aneuploids have not yet been reported from this region. This clearly reveals that the South Indian species are not so dynamic in the evolution of species as compared to their counterparts from North and Central India.

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MEIOSIS IN TRIPLOID TARO (*COLOCASIA ESCULENTA* (L.) SCHOTT)

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(Received 7 October 1992, accepted 7 January 1993)

SUMMARY

Meiosis was studied in 8 accessions in 5 triploid morphotypes of taro (*Colocasia esculenta*). Meiosis was irregular characterised by the formation of trivalents, bivalents and univalents in varying frequencies and consequent inibalance in anaphase segregation resulting in a considerable fall in pollen fertility which showed recognizable differences among the morphotypes. The morphotype which exhibited highest frequency of trivalents (10) showed the least pollen fertility (6.8%) and the one with least frequency of trivalents showed the maximum pollen fertility (33.1%).

Based on meiotic behaviour supported by karyomorphological data, the present triploid taros are considered to be of autoploid nature, possibly arisen by union of reduced and unreduced gametes of natural diploid forms. The variation noticed in the magnitude of pollen fertility among the morphotypes studied is discussed, and it is suggested that those with a higher frequency of trivalents and with a very low pollen fertility to be of relatively recent origin and the others with a lower trivalent frequency and a higher pollen fertility to be of earlier origin.

Key Words: Taro, triploid, meiosis, pollen fertility.

INTRODUCTION

Taro (*Colocasia esculenta*) is an economically important tuber crop grown throughout the humid tropics for the edible corms, cormels and leaves. Great bulk of the research work in the crop is on its cytology, mostly chromosome number reports (Fukushima et al. 1962, Yen & Wheeler 1968, Ramachandran 1978, Sreekumari & Mathew 1989) and a few karyotype studies (Sharma & Das 1954, Jos & Magoon 1970, Vijaya Bai et al: 1971, Kuruvilla & Singh 1981, Coates et al. 1988, Sreekumari & Mathew 1991). The available chromosomal data indicate that the species exists in nature at 2 levels of ploidy, diploids with 2n = 28 and triploids with 2n = 42. In addition, a number of numerical variants are also known. The information on meiotic behaviour in the plant, particularly on the triploid forms is very sparse (Vijaya Bai et al. 1971) Triploid taros are found to be highly sterile, and among the different populations, a wide variation in the degree of pollen fertility has been noticed. Detailed information of meiotic behaviour in different triploid forms may yield clues on the possible cause of this variation in addition to shedding light on the nature, mode of origin and evolution of triploid forms. The present report concerns the results of meiotic study in 8 triploid accessions belonging to 5 morphotypes.

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MATERIALS AND METHODS

One hundred and forty triploid germplasm accessions of taro are maintained at the Central Tuber Crops Research Institute, Trivandrum, and based on recognizable morphological differences they were grouped under 24 morphotypes, and were screened for flowering during 1989 and 1990. Meiosis in all 8 accessions belonging to 5 morphotypes which flowered during the year was studied. Young male flower buds were fixed in Carnoy's fluid. Chromosome preparations were made by acetocarmine smear technique. The frequency of chromosome associations at meiosis was scored from observations in 50 PMCs each from 5 plants per accession. Pollen fertility was assessed on the basis of stainability of pollen grains by smearing mature anthers in 1:1 mixture of 1% acetocarmine and glycerine, stained ones being scored as fertile and unstained ones as sterile. Frequency of this was calculated from data of 2000 pollen grains from 5 random fields in 10 slides each prepared from 5 plants per accession.

OBSERVATIONS

All the morphotypes studied here showed the chromosome number of n = 21.

Morphotype - 1

The number of trivalents in each PMC ranged from 5-8 with a mean of 6.74 ± 0.07. Cells with 6 trivalents in each cell showed the highest frequency. The number of bivalents and univalents in each cell ranged from 5 to 7. Anaphase I separation was irregular with varying numbers of chromosomes separating to the two poles. The most frequent segregation type was 16:26. There was significant reduction in pollen fertility (23.5%).

Morphotype - 2

The number of trivalents in each PMC ranged from 407 with a mean of 5.82 ± 0.08. Cells with 5 trivalents in each were more frequent. The number of bivalents and univalents in a PMC ranged from 6-9 and 3-9 respectively. The most frequent A I segregation type was 18:24. Only 20.7% of the pollen grains were found to be fertile.

Morphotype - 3

The frequency of chromosome associations into trivalents, bivalents and univalents was comparable in both the accessions studied. The number of trivalents in each PMC ranged from 4-9 with a mean of 6.36 ± 0.08. Cells with 6 trivalents in each cell showed the highest frequency. The number of bivalents and univalents per PMC ranged from 4-7 and 4-8 respectively. Anaphase I separation was highly irregular with chromosomes varying in number from 15-27 at each pole. Mean pollen fertility was 15.8%.

Morphotype - 4

In this, the number of trivalents per PMC ranged from 3-6 with a mean of 4.21 ± 0.05. Cells with 4 trivalents in each showed the highest frequency. The number of bivalents and univalents in each PMC ranged from 9-10 and 3-6 respectively. Anaphase I separation was irregular with varying number of chromosomes separating to the 2 poles, of which the most frequent segregation type was 19:23. Pollen fertility in this morphotype was much higher (33.1%) than that observed in the other morphotypes.

Morphotype - 5

The highest number of trivalents was observed in this morphotype. The number of trivalents in a PMC ranged from 9-12 with a mean of 10.0 ± 0.05. PMCs with 10 trivalents in each showed the highest frequency. The number of bivalents per cell ranged from 3-5 and univalents from 2-6. The most frequent A I segregation type was 12:30. Thus anaphase I separation was highly irregular resulting in a significant reduction in pollen fertility (6.8%).

DISCUSSION

In the 8 triploid accessions belonging to 5 distinct morphotypes of taro studied here, meiosis was abnormal due to the formation of trivalents in a fairly high frequency. This together with incidence of univalents in varying frequencies and their lagging at anaphase I has led to irregular segregation of chromosomes resulting in a considerable fall in pollen fertility which showed a notable difference among the different morphotypes. The lowest frequency of pollen fertility was in morphotype-5 (6.8%) and the highest in morphotype-4 (33.1%).

Reduction in pollen and seed fertility is known to be a universal effect of autopolyploidy. Stebbins (1971) has pointed out that the principal cause of sterility in autopolyplids is a series of disharmonies at various stages of cell cycle, of which meiotic abnormalities such as the formation of multivalents and consequent irregular segregation of chromosomes are among the most important. Multivalent frequency in autopolyploids varies considerably from species to species for diverse reasons. Some authors (Morrison & Rajhathy 1960), based on observations on autopolyploids belonging to various plant taxa, have postulated that the average number of multivalents in them should be two-third of the total possible number. On such a consideration, in the triploid *Colocasia* with 2n = 42 in which the maximum possible number of trivalents is 14, the average number must be a little over 9. Among the triploid taro types studied here, the number of trivalents ranged from 3-12 in the different morphotypes and the mean ranged from 4.2 - 10. Morphotype - 5 showed the highest frequency of trivalents (9-12) wherein PMCs each with 10 trivalents predominated. But morphotype - 4 showed the lowest frequency of trivalents (3-6) with a mean of 4.2 (Table 1). Gottschalk (1978) has cited examples of plants in which autotetraploids immediately after formation have shown higher frequency of quadrivalents, and their frequency diminishing in subsequent generations ultimately leading to alterations in meiotic behaviour. In the present instance, such of the triploids which showed higher frequency of trivalents may be the ones of relatively recent origin which possessed 3 strict homologues for each chromosome, while the other with fewer trivalents might be of older origin in which during the course of evolution, structural alterations may have occurred leading to loss of strict homology among the 3 chromosomes. Under such a situation, preferential pairing takes place only between strict homologues resulting in corresponding reduction in trivalent frequency. Results of karyomorphological studies made recently (Sreekumari, unpubl.) appears to support this possibility. Karyomorphological analysis showed that in the morphotypes with more trivalents all the different chromosomes were homomorphic, while in the ones with lower trivalent frequency, some of the chromosomes were structurally heteromorphic.

In all the morphotypes studied here, irrespective of the number of trivalents, anaphase I separation was irregular with unequal distribution of chromosomes. The number of chromosomes at

any one pole in the different morphotypes varied, which in morphotype-5 showed the maximum deviation in which the most frequent segregation type was 12:30 whereas the minimum unbalanced number observed was 19:23 (morphotype-4). It may be noted that the extent of meiotic abnormality in the different morphotypes expressed in terms of the frequency of trivalents and magnitude of unbalanced segregation of chromosomes at anaphase I is seen to be correspondingly reflected in the frequency of fertile pollen grains in them which is lowest in morphotype-5 (6.8%) as against 33.1% in morphotype-4.

TABLE 1 : Summary of chromosome associations, anaphase segregation, laggards and pollen fertility in five triploid morphotypes of taro. (Figures in the first line represent the mean and those of the second line represent the range in each morphotype).

Morpho-types	Trivalents	Bivalents	Univalents	Laggards	A I separation (most frequent)	Pollen fertility (%)
1	6.7 ± 0.07	7.8 ± 0.01	5.8 ± 0.02	4.0 ± 0.01	16 : 26	23.5
	5 - 8	6 - 9	3 - 9	3 - 6		
2	5.8 ± 0.07	7.0 ± 0.02	5.1 ± 0.07	4.0 ± 0.03	18 : 24	20.7
	4 - 7	6 - 9	3 - 9	3 - 6		
3	6.4 ± 0.08	6.1 ± 0.05	6.0 ± 0.01	5.1 ± 0.06	15 : 27	15.8
	4 - 9	4 - 7	4 - 8	6 - 8		
4	4.2 ± 0.05	9.2 ± 0.04	4.0 ± 0.02	2.6 ± 0.03	19 : 23	33.1
	3 - 6	9 - 10	3 - 6	2 - 3		
5	10.0 ± 0.05	4.0 ± 0.07	4.6 ± 0.09	6.1 ± 0.01	12 : 30	6.8
	9 - 12	3 - 5	2 - 6	6 - 8		

In all the triploid morphotypes, the number of bivalents observed at meiosis was more than expected and univalents correspondingly become reduced in number in relation to the number of trivalents. More than the expected number of bivalents noticed could be the result of intragenomic pairing which is suggestive of possible ancestral homology between chromosomes belonging to the haploid set with 14 chromosomes. The magnitude of incidence of trivalents and the consequent meiotic abnormalities culminating in conspicuous fall in pollen fertility observed in general in different morphotypes is very much suggestive of their autoploid nature. The possible autoploid nature of triploid taro appears to be particularly evident from their karyomorphological data. It was found that in most of the triploids each chromosome is seen to be present in three doses in the respective karyotypes as reported in some natural autotetraploids where each chromosome is seen in four doses (Nijalingappa, 1974,1977). In nature, triploids arise through chance hybridization between diploids and tetraploids and also by union of unreduced and reduced gametes. In the case of taro, natural tetraploids are virtually absent and hence the triploid strains which occur natually must have arisen through union of unreduced and reduced gametes of diploids making them autoploid in nature. The successful survival of the triploids depends largely on their reproductive efficiency. In many crop plants, natural as well as artificial triploids have been preserved through vegetative propagation on account of several

factors including *gigas* nature of several of their morphological characters (Marks 1966). This is apparently true of the present autotriploid *Colocasia* forms also.

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GENOTOXIC EVALUATION OF FURFURYL ALCOHOL IN MOUSE MEIOCYTE TEST SYSTEM

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SUMMARY

Genotoxic evaluation of the dietary furan furfuryl alcohol has been made following oral administration of 1000, 2000 and 4000 ppm of the compound to Swiss albino male mice as single and multiple doses and using meiotic test system and the standard cytogenetic parameters after 24 h and weekly intervals up to fifth week following the last day of administration. Chromosome mutational activity was assessed by scoring structural aberrations and mitoclasic effects by screening cells with polyploid counts. Appropriate statistical tests were applied to the data to evaluate their significance. Multiple doses were more effective in causing the anomalies. There was a preponderance for the induction of sex chromosomal univalents in treated series over their respective controls. The results indicate a very mild chromosome mutational activity. Increased incidence of polyploids suggested the mitoclasic action of the compound. Abnormal sperms were recorded in both series, but to an insignificant extent.

Key Words: Furfuryl alcohol, genotoxicity, clastogenicity, mouse sperm, head morphology.

INTRODUCTION

Furfuryl alcohol is a dietary furan found in coffee, peanuts, popcorn, caramelized sugar, cooked *Asparagus* and in a variety of nonvegetarian foods (Fishbein 1984). It has wide industrial applications in the manufacture of cement, furoic resins, corrosion resistant polymers, high temperature laminates as well as a solvent in textile industry (Burka et al. 1991).

The information available on the mutagenic activity of furfuryl alcohol is highly fragmentary. Stich et al. (1981) reported its clastogenicity in CHO cells in the presence of S9 mix. However, it did not induce SCEs in human leucocytes in vitro and in occupationally exposed persons (Gomez-Arroyo & Souza 1985). Since the knowledge on mutagenic potentials of the compound is scanty, the present investigation was envisaged by employing meiotic test system and sperm head toxicity assay. These tests involving germ cells are highly recommended protocols for genotoxic safety evaluation especially since the effects induced in them can be transmitted to future generations (Wyrobek et al. 1984).

MATERIALS AND METHODS

Eight- to 10-week-old pure bred Swiss albino male mice with an average weight of 25 g were orally administered with 1000, 2000 and 4000 ppm of furfuryl alcohol (99% pure, Oxoid Ltd., Basingstoke, England) in 0.5 ml volumes of sterile double distilled water. These concentrations were computed on the basis of clastogenic doses of furans which ranged from 100 to 3900 ppm (Stich et al. 1981). Two sets of experiments were conducted. In the single dose series, the compound was administered only once to the animals, whereas in multiple dose experiments, the animals were given the compound 5 times at 24 h intervals to simulate a human situation. Adequate controls were maintained under exactly identical conditions in relation to their experimental counterparts. Animals of both groups were sacrificed for testes and epididymis after 24 h and weekly intervals up to

5 weeks corresponding to one spermatogenetic cycle (Adler 1982). A minimum of two animals per dose per period were employed.

The standard techniques of Evans et al. (1964) and Wyrobek & Bruce (1978) were followed for making chromosome preparations in spermatocytes and sperm suspensions from epididymis respectively. While 200 well spread and brightly stained stages were scored for chromosome abnormalities, 2000 sperms were recorded for sperm head abnormalities. Statistical analysis was carried out by applying modified Wolff's Chi-square test of Li (1961) to the data on chromosome abnormalities and 2 x 2 contingency test of Pillai and Sinha (1968) for assessing sperm head abnormalities.

RESULTS AND DISCUSSION

The genotoxic response of furfuryl alcohol has to be discussed in the context of its metabolism. It gets oxidised to furfural and further to furoic acid and finally gets converted to pyromicuric acid by conjugation with glycine (Castellino et al. 1963).

The recommended cytogenetic assays employing structural, numerical and behavioural chromosome aberrations as cellular dosimeters are used in the present study for evaluating the action of furfuryl alcohol (Adler 1984). Quantitative data on the aberrations induced by furfuryl alcohol are furnished in Tables 1 and 2. Multiple doses were more effective in producing abnormalities than single dose administration.

Among the induced aberrations, behavioural abnormalities (Lin et al. 1971) in the form of univalents, especially with sex chromosomes, showed preponderance over others. In treated groups, they were in the range of 3-14% in single and 3-24% in multiple dose series. Since univalents are reported to occur spontaneously in untreated animals (Lin et al. 1971, Purnell 1973) enough care was exercised to employ animals of the same age and weight in experimentation and evaluate their statistical significance with reference to their respective controls. They were significant with 2 higher doses after first to third week and with the highest dose after fourth and fifth weeks (Table 2). Autosomal univalents were low when compared to XY univalents and they were insignificant. The mode of origin, importance and significance of these univalents have been already discussed elsewhere (Subramanyam & Jameela 1977, Laxminarayana et al. 1980). The consequences of the induction of this phenomenon especially for the sex chromosomes are rather disquieting, since they form aneuploid gametes and can end up with aneuploid syndromes. Hence, the concentrations and periods discussed above have to be reckoned as of importance and consequence from the point of view of genotoxicity.

Numerical aberrations in the form of cells with polypoid counts were recorded for most of the doses and periods. They are reported in untreated animals to the extent of 2.69 ± 0.29 at pachytene and 3.23 ± 0.18 at metaphase. Their origin has been traced to nuclear fusion of 2 or more spermatocytes or by incomplete division of spermatogonia (Lin et al. 1971). In the present study, however, a maximum of 2% was observed in controls and 1-8% in treated groups. They were significant with the highest dose after 24 h in single and up to third week in multiple dose series (Tables 1 & 2). These indicate the activity of the compound and/or its metabolite(s) up to 3 weeks following multiple administration in inducing polyploidy to a significant extent especially with reference to their respective controls.

Burka & Boyd (1985) have suggested the production of electrophilic intermediates in the metabolism of furans and their probable interactions with proteins, nucleic acids and other macromolecules. The significant induction of polyploids in the present study might be due to the

TABLE 1 : Response of mouse meiocytes to single doses of furfuryl alcohol.

Period (Week)	Dose (1000 ppm)	Structural aberrations (%)				Poly-ploidy (%)	Univalents (%)		SHA
		Gaps	Frag-ments	Translo-cations	Total without gaps		XY	Auto-somal	
24 h	C	0	0	0	0	0	3	6	10
	1	0	0	0	0	3	3	7	12
	2	0	0	0	0	3	4	3	12
	4	1	1	0	1	6*	8	2	18
I	C	0	0	0	0	0	5	3	11
	1	0	0	0	0	0	5	8	14
	2	0	0	0	0	0	4	3	12
	4	0	0	0	0	2	8	4	20
II	C	0	0	0	0	1	9	3	10
	1	0	0	1	1	3	14	4	10
	2	0	0	2	2	4	4	5	14
	4	0	0	1	1	1	8	5	21
III	C	0	0	0	0	1	5	4	13
	1	0	0	0	0	0	7	6	17
	2	0	0	0	0	0	8	4	16
	4	0	0	0	0	4	9	4	18
IV	C	0	0	0	0	2	6	6	12
	1	0	0	0	0	0	7	6	18
	2	0	0	0	0	1	6	5	20
	4	0	0	0	0	4	10	8	22
V	C	0	0	0	0	2	6	4	14
	1	1	0	1	1	0	6	6	19
	2	0	0	0	0	2	9	6	26
	4	0	0	1	1	4	9	5	28

* Significant at 5% level ; C, Control; SHA, Sperm head abnormalities.

interaction of these reactive metabolites with proteins required for the construction of microtubules of the spindle (Subramanyam et al. 1984). The biological significance of these polyploids in terms of viability and motility of diploid sperms is not yet fully understood. However, it is suggested that during fertilization such sperms fail to compete with haploid ones (Lin et al. 1971).

Structural aberrations in the form of gaps, fragments and translocations were recorded. They were the lowest among all induced aberrations. In multiple dose series, the frequency of fragments was comparatively more and they were significant with the highest dose after fifth week (Table 2). Translocations which are cellular indicators of chromosome mutations were rare in both series. In

TABLE 2 : Response of mouse meiocytes to multiple doses of furfuryl alcohol.

Period (Week)	Dose (in 1000 ppm)	Gaps	Structural aberrations (%)			Poly-ploidy (%)	Univalents (%)		SHA
			Frag-ments	Trans-locations	Total without gaps		XY	Auto-somal	
24 h	C	0	0	0	0	0	5	2	10
	1	0	0	0	0	0	7	4	13
	2	1	0	1	1	2	9	3	14
	4	2	0	1	1	4*	9	3	14
I	C	0	0	0	0	1	4	3	11
	1	1	0	0	0	3	9	4	16
	2	1	0	1	1	5	14*	6	17
	4	0	2	0	2	7*	18*	5	21
II	C	0	0	0	0	1	6	2	10
	1	2	1	0	1	3	9	3	14
	2	1	1	1	2	5	19*	3	17
	4	0	2	1	3	8*	24*	4	20
III	C	0	0	0	0	1	4	3	11
	1	0	1	0	1	4	6	3	15
	2	0	2	0	2	5	12*	2	20
	4	0	3	0	3	7*	13*	5	21
IV	C	0	0	0	0	2	5	4	17
	1	1	1	0	1	6	8	4	19
	2	0	2	0	2	4	10	6	20
	4	0	3	0	3	7	14*	3	24
V	C	0	1	0	1	1	4	4	11
	1	1	4	0	4	4	3	2	14
	2	0	5	0	5	3	8	4	17
	4	2	7*	0	7*	4	14*	3	23

* Significant at 5% level; C, Control; SHA, Sperm head abnormalities.

contrast to the negative mutagenic response reported by Arnaiz et al. (1989) in Drosophila melanogaster using sex linked recessive lethal assay, these results indicate a mild chromosome mutational activity of furfury alcohol and/or its metabolite(s) in meiotic test system.

Data on sperm head morphology assay are also furnished in Tables 1 and 2. In general, a dose dependent increase in the induction of abnormal sperms like amorphous and hookless sperms was recorded for almost all periods in both series. These altered sperms can lead to embroyonic death or transmit genetic aberrations to live progeny (Wyrobek et al. 1984). Even though they were not statistically significant, the periods up to fifth week in both series have to be reckoned as important

from the point of view of possible genetic hazard since they cause alteration in the genes which control sperm head morphology as they are not detected at the chromosomal level (Beaula Helen & Subramanyam 1991).

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IDENTIFICATION OF GENES CONTROLLING SEED SURFACE IN GRAM (*CICER ARIETINUM* L.)

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SUMMARY

In the present study, 8 parents, differing in respect of seed surface were selected and 7 triangular crosses were made. Their F_1, F_2 and F_3 generations were studied to know the inheritance of seed surface and genes involved. The dominance of rough seed coat was observed over smooth and was found to be controlled by 3 complementary duplicate genes. $R_{SS_{n1}}R_{SS_{n2}}$ and $R_{SS_{n3}}$.

Key Words : Triangular cross, seed surface, gram, inheritance.

INTRODUCTION

Genetics of several morphological characters including seed surface have been studied in gram by several earlier workers. (Singh & Ekbote 1936, Ayyar & Balsubrahmanyam 1937, Deshmukh et al. 1972, More & D'Cruz 1976, Nayeem et al. 1977, Phadnis 1977, Reddy & Nayeem 1978, Pawar & Patil 1979, Ghatge & Kolhe 1985). The present investigation was undertaken in order to identify the genes controlling seed surface in gram from triangular crosses.

MATERIALS AND METHODS

The hybridization programme was undertaken among the 8 parents selected from a collection of 135 gram types maintained in botanical garden of College of Agriculture, Pune. The selected parents were having constrasting characters in respect of seed surface. The crosses were effected and their F_1, F_2 and F_3 generations were studied in order to know the inheritance of seed surface. Seven triangular crosses involving 8 parents, were studied in F_2 generation (Table 2) and the results obtained in F_2 generation were confirmed by studying the behaviour of selected families in F_3 generation (Table 3).

Chi-square test (X^2) was applied for testing goodness of fit for segregation of individual character (Fisher 1941). The genes governing the seed surface in gram were symbolized as suggested by Tanaka (1957).

OBSERVATIONS

Table 1 shows the seed surface character possessed by selected 8 parents as well as the F_1s of 12 crosses. It is further revealed that rough seed coat is dominant over smooth in all the 12 crosses. The segregation for seed surface was studied in F_2 generation (Table 2) of 12 crosses which showed that seed surface was controlled by 3 genes having complementary duplicate gene action. The results obtained in F_2 generation of 12 crosses were confirmed from breeding behaviour of selected F_3 families (Table 3).

TABLE 1 : Seed surface of parents and the F_1s in *Cicer arietinum*.

Name of parent / cross	Seed surface
Tiny Leaf (TL)	Smooth
Green Bold (GB)	Rough
Bronze Leaf (BL)	Smooth
White Flower White Grained II (WFWG)	Rough
Spreading (SP)	Smooth
Chrysanthifolia (bl) Chy (bl)	Rough
N_31	Rough
Pusa 83 DP (PDP-83)	Rough
TL x BL	Rough
GB x TL	Rough
GB x BL	Rough
TL x WFWG	Rough
TL x Chy (bl)	Rough
Sp x WFWG	Rough
BL x N-31	Rough
TL x N-31	Rough
TL x PDP - 83	Rough
BL x PDP -83	Rough
PDP -83 x N-31	Rough

TABLE 2 : Segregation of F_2 population of 12 crosses for seed surface in *Cicer arietinum*.

Cross	Ratio	Seed surface		X^2	P	Gene symbols
		Rough	Smooth			
TL x BL	9:7	277	211	0.09	0.7 - 0.8	Rss_{a2}, Rss_{c2}
GB x TL	3:1	332	114	0.07	0.7 - 0.8	Rss_{a2}, (Rss_{c2})
GB x BL	3:1	116	42	0.21	0.5 - 0.7	Rss_{c2}, (Rss_{a2})
SP x WFWG	9:7	208	164	0.02	0.8 - 0.9	Rss_{b2}, Rss_{c2}
TL x WFWG	3:1	214	70	0.01	0.8 - 0.9	Rss_{b2}, (Rss_{c2})
TL x Chy (bl)	3:1	402	130	0.09	0.7 - 0.8	Rss_{a2}, (Rss_{c2})
Chy (bl) x WFWG	15:1	315	25	0.70	0.3 - 0.5	Rss_{a2}, Rss_{b2}(Rss_{c2})
TL x N-31	54:10	525	101	0.12	0.7 - 0.8	Rss_{a2}, Rss_{b2} Rss_{c2}
BL x N-31	3:1	318	110	0.11	0.7 - 0.8	Rss_{b2} (Rss_{a2})

TABLE 2 : (Contd.)

Cross	Ratio	Seed surface		X^2	P	Gene symbols
		Rough	Smooth			
TL x PDP-83	3:1	392	126	0.12	0.7 - 0.8	Rss_{a2} (Rss_{c2})
BL x PDP-83	3:1	201	69	0.04	0.8 - 0.9	Rss_{c2}, (Rss_{a2})
PDP-83 x N-31	15:1	163	15	0.35	0.5 - 0.7	Rss_{b2}, Rss_{c2} (Rss_{a2})

TABLE 3 : Breeding bahaviour of F3 families in twelve crosses for seed surface in gram.

Cross	No. of families	B.T. Rough	F_2 behaviour				B.T. Smooth	X^2	P
			3:1	9:7	15:1	54:10			
TL x BL	58	7	16	12	-	-	23	3.96	0.3 - 0.5
GB x TL	63	20	29	-	-	-	14	1.53	0.3 - 0.5
GB x BL	59	17	29	-	-	-	13	0.55	0.7 - 0.8
SP x WFWG	56	5	12	16	-	-	23	1.30	0.7 - 0.8
TL x WFWG	61	18	29	-	-	-	14	0.67	0.7 - 0.8
TL x Chy (bl)	64	13	37	-	-	-	14	1.59	0.3 - 0.5
Chy (bl) x WFWG	60	25	15	-	10	-	8	6.50	0.2 - 0.3
TL x N-31	63	13	10	12	9	5	14	4.50	0.3 - 0.5
BL x N-31	52	17	23	-	-	-	12	1.65	0.3 - 0.5
TL x PDP-83	62	14	33	-	-	-	15	0.29	0.8 - 0.9
BL x PDP-83	48	15	19	-	-	-	14	2.12	0.3 - 0.5
PDP-83 x N-31	58	23	15	-	14	-	6	1.77	0.5 - 0.7

DISCUSSION

Monogenic dominance of rough seed coat over smooth was reported by Singh & Ekbote (1936), Deshmukh et al. (1972), More & D'Cruz (1970, 1976), Patil & Deshmukh (1975), Nayeem et al. (1977), Reddy & Nayeem (1978), Pawar & Patil (1979), Ghatge & Kolhe (1985).

In the present study, out of 12 crosses 7 (TLxWFWG, GBxTL, GBxBL, BLxN-31, TLxChy(bl), BLxPDP-83 and TL x PDP) gave a monogenic ratio of 3 rough : 1 smooth seed surface thus

confirming the reports of earlier workers. Similarly, in 2 crosses viz., SPxWFWG, and TLxBL, a complementary ratio of 9 rough : 7 smooth seed surface was obtained which was in agreement with the findings of Deshmukh et al. (1972), More & D'Cruz (1976) Reddy & Nayeem (1978) and Pawar & Patil (1979). However, Nayeem et al. (1977) studied spinnate nature of seed surface and recorded a 2 gene complementary ratio of 9 spinnate : 7 non-spinnate seed.

In another 2 crosses viz., Chy(bl) x WFWG and PDP-83XN-31 a duplicate ratio of 15 rough : 1 smooth was obtained while in TLxN-31, a trigenic ratio of 54 rough : 10 smooth seed was observed indicating that 3 complementary duplicate genes are involved in the inheritance of seed surface which has been reported for the first time (Fig.1).

The genes governing the seed surface in different parents have been symbolized and shown in Fig. 1 which further shows the behaviour of parents in triangular crosses for seed surface and the segregation observed in F_2 generation of 12 crosses.

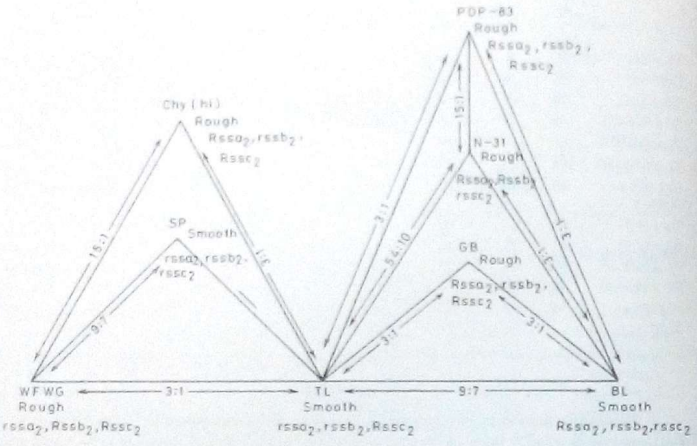

Fig. 1 : Triangular crosses showing the segregation for seed surface in F_2 with phenotypic ratios and genes governing seed surface.

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EFFECT OF GAMMA RAYS AND EMS ON BIOLOGICAL AND MUTAGENIC PARAMETERS IN CEREALS

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SUMMARY

Biological and mutagenic effects of gamma rays, EMS and their combination were studied in 2*x* rye, 4*x* and 6*x* wheats and 6*x* triticale. Higher lethality for germination and seedling height and higher values for mutagenic effectiveness, efficiency, factor of effectiveness and mutants per mutation were noticed in EMS treatments of both individual and combined treatments. The latter produced positive synergistic and less additive effects. Lower ploidy levels were more mutable than of higher ploidy and 6*x* triticale was found to be more sensitive than 6*x* wheat.

Key Words: Cereals, mutagenic effects.

INTRODUCTION

The use of induced mutations is supplementary to the recombination methods of plant breeding. The direct control of the mutation process has been long desired, in order to induce mutations at specific loci and to produce mutants with beneficial value. In order to use mutation breeding as an effective tool, a systematic study of induced mutagenesis in different crop plants using a variety of mutagens singly and in combination is essential. Biological parameters and chlorophyll mutations are extensively used to judge the optimum dose / concentration at which maximum mutations are achieved. The present paper reports the effect of gamma rays and EMS on certain biological and mutagenic parameters in different cereal crops which differ in ploidy level.

MATERIALS AND METHODS

Seeds of one variety each of 2*x* rye (*Secale cereale* L.) var. King II, 4*x* wheat (*Triticum durum* Desf.) var. Jairaj, 6*x* wheat (*T. aestivum* L. em Thell) var. WH 147, 6*x* triticale (X *Triticosecale* Wittmack) var. Carman were treated with gamma rays (GR) (15, 20, 25 kR) and 0.5% ethyl methane sulphonate (EMS) (8, 10, 12h) and combination of GR + EMS. Data on various biological parameters including seed germination, seedling height, stomatal frequency, chlorophyll variants and pollen sterility were recorded in M_1 generation. Data on chlorophyll mutation frequency recorded in M_2 generation were used to estimate 5 mutagenic parameters viz., mutagenic effectiveness, efficiency, factor of effectiveness, mutants/mutation and co-efficient of interaction. The methods for estimating the above parameters were described earlier (Reddy 1989, 1992).

OBSERVATIONS

The data on germination, seedling height and various mutagenic parameters, induced by GR, EMS and their combination in 4 different cereal crops (2*x* rye, 4*x* wheat, 6*x* wheat, 6*x* triticale) are presented in Table 1. The decrease in germination and a reduction in seedling height were noticed in all mutagenic treatments. Similarly, other biological parameters include seedling injury, stomatal frequency, abnormalities in stomata, chlorophyll variants and pollen sterility were also increased either

marginally or significantly. The decrease and increase was dose and duration dependent. The lethality was prominant in combined treatments followed by EMS and GR. The lethality was decreased with increase in ploidy level, while 6x triticale was found to be more sensitive than 6x wheat.

EMS was found highly effective, while the efficiency, factor of effectiveness and mutants per mutation were more in combined treatments. The values of all the mutagenic parameters were increased with dose of GR and duration of EMS. However, in combined treatments the increase was dependent on duration of EMS. Coefficient of interaction showed that in combined treatments both positive synergism and less additive effects are present.

DISCUSSION

Germination, survival and seedling growth are widely used as an index in determining the biological effects of various mutagens. The reduction of these parameters were prominent in EMS treatments either alone or in combination. Such an inhibitory effect of various mutagens were reported in several other crops (Reddy et al 1991a, 1992, Reddy & Aloka Saikia 1992, Pushpalatha et al. 1992). Reduction of these parameters has been attributed to various factors including changes in the balance of growth regulators and metabolic activity (Aman 1968), physiological changes include inhibition of DNA synthesis (Gordon 1957) or inhibition of mitotic proliferation (Sparrow 1961). Increase in seedling injury could be due to mitotic irregularities, while increase in stomatal index and abnormal stomata were explained due to stimulatory effect of mutagen causing changes in endogenous growth regulators (Sharma & Govil 1986). Chlorophyll chimeras in M1 could result is alteration of DNA in chloroplasts and EMS is well known to react preferentially with guanine. Such variants were also likely to arise from a dominant mutation or due to mitotic recombination. Sterility in pollen is mainly due to interchanges between non-homologous chromosomes and other detectable chromosomal aberrations, reported in our earlier study (Suganthi & Reddy 1992).

The effectiveness, which gives the frequency of mutations induced by a unit dose of mutagen was found higher at higher duration of EMS either alone or in combination supporting that chemical mutagens are more effective than GR. In individual treatments of GR, the effectiveness was more in 25 kR treatments followed by 15 kR in rye and in both the wheats but in triticale it is dose dependent. This non-random pattern of variation in mutagenic effectiveness suggest that the genotypic response to GR is of genetic origin. Increase in efficiency in all the mutagenic treatments suggests that the proportionate increase in the mutagenic level. The higher efficiency at higher mutagenic level is perhaps due to the fact that the biological damage did not increase with increase in the mutation rate was higher than the proportionate increase in the mutagenic level. The mutagenic parameter factor of effectiveness gives the number of mutations per 100 treated seeds. This method is superior over the other methods suggested by Gustafssan (1937) where mutation frequencies are calculated only on surviving plants. Results obtained by this method also supports the ability of EMS in producing more mutations. Mutants per mutation, is another mutagenic parameter and gives an idea of mutants produced due to a mutational event. Though mutants per mutation were dose and duration dependent, the total values (5.4-7.7) were comparatively lower than those reported earlier by Rao & Rao (1983) in rice (11%) and Reddy & Gupta (1989) in triticale (13%), which could be due to genotypic or dose/duration of mutagen. Coefficient of interaction (K) shows that both positive synergism and less additive effects were occured in the combined treatments. Higher values of efficiency, factors of

TABLE 1 : Effect of gamma rays and EMS on biological and mutagenic parameters.

	Difference over control		Effec-tive-ness	Effi-ciency	Factor of effect-iveness	Mutants / mutation	(K)
	Germi-nation (%)	Seedling ht. (cm)					
RYE (2x) King II							
GR							
15 kR	-29.5	-3.9	0.01	0.006	3	5.7	-
20 kR	-32.6	-4.3	0.01	0.006	3	5.9	-
25 kR	-35.7	-4.7	0.01	0.008	5	6.1	-
EMS							
8 h	-44.8	-4.9	0.15	0.013	8	6.3	-
10 h	-46.9	-5.1	0.17	0.018	10	6.6	-
12 h	-50.0	-5.7	0.18	0.022	13	7.1	-
GR + EMS							
15 kR+12 h	-59.1	-6.9	0.06	0.023	16	7.6	1.3
20 kR+10 h	-53.0	-5.5	0.03	0.018	11	7.0	0.9
25 kR+ 8 h	-51.0	-5.4	0.02	0.015	9	6.5	0.8
WHEAT (4x) Jairaj							
GR							
15 kR	-25.2	-0.8	0.00	0.005	2	5.7	-
20 kR	-28.4	-0.9	0.00	0.005	2	5.8	-
25 kR	-32.6	-1.0	0.01	0.009	4	5.9	-
EMS							
8 h	-38.9	-1.0	0.14	0.014	7	6.0	-
10 h	-43.1	-1.1	0.16	0.018	9	6.4	-
12 h	-46.3	-1.3	0.20	0.026	11	6.5	-
GR + EMS							
15 kR+12 h	-51.7	-1.4	0.06	0.026	12	6.9	1.0
20 kR+10 h	-48.4	-1.3	0.03	0.019	10	6.9	0.9
25 kR+ 8 h	-47.3	-1.2	0.02	0.016	8	6.3	0.8
WHEAT (6x) WH 147							
GR							
15 kR	-23.9	-0.1	0.00	0.003	2	5.4	-
20 kR	-26.0	-0.3	0.00	0.004	2	5.5	-
25 kR	-29.1	-0.4	0.00	0.007	4	5.7	-
EMS							
8 h	-36.4	-1.0	0.08	0.009	5	5.8	-
10 h	-41.6	-1.2	0.12	0.014	8	6.2	-
12 h	-43.7	-1.3	0.12	0.017	10	6.4	-
GR + EMS							
15 kR+12 h	-48.9	-1.5	0.04	0.018	11	6.5	1.0
20 kR+10 h	-42.7	-1.4	0.02	0.017	8	6.3	1.0
25 kR+ 8 h	-39.5	-1.3	0.02	0 .015	7	6.1	1.1

TABLE 1 (Contd.)

	Difference over control		Effective-ness	Efficiency	Factor of effectiveness	Mutants / mutation	(K)
	Germination (%)	Seedling ht. (cm)					
TRITICALE (6x) Carman							
GR							
15 IR	-24.4	-0.0	0.01	0.008	4	5.5	-
20 kR	-29.7	-0.1	0.02	0.014	6	5.7	-
25 kR	-34.0	-0.3	0.02	0.017	8	5.9	-
EMS							
8 h	-36.1	-1.3	0.19	0.021	10	5.9	-
10 h	-41.4	-1.4	0.19	0.023	12	6.3	-
12 h	-43.6	-1.6	0.23	0.021	16	6.5	-
GR + EMS							
15 kR+12 h	-51.0	-1.6	0.07	0.011	18	6.8	1.0
20 kR+10 h	-54.2	-1.5	0.07	0.033	21	6.8	1.3
25 kR+ 8 h	-45.7	-1.1	0.04	0.027	15	6.3	0.8

effectiveness and mutants per mutations in combined treatments were earlier attributed to synergistic effects in triticale, barley, wheat (Reddy 1992) and in rice (Mohan Rao 1972).

Higher lethality and higher values for all mutagenic parameters at lower ploidy level (2x rye followed by 4x wheat) indicates more resistance of hexaploids to mutagens than tetraploids and the latter being more resistant than the diploids. These results are in conformity with earlier reports in several cereals (Bhaskaran & Swaminathan 1961, Suganthi & Reddy 1992). Among hexaploids, triticale was found more sensitive and mutable than wheat and this could be attributed to unstable genomic condition of triticale (Reddy 1991).

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INDUCED VARIABILITY FOR SEED YIELD AND SEED PROTEIN IN FRENCH BEAN (*PHASEOLUS VULGARIS* L.)

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SUMMARY

Seeds of french bean varieties Jwala and Top crop were used for mutation induction by gamma rays. Dry seeds were subjected to 5, 10, 15, 20 and 25 kR of gamma rays. Data on seeds per plant were recorded in M_1 and M_2 generations. The M_1 and M_2 seeds were analysed for seed protein content and protein profile. Increase in mean, coefficient of variation and higher heritability for both the traits in M_2 generation suggest induction of substantial genetic variation in M_2 generation of mutagenized population. A comparision of protein profile of normal and mutagenized seeds confirm that alteration in protein has been achieved both at quantitative and qualitative levels.

Key Words : French bean, mutation, seed protein, electrophoretogram.

INTRODUCTION

Grain legumes are the chief source of protein and dietary amino acids for man and farm animals. These crops form a major source of protein for vegetarian and economically backward people of Third World countries (Lush & Rachie 1979). *Phaseolus vulgaris* L. (french bean) is a major pulse crop of tropical and sub-tropical countries and occupies a significant place in human diet as pulse or green vegetable. The present paper reports the effects of induced mutagenesis on seed protein content, seed yield per plant and protein profile in 2 varieties *P. vulgaris* viz. Top crop and Jwala.

MATERIALS AND METHODS

Seeds of *P. vulgaris* L. var. Jwala and Top crop having nearly 13% moisture were subjected to 5, 10, 15, 20 and 25 kR of gamma rays. The mutagenized seeds along with their respective control were sown in randomised complete block design in 3 replicates to raise M_1 generation. Seeds from each M_1 plant were harvested separately and sown to raise M_2 generation as single plant progenies. Seeds from 50 plants selected randomly from each treatment in M_1 and their M_2 progenies were analysed for total seed protein content by microkjeldahl method (McCkenzie & Heather 1953). Analysis of variance, genotypic and phenotypic coefficient of variances, heritability and genetic advance were computed for total seeds per plant and seed protein in each variety. Protein for electrophoresis was extracted in 0.2 M phosphate buffer. The homogenate was centrifuged and supernatant was used for electrophoresis. Polyacrylamide disc gel electrophoresis was carried out in 5 x 60 mm glass tubes using 7.5% acrylamide gels. The protein was fractioned in anodic system using tris-glycine buffer at pH 8.3 with bromophenol blue as tracking dye (Davies 1964). After electrophoresis, the gels were stained in 0.2% comassie brilliant blue for 30 min and destained in 7% acetic acid.

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RESULTS AND DISCUSSION

The data recorded on seed protein and total seeds per plant in M_1 and M_2 generations after gamma irradiation of seeds are presented in Table 1. The increase in mean seed protein content and decrease in total seeds per plant with appreciable increase in coefficient of variation as compared to control have been recorded in M_1 generation of both the varieties. In M_2 generation, however, the seed yield per plant increased significantly as compared to the marginal change in the mean seed protein content. The coefficient of variation in M_2 generation was higher than the control but lower than the M_1 generation for both the characters (Table 1). Analysis of variance, heritability, component of variance, genetic advance and GA % mean for both the characters in M_2 generation have been presented in Table 2. Statistically significant 1-*f*-value suggest induction of larger variation in the mutagenized population. Phenotypic coefficient of variation was found to be higher than genotypic coefficient of variation in both the varieties for both the characters. This suggests that the characters are influenced by environmental factors. Moderately high heritability for both the traits and higher GA for seeds per plant as compared to seed protein have been recorded. Higher heritability along with high genetic gain are most useful in predicting the effect for selecting the better individuals. If the heritability is mainly owing to non-additive gene effect the expected genetic gain would be low, but if it is owing to additive gene effect a high genetic advance may be expected. Higher heritability (more than 50%) for seed protein and total seeds / plant in Top crop and only for total protein in Jwala in present study suggest the presence of higher proportion of genetic variance in the treated population. Thus selection for these traits would be fairly easy. Nevertheless, higher heritability has not been found to be associated with high genetic advance for seed protein in both the varieties.

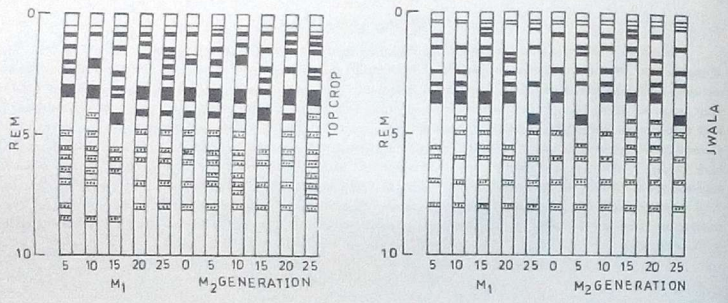

Fig. 1. : Protein profile of buffer soluble seed protein of normal and and mutagenized seeds in M_1 and M_2 generations of *P. vulgaris* var Top crop and Jwala.

TABLE 1 : Mean, standard deviation and coefficient of variation for seed protein (mg/100mg seed meal) and seeds per plant in M_1 and M_2 generations in Top crop and Jwala varieties of *Phaseolus vulgaris*.

Dose (kR)		M_1 generation			M_2 generation		
		Mean	± S.D.	C.V.	Mean	± S.D.	C.V.
TOP CROP	WP	22.2	± 0.6	3.0	22.4	± 0.4	2.1
Control	TS	18.7	± 1.5	8.4	16.3	± 1.6	10.1
5	WP	23.6	± 0.5	2.4	24.8	± 0.3	1.3
	TS	15.4	± 3.9	25.1	22.7	± 5.8	29.5
10	WP	24.7	± 0.9	3.6	23.3	± 0.6	2.4
	TS	15.2	± 3.7	24.8	26.1	± 5.1	19.5
15	WP	25.9	± 1.3	5.1	25.5	± 0.4	1.7
	TS	11.9	± 2.7	22.8	20.9	± 3.7	18.5
20	WP	26.5	± 1.4	5.2	26.9	± 1.8	6.6
	TS	15.5	± 2.7	17.7	26.9	± 5.7	21.2
25	WP	27.8	± 3.2	11.7	27.7	± 0.3	1.1
	TS	13.2	± 1.0	8.1	27.7	± 7.5	28.2
JWALA	WP	22.2	± 1.1	4.5	23.0	± 1.0	4.3
Control	TS	9.9	± 1.9	19.5	12.5	± 1.9	15.92
5	WP	23.6	± 1.2	5.1	24.1	± 1.1	4.9
	TS	13.0	± 4.2	31.2	15.9	± 3.9	24.9
10	WP	24.7	± 1.2	5.1	24.3	± 1.2	4.9
	TS	10.9	± 4.7	43.5	19.2	± 4.9	25.5
15	WP	25.1	± 1.3	5.3	25.0	± 1.2	4.9
	TS	8.5	± 3.1	36.7	17.9	± 5.9	33.2
20	WP	26.7	± 1.4	5.2	25.4	± 1.2	4.7
	TS	8.1	± 2.7	33.2	21.3	± 5.7	26.7
25	WP	28.7	± 1.5	5.2	28.7	± 1.3	4.6
	TS	9.2	± 1.8	19.4	25.91	± 3.7	14.3

Seed protein profile of the normal and mutagenized population of Top crop and Jwala is presented in Fig. 1. Variation in the present and/or absence of specific protein bands in relation to control could be discerned in the mutagenized population. Shift in main protein band present at REM 3.4 in control in both the varieties is conspicuous at 15 kR in Top crop and 5 and 25 kR in Jwala in M_2 generation. Presence of an extra band at REM 0.7, 1.4, 1.7, 2.9, 4.5, 5.6, 6.5 and 7.5 in Top crop and 0.7, 1.4, 2.0, 6.5 and 7.5 in Jwala in M_2 generation is apparent in the zymograms. Similarly, absence of bands at REM 1.7 (at 5 kR) and 5.0 (5 kR) in Jwala in M_2 generation were noted. Electrophoretic analysis of seed storage protein have been utilised for studying the genetic diversity in natural as well as mutagenized populations (Pandey & Purohit 1979, Jope & Jana 1980). Variation in banding pattern observed in these 2 varieties suggests genotypically controlled biochemical diversity.

Appearance of new bands, disappearance of bands present in the protein profile of control seeds and shift in the position of bands in the zymogram of the mutagenized population confirms the alteration in the polypeptide of seed storage protein due to gene(s) mutation.

TABLE 2 : Analysis of variance, estimates of CV_g, CV_p, heritability and genetic advance for seed yield and seed protein in M_2 generation in *Phaseolus vulgaris* var. Top crop and Jwala.

	Top crop		Jwala	
	WP	TS	WP	TS
Mean square	8.34	85.30	11.62	63.38
F - value	7.51**	5.96**	4.38*	3.67
CV_g	6.89	20.95	6.18	20.91
CV_p	15.1	30.45	8.06	26.48
Heritability	53.01	47.09	63.46	62.32
Genetic Advance (K = 2.06)	6.15	31.73	4.96	48.73
GA % mean	24.55	168.97	12.76	209.51

*, ** significant at 5% and 1% level respectively
CV_g and CV_p genotypic and phenotypic coefficient of variance respectively WP and TS whole seed protein and total seeds per plant respectively.

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CLASTOGENIC POTENTIAL OF DE-OILED NEEM CAKE

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SUMMARY

De-oiled neem cake is widely used as manure in agricultural practices to supply the essential nutrients. Neem cake has gained importance in recent years as a broad spectrum repellant and insecticide. Investigations carried out during the present study showed that neem cake is clastogenic and can induce chromosome clumping, c-mitoses and other abnormalities in cell divisions. The results indicate that excessive application of neem cake in soil may exert hazardous effects on plants.

Key Words: Neem cake, clastogenecity, *Allium cepa*.

INTRODUCTION

It is well known that many plant species are capable of synthesizing toxic chemicals to defend themselves against insect pests. A search for these natural pesticides is in progress in several countries to replace the popular synthetic pesticides which exert harmful effects on human beings.

Neem (*Azadirachta indica* Juss.) belonging to the family Meliaceae has in recent years received attention both as a broad spectrum repellant and insecticide. The seed cake, residue of the oil extract, is considered as a better source for the supply of nitrogen than farmyard manure and is widely used in agricultural practises to supply the essential nutrients. Coating of urea with neem cake has been found to increase the efficiency of urea by 19-20%. Due to the insect repellant properties of neem cake it is widely used in agricultural practises to supply the essential nutrients and also to act as a systemic pesticide.

Studies on isolated compounds reveal that most of the active components in *A. indica* belong to limonoids, flavonoids, macromolecular substances and to a lesser extent sulphurous compounds (Van der Nat et al. 1991). Neem seed fractions are known to adversely affect the growth and morphogenesis of several insect species. However, enquiry into the nature of such changes that might occur in plants grown in soil containing neem cake has not received attention yet. The aim of the present investigation has been to evaluate the clastogenic potential of neem cake.

MATERIALS AND METHODS

De-oiled neem cake used for the studies was obtained from India phosphate company. In order to study the clastogenic potential of neem cake healthy bulbs of *Allium cepa* were grown in sand mixed with neem cake in different proportions (1:10, 1:15, 1:20, 1:25, 1:30). After about 3-6 days, when roots began to develop, root tips were fixed in Carnoy's fluid without any pre-treatment. For cytological studies, the acetocarmine squash technique was followed. Root tips fixed from bulbs grown in pure sand without adding any neem cake served as control. In each experiment, 3 roots were examined per treatment to score the abnormalities in division. Three replications were made and three hundred cells in division were analysed per treatment.

RESULTS AND DISCUSSION

The normal somatic complement of *Allium cepa* analysed in the control plants showed 16 chromosomes at the metaphase stages of divisions. Only 2.38 per cent of the root tip cells showed abnormalities in division. A wide spectrum of chromosomal aberrations was observed in the roots examined from the different treatments (Fig.1). Statistically significant differences from controls were observed. As many as 10 fragments per cell were observed in the highest treatments. The data presented in Fig.1 show that clastogenic effects tend to increase in frequency as the concentration of

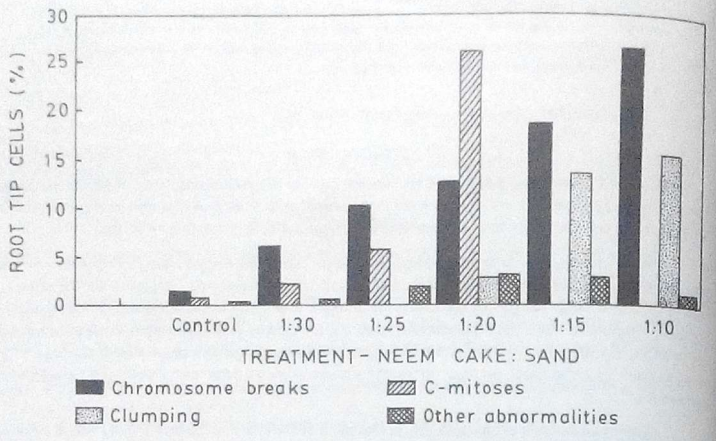

Fig. 1 : Histogram showing cytological abnormalities in root tip cells of *Allium cepa* grown in sand containing neem cake.

the causative substance in the soil increases. Clumping of chromosomes was evident in higher treatments only. C- mitotic divisions were present in lower treatment. The occurrence of multipolar spindles in addition to laggards and bridges at anaphase were the other abnormalities observed in different treatments.

The present study has shown that neem cake is clastogenic and can produce a variety of abnormalities in cell divisions. Previous studies have shown that decaying organic substances like compost and cowdung used as manures can induce mutations of different mangitudes in plants (Abraham 1965, 1974). Magnesium sulphate used as fertilizer and as an ingredient in the preparation of nutrient media for in vitro cultures has been found to induce chromosome breakage and mixoploidy in root tip cells and callus tissues (Abraham & Nair 1988, Abraham et al. 1992).

It is well known that in recent years de-oiled neem cake is widely used as manure and as a systemic pesticide. The antitumor, antiulcer, antimalarial, antipyretic and antimicrobial properties of *A. indica* has been reviewed by Van der Nat et al .(1991). The insecticidal properties of almost every part of the tree have been studied in detail by different workers. Neem seed kernel extracts have been found to inhibit normal pupal-adult development in *Trogoderma granarium* (Chellayan & Karnavar 1990). Sterlizing effects of neem fractions were studied by Schmidt & Pesel (1987) in ants. The antifertility effect of *A. indica* seed oil was reported by Sinha et al. (1984). The leaves of *A. indica* are known to cause toxic effects in goats and guinea pigs (Ali 1987).

In view of the complex chemical composition of neem extracts more studies on isolated compounds are required for understanding the mechanism of action. Considering the rising prices of synthetic pesticides and their threat to public health, agricultural scientists over the world are opting for plant products as natural pesticides. It is necessary to encourage use of neem cake as manure as it possesses insect repellant and pesticidal properties. However, it should be borne in mind that excessive and indiscriminate use of neem cake can produce abnormalities in cell divisions in exposed plants.

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SPONTANEOUS MEIOTIC ABNORMALITIES AND STERILITY OF POLLEN IN *JASMINUM*

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SUMMARY

Nine species of *Jasminum* studied here exhibit a wide spectrum of meiotic abnormalities such as chromosome clumping, cytomixis, unequal segregation at anaphases, lagging, precocious chromosome movement and tri- to polypolar chromosome orientation. This results in the production of pollen grains with less and more than the gametic number. Due to under and overdose of chromatin materials the pollen grains become sterile. The sterility of medium-sized pollen grains with normal gametic chromosome number is, however, attributed to be genic.

Key Words: *Jasminum*, meiosis, pollen sterility.

INTRODUCTION

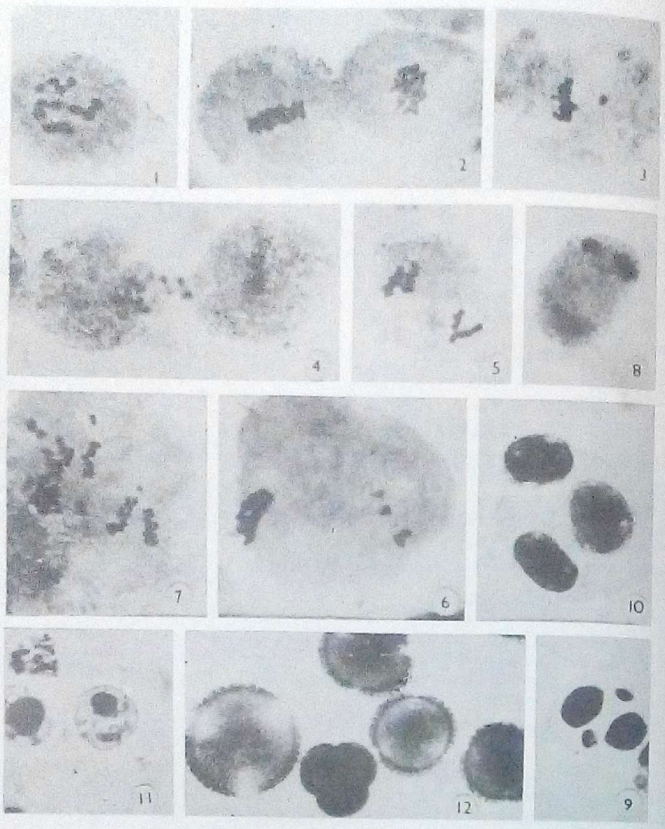
The jasmines (Oleaceae) constitute a small set of fascinating money crops grown chiefly for their fragrant flowers and sweet-scented perfume. Despite their commercial importance, they remained least investigated due to the extreme smallness of their chromosomes and lack of proper staining techniques. However, specially suitable cytological staining techniques have been developed for their investigations (George & Geethamma 1983a, 1984). Being pollen and seed-sterile, their propagation is entirely through vegetative means. This paper gives an account on spontaneous meiotic aberrations and consequent polymorphic forms and sterility of pollen in 9 species of *Jasminum* (Table 1).

MATERIALS AND METHODS

The flower buds of proper sizes from 9 species of *Jasminum* were fixed in Carnoy's fluid (3 alcohol: 1 acetic acid:1 chloroform) to which a few drops of ferric acetate were added. The anthers were smeared in propionocarmine. Photomicrographs of the required stages were taken from fresh preparations. Pollen fertility is determined by the stainability of pollen in 1:1 acetocarmine- glycerine mixture.

OBSERVATIONS

In all the 9 species of *Jasminum* under investigation the process of meiosis was highly irregular in majority of the PMCs (an average of 66.6% cells) excepting a few with countable bivalents (Fig. 1). The noteworthy abnormalities to be mentioned were degeneration of PMCs, clumping of the entire set of chromosomes (Fig. 2) or partial clumping of chromosomes into unequal masses (Fig. 3), cytomictic communicating channels between adjacent PMCs with migrating chromatin material (Fig. 4), precocious chromosome movement at anaphase (Fig. 5), unequal segregation (Fig. 6) tripolar orientation (Fig. 8), spindle arrest and consequent chromosome scattering (Fig. 7), formation of tetrads with unequal microspores (Fig. 9), triads (Fig. 10), diads and monads (Fig. 11). The pollen grains thus



Figs. 1-12: Meiotic aberrations in *Jasminum* (all x 1200). 1-5. *J. pubescens*. 1, PMC with n=13; 2, PMC with clumped chromosomes; 3, PMC with partial clumping of chromosomes leaving a bivalent apart; 4, Cytomictic communicating channels between adjacent PMCs with migrating chromatin materials; 5, Anaphase II with precocious movement of chromosomes. 6,7. *J. angustifolium*. 6, Unequal chromosome segregation at anaphase; 7, Spindle arrest with chromosome scattering. 8, *J. grandiflorum*. tripolar orientation. 9, *J. calophyllum*. Tetrad with unequal sized microspores. 10,11. *J. sambac*. 10, Triad; 11, Monad. 12. *J. pubescens*. micro-, medium-and mega-pollen grains.

resulted are micro, medium- and mega-types (Fig. 12). Data on the species investigated and percentage of pollen sterility are given in Table 1.

TABLE 1 : Data on collection, meiotic abnormalities and pollen sterility in *Jasminum*.

Species	Locality	Average abnormality (%)	Pollen sterility (%)
Jasminum sambac Ait.	Trivandrum	85.5	81.5
J. pubescens Willd.	Trivandrum	96.3	93.5
J. rottlerianum Wall.	Kallar	15.5	5.7
J. angustifolium Vahl.	Trivandrum	20.2	15.8
J. nitidum Skan.	Bangalore	88.2	85.3
J. calophyllum Wall.	Coimbatore	22.1	15.5
J. grandiflorum L.	Trivandrum	90.2	88.3
J. sinense L.	Ooty	88.5	87.8
J. officinale Vahl.	Trivandrum	93.2	88.9

DISCUSSION

A careful cytological screening reveals that various meiotic abnormalities mentioned hitherto especially cytomixis are chiefly responsible for sterility of pollen in *Jasminum*. Such abnormalities appear regularly and spontaneously from early meiotic stages and cytomixis correspondingly occurs in later stages in majority of cases. Hence, it is reasonable to assume that meiotic abnormalities and cytomixis are regularly spontaneous and widespread in most of the species of *Jasminum* and appear to be originating from a peculiar gene action or defective gene function bringing about a pathological condition in the cells (George & Geethamma 1983b). Owing to such defective gene function the chromosomes are unable to carry on the dreary process of duplication and movement and lag in condensation and separation resulting in their clumping into entire or unequal masses consequently incomplete cytokinesis which appear like cytoplasmic extrusions as in what is called cytomixis (Gates 1911). The transfer of a whole or a part of chromatin materials from one cell leaving the adjacent cell empty or with only a few chromosomes was more frequent. This ultimately resulted in empty/almost empty nonstainable and much smaller, sterile micro-pollen and a highly stained mega-pollen.

The complete suppression of the entire meoitic process gives birth to monads, while that of meiosis II to diads. Tara & Namboodiri (1974) recorded such instances in *Impatiens sultani*. Cases where the second division occurs in any one set of chromosomes on either pole result in triads. Precocious chromosome movement, laggards and unequal segregation too contribute largely to sterile pollen grains with hypoploid chromosome constitution. Mega- and micro-pollen with hyper- and hypoploid chromosome numbers respectively become sterile possibly due to over- and underdose of chromatin materials. The sterility of medium sized pollen grains with normal gametic number is attributed to be genic.

Malavya & Shukla (1983) advocate any change in the nucleocytoplasmic equillibrium in the PMCs responsible for the meiotic abnormalities to occur. However, in *Jasminum*, continuous mode of vegetative propagation makes its genetic system more plastic and susceptible to various adverse environmental conditions which result in defective gene function and peculiar gene action causing abnormal meiosis, failure of cytokinesis, appearance of cytoplasmic extrusions, sterility of pollen grains and absence of seed-set. These abnormalities have become accumulated through continuous mode of vegetative propagation in these plants.

ACKNOWLEDGEMENTS

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KARYOLOGICAL STUDIES IN *MICRASTERIAS*

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SUMMARY

The present investigation deals with the karyology of 4 species of *Micrasterias* belonging to Conjugales (Chlorophyceae). Chromosome count for *M. zeylanica* Fritsch (n=20) is the first report of chromosome number for this species. In *M. rotata* (Greville) Ralfs. (n=19), *M. truncata* (Corda) Breb. (n=17) and *M. americana* var. *boldtii* (Ehrenberg) Ralfs. (n=14) the chromosome numbers are, however, new additions to the earlier reports.

Key Words : Desmids, *Micrasterias*, karyology.

INTRODUCTION

Cytological studies on desmids (Conjugales) are meagre inspite of their abundance and world-wide distribution. Cytological investigations on desmids were attempted for the first time by van Wisselingh (1912). Waris (1950) reported chromosome numbers of 3 species of *Micrasterias*. The first Indian contribution in this regard was by Saraswathi (1946) who studied the nuclear division of *Micrasterias denticulata* var. *notata*. Later, Vedajanani & Sarma (1978) and Chowdhury & Sarma (1984) studied the nuclear cytology of 3 species of *Micrasterias* and reported their chromosome counts.

In view of the paucity of information on the karyology of Indian desmids, the present study on the nuclear cytology of four taxa of the genus *Micrasterias* viz., *M. zeylanica, M. rotata, M. truncata* and *M. americana* var. *boldtii* was investigated and results were recorded.

MATERIALS AND METHODS

Materials were collected from different localities of Varanasi. Unialgal cultures of the taxa were raised by isolating cells, using the methods described by Pringsheim (1951). Modified Chu 10 medium (Chu 1942) supplemented with 8-10% soil-extract was found to be the best for multiplication and growth of desmids in the present investigation. Cultures were grown under illumination of ca.2500 lux for 16 h daily and maintained at 21±2°C. Carnoy's fluid as modified by Cave & Pocock (1951) was used for fixing the materials. Before fixation the materials were first subjected to 1-2 drops of iodine solution (1%) to enhance nuclear and chromosomal stain intensity. For cytological studies a concentrated drop of fixed material was drawn on a glass slide and allowed the fixative to evaporate, so that the material stuck to the slide. It was then treated with 2-3 drops of acetocarmine (2%) over which a cover glass was placed. Material was squashed while it was still warm. Photomicrographs were taken from temporary preparations.

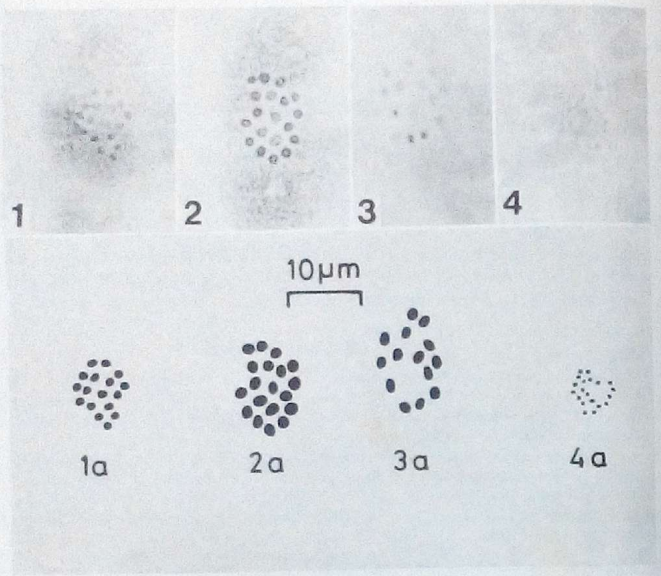
Materials were identified with the help of the monograph of West et al. (1933) and Croasdale & Scott (1976).

OBSERVATIONS

***M. truncata* (Corda) Breb.**

The material was found in Agriculture farm pond of Banaras Hindu University, Varanasi, in November 1991, mixed with *Spirogyra* and *Zygnema* spp. The cells of this taxon are 100-110 µm long and 95- 110 µm broad.

The interphase nucleus is oval to globular, 6.8 to 7.8 µm in diameter, with a centrally placed nucleolus measuring 2.5-3.0 µm across and devoid of vacuoles. The nucleolar track is not clear and a variable number (2-8) of dot-like chromocentres is observable. At prophase the nucleus enlarges about 1.5 times. At metaphase, the chromosomes organize themselves into a metaphase plate (10.0-11.0 µm in diameter), which is more or less of the same size as those of prophase nuclei. The chromosomes are



Figs. 1-4 : Karyology of *Micrasterias*. 1,1a, *M. truncata*. Photomicrograph and camera lucida drawing of metaphase showing n=17. 2,2a, *M. rotata*. Metaphase showing n=19. 3,3a, *M. americana*. Metaphase showing n=14. 4,4a, *M. zeylanica*. Metaphase showing n=20.

embedded inside nucleolar substance, extremely small, thick, dot-shaped and 0.5-1.2 µm in diameter. The chromosome number ascertained at metaphase is n=17, chromatids are not distinct and the nucleolar organizing (N.O.) chromosomes not clear (Figs. 1,1a). Since the size of the chromosomes is small, the position of centromeres is not discernible.

***M. rotata* (Greville) Ralfs.**

This species was collected from the freshwater tank of Botany Department, Banaras Hindu University, Varanasi, in March 1992, mixed with *Spirogyra*, diatoms and other desmids. The cells are 265-275 µm long and 230-240 µm broad.

The resting nucleus is small, elliptical and 8.0 to 12.5 µm in diameter. The prophase nuclei are approximately one- and -a- half times larger than the interphase ones. Single nucleolus (4.0-4.5 µm across and devoid of vacuoles) is located in the central region of the nucleus. The nucleolar track is clear and 4-8 dot-like chromocentres are observed. The diameter of metaphase plate is 11.0-13.0 µm. The chromosomes are embedded inside the dense nucleolar substance and become distinct by the end of prophase. Metaphase chromosomes (n=19) appear small rod-shaped, ranging from 1.6-2.0 µm long (Figs. 2,2a). Nucleolar organizing chromosomes are not clear. Chromosomes are probably polycentric at least in some cases, as revealed by parallel orientation of the sister chromatids end to end at metaphase and during anaphasic separation.

***M. americana* var. *boldtii* (Ehrenberg) Ralfs.**

This was collected in January 1992 from a freshwater permanent tank of Birla Temple, Banaras Hindu University, Varanasi. It was found mixed with Chlorococcales, *Spirogyra* spp. and other desmid taxa. The cells of *M. americana* are 120-130 µm in length and 95- 110 µm in breadth.

The metaphase nucleus is globular in shape measuring 7.5-8.8 µm in diameter. The nucleolus is simple, centrally placed, 3.0-3.5 µm across and devoid of vacuoles. The nucleolar track is also not clear. The chromocentres are 2-10 in number and dot-like. The chromosomes are small, short, rod-shaped, n=14 in number and 0.8-1.8 µm in length (Figs. 3,3a). They organize themselves into a plate at metaphase measuring 9.0-12.0 µm in diameter. Nucleolar organizing chromosomes could not be distinguished. Since the size of chromosomes is small, it is difficult to locate the exact position of the centromeres.

***M. zeylanica* Fritsch**

This desmid was collected in November 1991 from a temporary pond on Lanka-DLW Road, Varanasi growing mixed with *Spirogyra* spp. and some euglenoid flagellates. The cells are 48-52 µm in length and 50-52 µm in width.

The resting nucleus is small and oval to globular in shape ranging from 7.9-9.6 µm in diameter. The prophase nuclei are approximately one-and-a-half times larger than the interphase ones. A single nucleolus measuring 4.5-4.8 µm across is centrally located and is devoid of vacuoles. Nucleolar track is not clear. Chromocentres are dot-like and 2-6 in number. Metaphase plate is 10.0- 11.2 µm in

diameter. The chromosomes are embedded inside scanty nucleolar substance. The chromosomes (n=20) are dot- to rod-shaped and 0.5-1.4 µm long (Figs. 4,4a). No nucleolar organizing chromosomes are observed. These small chromosomes do not give any clue about the position of their centromeres, neither at metaphase nor during anaphasic separation into daughter nuclei.

DISCUSSION

As far as the authors are aware the chromosome counts obtained here for *M. truncata* (n=17), *M. americana* (n=14) and *M. rotata* (n=19) are new additions to earlier records of desmids. The n=20 chromosomes exhibited by *M. zeylanica* presents a first report for the species. The present chromosome counts of n=19 made for *M. rotata* differs from the earlier counts of Waris (1950) (n=c.200), King (1960) (n=172) and Kasprik (1975) (n=159). Similarly, the chromosome numbers recorded for *M. americana* var. *boldtii* (n=14) and that of *M. truncata* (n=17) differ from the earlier counts of Kasprik (1975) as n=88 and n=180 chromosomes, respectively. Kasprik (1975) studied the karyotypes of 23 species of *Micrasterias* and explained that they differed not only in chromosome number but also in chromosome morphology. Chromosome numbers for different species recorded in this study are comparatively very low as compared to those of earlier reports. Godward (1966) and Kasprik (1975) suggested on the basis of various cytological investigations that within individual desmid species the chromosome numbers often vary considerably, thus forming aneuploid series rather than polyploid ones. This view is not supported by the authors of present communication because without polyploidy such large variations in chromosome numbers do not seem to be possible. Perusal of chromosome numbers indicate that a combination of euploidy, aneuploidy and fragmentation of chromosomes individually or coupled with other mitotic irregularities appear to be responsible for such variations in chromosome number which have arisen spontaneously during the course of evolution (Kasprik 1975, Chowdhury & Sarma 1984).

Chromosomes are dot-like in *M. truncata* and *M. zeylanica,* while those of *M. rotata* and *M. americana* are small, rod-like besides dot-like ones. The small, rod-like chromosomes clearly revealed chromatids, but the individuality of chromatids could not be made out where chromosomes are dot-like. Nucleolar organizing chromosomes were not observed in any species of *Micrasterias* studied. The size of chromosomes in the taxa studied here varies from 0.5-2.0 µm. Nucleolar substance is present in all the species studied, difference being only in the degree of stainability. Polycentric nature of chromosomes could be assumed only in *M. rotata,* where the chromosomes were in the form of short rods. In species with dot-like chromosomes, it is rather difficult to say anything about the centromeric organization of chromosomes.

Variation in chromosome numbers can be attributed to the existence of chromosomal races in species of *Micrasterias* as already reported in various other desmid taxa (Brandham 1965, Chowdhury & Sarma 1984) and several other eukaryotic algae (Sarma 1978). The variation in chromosome numbers within the clone are attributable to polycentric nature of chromosomes which are prone to fragmentation. After fragmentation (i.e. a transverse division) of such chromosomes the fragments move polewards independently, in contrast to chromosomes with localized centromeres in which such fragments are lost. Such an event changes the chromosome without any loss of structural genes (Coesel & Menken 1986).

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REVERSAL OF ALKALOID INHIBITED CYTOKINESIS IN *CYCLOTELLA MENEGHINIANA* KUETZ. F. *UNIPUNCTATA* A. CL.

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(Received 28 October 1992, accepted 5 March 1993)

SUMMARY

Methyloxypurine alkaloids viz., caffeine, theobromine and theophylline inhibited cytokinesis in *Cyclotella meneghiniana* Kuetz. f. *unipunctata* A. Cl. and led to formation of binucleate cells. Caffeine is potent inhibitor of cell plate formation followed by theophylline and theobromine. Effect of alkaloids up to 3 mM was successfully reverted by 10 mM calcium.

Key Words: Caffeine, theobromine, theophylline, calcium, cytokinesis, *Cyclotella*.

INTRODUCTION

Methyloxypurine alkaloids such as caffeine, theobromine and theophylline are known to inhibit plant cytokinesis (Kihlman 1977). Although their effect on plant cells has been studied by various workers but, the exact mechanism of inhibition is yet to be understood (Bonsignore & Hepler 1985, Encina & Becerra 1986). Most of the studies so far deal with higher plants and very little is known of these chemicals on algae except for the reports of Sarma & Tripathi (1973), Sarma & Shyam (1975) and Sarma & Chaudhary (1977). The present paper deals with the effect of 3 methyloxypurine alkaloids (caffeine, theobromine and theophylline) on the division process of *Cyclotella meneghiniana* Kuetz. f. *unipunctata* A. Cl.

MATERIAL AND METHODS

C. meneghiniana f. *unipunctata* was grown in Chu-10 medium under standard culture conditions described elsewhere (Giri 1992). Material from fully grown 15-day-old cultures were reinoculated aseptically in fresh medium and allowed to grow for 48 h to conduct the following experiments.

(i) Actively growing 48 h-old cells were treated with different concentrations of caffeine, theobromine and theophylline separately ranging from 1 to 5 mM for 2 to 12 h in each concentration. Subsequently, cells were washed in fresh medium devoid of alkaloid to remove the traces of chemical and allowed to complete the remaining part of the photoregime. Cells were fixed in 1:3 acetic-alcohol at the end of dark period and stained in 1.5% acetocarmine.

(ii) Exponentially growing cells were treated for 6 h with different levels of alkaloids in the presence of 10 mM calcium and processed for cytological observations as mentioned above.

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OBSERVATIONS

Caffeine, theobromine and theophylline excised cytokinesis in the dividing cells of *C. meneghiniana* f. *unipunctata* and resulted in the formation of binucleate cells. Binucleate cells up to 21% were induced by 10 mM caffeine after a treatment period of 6 h followed by theophylline (17%) and theobromine (14.6%) at the same concentration and treatment period. The number of binucleate cells increased linearly with increase in concentration and treatment period up to 6 h (Fig. 1). In the second experiment, the number of binucleate cells induced by 1-3 mM alkaloid in the presence of 2.5-10 mM calcium respectively remained as equal as in control (Fig.2).

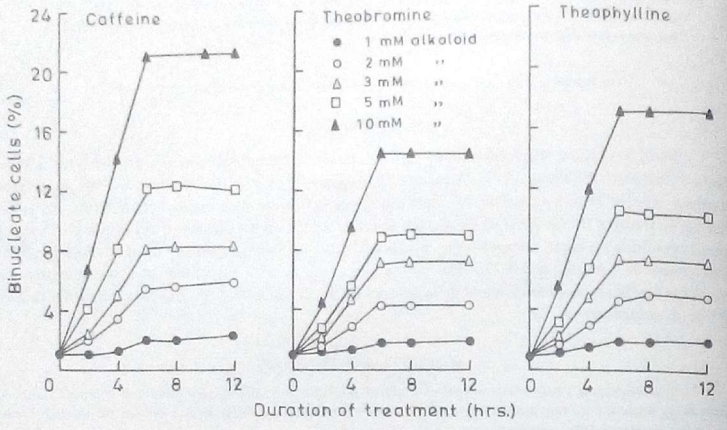

Fig. 1 : Effect of methyloxypurines on the division process of *Cyclotella meneghiniana* f. *unipunctata*.

DISCUSSION

Cytokinesis in *C. meneghiniana* f. *unipunctata* did not proceed in the presence of caffeine, theobromine or theophylline rather resulted in the formation of binucleate cells. Inhibition of cytokinesis by caffeine and related alkaloids was also reported by Bonsignore & Hepler (1985) and Encina & Becerra (1986). Caffeine was found to be potent inhibitor of cytokinesis followed by theophylline and theobromine. Methyloxypurines disrupted cytokinesis variously in different organisms. Cytokinesis in 20% of cells was affected by caffeine as well as theobromine in *Gymnodium inversum* (Sarma & Shyam 1975) and *Oedogonium acmandrium* (Sarma & Tripathi 1973) whereas only 6% of dividing cells were affected in *Allium cepa* (Becerra & Lopez-Saez 1978). Maximum effect of the 3 alkaloids was registered after a treatment period of 6 h. Accordingly, Sarma & Shyam (1975)

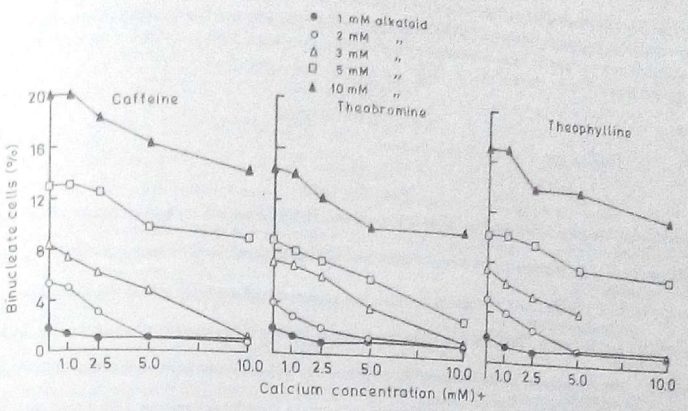

Fig. 2 : Reversion of alkaloids' effect by calcium.

and Sarma & Chaudhary (1977) also recorded maximum effect of caffeine in *Gymnodium inversum* and *Sphaeroplea annulina* respectively after 6 h exposure to the chemical. However, Sarma & Tripathi (1973) recorded maximum effect of caffeine and theobromine after 8 h in *Oedogonium acmandrium* whereas caffeine induced maximum binucleate cells in *Allium cepa* after 4 h treatment (Encina & Becerra 1986).

Paul & Goff (1973) reported that caffeine inhibits vesicle coalescence by blocking Ca^{2+} which is an absolute requirement during cell plate formation. Giri(1989) found that minimum of 10^{-6} M calcium is required by the dividing cells for successful completion of cytokinesis. Further, binucleate cells were also formed in *C. meneghiniana* f. *unipunctata* when this alga was grown in Ca^{2+} depleted medium. Similar condition was reported earlier in *C. cryptica* by Badour (1968). Thus, the action of caffeine and related alkaloids is related to calcium depletion from the cells. Similarly, Hepler & Wayne (1985) reported that caffeine disrupts balance in cellular calcium associated with membrane formation.

Binucleate cells induced by 1-3 mM alkaloid reduced significantly when the cells were supplemented with calcium ranging from 2.5-10 mM. In an earlier study Becerra & Lopez-Saez(1978) found that calcium, magnesium and other berylium compounds can either revert or lower caffeine's effect. Reduction of binucleate cells in *C. meneghiniana* f. *unipunctata* in the presence of Ca^{2+} could be due to availibility of sufficient cellular Ca^{2+} to the dividing cells. Hepler & Wayne (1985) were of opinion that reversal of caffeine's effect by calcium is brought by competetive inhibition. However, binucleate cells produced by 5 and 10 mM alkaloid were partially reduced even in the presence of 10 mM calcium. Effects of theobromine and theophylline were alleviated easily than caffeine. Cytokinesis

in caffeine and theobromine affected cells of *Oedogonium acmandrium* (Sarma & Tripathi 1973) and *Gymnodium inversum* (Sarma & Shyam 1975) could not be restored but further nuclear divisions continued in a normal fashion resulting in multinucleate condition. However, such a type of mitoses were not noticed in the present study.

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INTERSPECIFIC VARIATIONAL PATTERN OF FLAVONOIDS, SOLUBLE PROTEINS, ESTERASE AND PEROXIDASE ISOZYMES IN *SORGHUM*

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SUMMARY

Interspecific variation in respect of flavonoids and soluble protein patterns along with peroxidase and esterase isozyme profiles was recorded and compared in 6 species of *Sorghum*, namely, *S. caudatum* (Hack.) Stapf, *S. cernuum* Host, *S. conspicuum* Snowden, *S. membranaceum* Chiov., *S. subglabrescence* Schweinf. & Aschers. and *S. verticilliflorum* (Steud). Stapf. The patterns were observed to be species-specific.

Key Words: Chemotaxonomy, *Sorghum*, flavonoids, proteins.

INTRODUCTION

The genus *Sorghum* of family Poaceae comprises 60 species which are distributed in tropical and subtropical regions (Willis 1973). Present investigation pertain to 6 species, namely, *S. caudatum*, *S. cernuum*, *S. conspicuum*, *S. membranaceum*, *S. subglabrescence* and *S. verticilliflorum*. Cytologically, all of these revealed a haploid count of n=10. The course of meiosis was perfectly normal in all the taxa resulting in the formation of fertile pollen. In a previous communication we presented an assessment of both qualitative and quantitative variation in fatty acids and free amino acid profiles revealed by these species (Sood et al. 1991). The present study depicts the interspecific variational pattern in respect of flavonoids, soluble proteins, peroxidase and esterase isozymes. The usefulness of such information in taxonomic and evolutionary studies is now well documented (Sachdeva & Bhatia 1980, Sachdeva & Kals 1981, Harborne & Turner 1984, Bala & Sachdeva 1990).

MATERIAL AND METHODS

Plants of the six species were raised from seed in the experimental area of the University. Leaf material was sampled just at the time of emergence of the inflorescence. The technique of Mabry et al. (1970) was used for the study of flavonoid patterns. For analysing soluble proteins, peroxidase and esterase isozyme patterns, the following procedure was adopted:

Extraction procedure

Leaf extracts were prepared by grinding weighed samples in chilled pestle and mortars using acid washed sand with 62 mm Tris-HCL buffer (pH 7.6; 1:25 g/ml, w/v) containing 5 mM β- mercaptoethanol, 5 mM ethylenediaminetetra acetic acid (EDTA) and 4 per cent polyvinylpyrrolidone (PVP) for solubilizing proteins and isozymes of esterase and peroxidase. All operations were carried out at 4°C. Cell suspension paste was filtered and squeezed through two layers of muslin cloth and then centrifuged at 15,000xg for 30 min at 0°C. The supernatant was used in gel electrophoresis. Protein concentration was determined by the method of Lowry et al. (1951).

Separation of proteins

Polyacrylamide gel electrophoresis was used for studying soluble proteins and isozymes of peroxidase and esterase. For this the anionic system of Davis (1964) was adopted. 7.5% polyacrylamide gel was used. Samples containing 150-200 mg protein were layered above the stacking gel. To each sample, 1-2 drops of 40 per cent sucrose and one drop of 0.002 per cent

bromophenol blue solution was added as a tracking dye. Electrophoresis was conducted in the cold (about 4°C). Initially 2 mA current was applied for 15 min; this was increased to 3 mA current per gel tube until the tracking dye (bromophenol blue) entered into the resolving gel. After the completion of electrophoresis, which was indicated by the movement of tracking dye to the bottom of gels, the gels were removed from the tubes and stained for proteins (Weber & Osborne 1969), peroxidases (Kuhns & Fretz 1978) and esterases (Tripathi et al.1983).

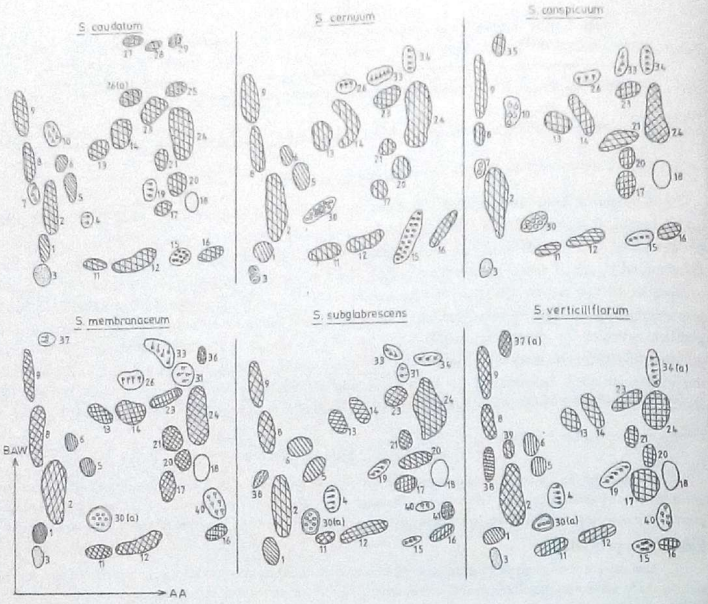

Fig. 1 : Flavonoid patterns in *S. caudatum, S.cernuum, S.conspicuum, S.membranaceum, S.subglabrescence* and *S.verticilliflorum*. Colour of spots under uv, uv+NH_3; 1,LY/FY; 2,FY/FY; 3,LBl/FBl; 4,LBl/FBl; 5,LBl/FBl; 6,LBl/FBl; 7,LBl/FBl; 8,FY/FY; 9,FY/O; 10,LBl/FBl; 11,DBr/Br; 12,DBr/Br; 13,LBl/FBl; 14,LBl/FBl; 15,Bl/FBl; 16,Bl/FBl; 17,Bl/FBl; 18,Bl/FBl; 19,LBl/FBl; 20,LBl/FBl; 21,LBl/FBl; 23,LBl/FBl; 24,LBl/YG; 25,LBl/YG; 26,Bl/FBl; 26(a),Bl/YG; 27,Bl/FBl; 28,Bl/FBl; 29,Bl/FBl; 30,LBl/FBl; 30(a),LBl/YG; 31,LBl/YG; 33,LBl/FBl; 34,LBl/FBl; 34(a).LBl/YG; 35,Br/O; 36,LBl/FBl; 37,LBl/FBl; 37(a),LBl/YG; 38,-/FBl; 39,-/FBl; 40,LBl/FBl; 41,-/FBl.
(Bl-Blue; LBl-Light blue; FBl-Fluorescent blue; FY-Fluorescent yellow; O-Orange; DBr-Dark brown; Br-Brown; YG-Yellow green)

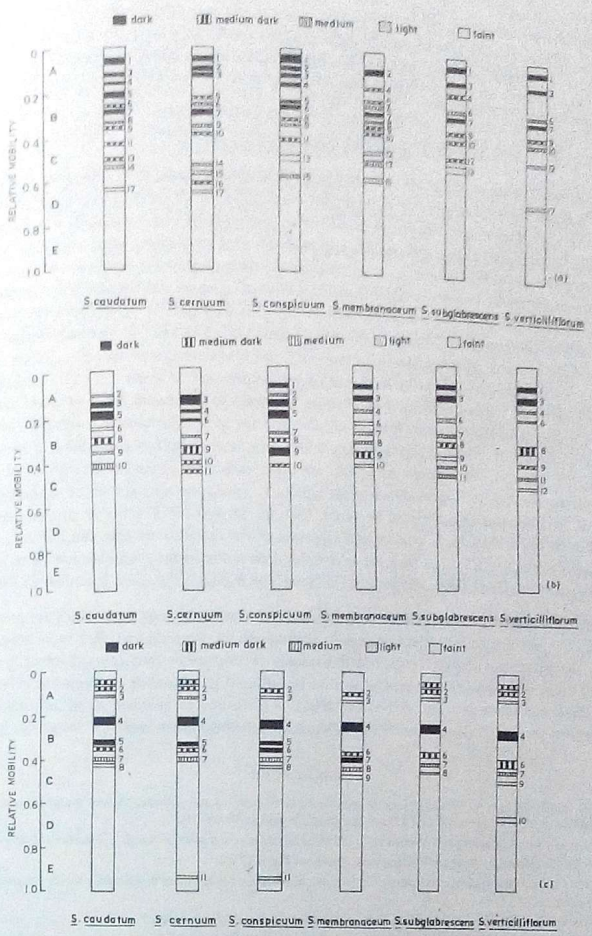

Fig. 2 : Zymograms of electrophoretric banding patterns of total proteins (a) esterase, (b) peroxidase and (c) isozymes in six species of *Sorghum*.

Gel comparison

All comparisons between gels were normalised to Relative migration (Rm) between 0 and 1. For ease of visual examination, the zymograms were divided into five zones of differing mobilities relative to the distance migrated by the tracking dye front. Zone A represents Rm values from 0-0.02, Zone B from 0.2-0.4, Zone C from 0.4-0.6, Zone D from 0.6-0.8 and Zone E from 0.8-1.0.

RESULTS AND DISCUSSION

The two-dimentional thin layer chromatographic flavonoid patterns exhibited by 6 species are depicted in Fig.1. the total number of spots ranged from 22 to 28. Of these, 13 spots (all black) were noticed to be shared by all species. They could easily be distinguished from each other by the presence or absence of certain spots. *Sorghum caudatum* exhibited a total of 28 spots. Five spots (25, 26a, 27 28 and 29) were specific to this species. In *S. conspicuum* 23 spots were noticed and spot 35 was observed to be species-specific. *S. membranaceum* revealed a total of 25 spots and spot 36 was observed to be species-specific. *S. subglabrescence* had a total of 27 spots and spot 41 served as a species marker spot. *S. verticilliflorum* also exhibited 27 spots with 34a, 37a and 39 serving collectively as species-specific spots. *S. cernuum* showed a total of 22 spots and though no species- specific spot was observed yet the species could easily be identified by the presence of spots 1,5 and 6 accompanying absence of spot 18. Certain additional spots were observed to be shared by 2 or more species. *S. conspicuum* clearly stands out quite apart from other species as it lacks spots 1,5 and 6 present in the other 5 species but still reflects a closer affinity with *S. cernuum* as both of these have in common the spots 26, 33 and 34. The closer affinities are also indicated amongst *S. verticilliflorum*, *S. membranaceum* and *S. subglabrescence* with which *S. caudatum* appears to be distantly related because of the presence of additional spots 25, 26a, 27 28 and 29. Flavonoid patterns have been observed to be under the genetic control and a perusal of the literature reveals that not only are such spots helpful at species level but they are of considerable value in the identification of intraspecific cytological variants (Grant 1968, Harborne 1975, Sachdeva & Kals 1981, Bala & Sachdeva 1990).

The total protein banding patterns and esterase and peroxidase isozyme profiles are presented in the Fig.2. The patterns were distinct enough to distinguish the 6 species as they were highly taxon specific. The differences observed presently in number, electrophoretic mobility and intensity of bands paralleled those noticed in closely related species in different genera, such as *Digitaria* (Hayward & Hacker 1980) and *Hordeum* (Rao & Sharma 1985). It is desired to generate more information from related species for an overall assessment of phylogenetic relationship amongst species of this genus.

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J. Cytol. Genet. 28: 59-66 (1993)

KARYOLOGICAL INVESTIGATIONS ON FOUR SPECIES OF ELATERID BEETLES (POLYPHAGA : COLEOPTERA)

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SUMMARY

Cytological investigations were carried out on 4 species of elaterid beetles belonging to subfamilies Cardiophorinae and Elaterinae viz., *Cardiophorus haridwarensis* Vats & Chauhan (2n=21), *C. limbatus* Candeze (2n=21), *Melanotus kumaunensis* Vats & Chauhan (2n=12) and *M. tenebrosus* Erichson (2n=19). With the exception of *M. kumaunensis*, which possesses XY male sex chromosome system, all other species uniformly depict X0 male sex chromosome mechanism. Karyotypic details and behaviour of chromosomes during mitosis and meiosis in the 4 species are presented.

Key Words : Coleoptera, polyphaga, Elateridae, sex chromosomes, karyotype.

INTRODUCTION

Our knowledge of coleopteran chromosomes has accumulated rapidly in the last two decades but as far as family Elateridae is concerned, though taxonomically it has 7000 recorded species (Richards & Davies 1979), it occupies very little space on the cytological map. The larvae of *Agriotes, Limonius, Athous* etc., commonly known as 'wireworms', are root-feeders and are exceedingly injurious to agriculture. As such, these beetles have much economic importance. Even then, chromosomal data of only 89 species, including 15 species of Indian origin, are available (Smith 1953, 1960, Manna & Lahiri 1972, Dasgupta 1977, Smith & Virkki 1978, Ferreira et al. 1984, Virkki & Denton 1987). *Chalcolepidius zonatus*, a pyrophorine, possesses the lowest diploid chromosome number, 4. This is indicative of several changes having taken place during the evolution of karyotype in elaterids. To have more data for a better understanding of the evolutionary phenomenon, a planned and comprehensive cytological study of this family was undertaken (Yadav & Vyas 1992). The present report deals with the karyological details in 4 species of elaterid beetles 2 of which are new additions to the cytology of Coleoptera.

MATERIALS AND METHODS

Adult individuals of 4 species of 'click beetles' collected from natural population were utilized for the present investigations. A classified list of the species, collection data and other cytological details are presented in Table 1. Karyological preparations were made from male germ cells according to the technique of Yadav & Lyapunova (1983).

OBSERVATIONS

***Cardiophorus haridwarensis* Vats & Chauhan** 2n=21 (10+X0 male), N.F.a = 40

The diploid complement revealed by spermatogonial metaphase is 21 (Fig. 1). The karyotype comprises 8 pairs of metacentric (pairs 1, 3-5, 7-9) and 3 pairs of submetacentric (pairs 2, 6, 10)

TABLE 1 : Locality, period of collection and other cytological details of the elaterid beetles.

Species	Locality	Period of collection	No. of individuals sacrificed	$\bar{X}$	T.C.
Cardiophorus haridwarensis	Ram Nagar	July 1991	5	15.76	0.85
C. limbatus	- do -	July 1992	7	18.92	0.81
Melanotus kumaunensis	Kurukshetra	April 1992	3	4.8	0.78
M. tenebrosus	Dehradun	July 1991	4	21.24	0.78

$\bar{X}$ Mean chiasma frequency per nucleus at metaphase I; T.C. Terminalization coefficient at metaphase I.

autosomes and a metacentric X chromosome (Fig. 2). Whereas the autosomes show a gradual decrease, the X is sixth in order of size (Table 2). Metaphase I comprises 10 ring and rodshaped autosomal bivalents and the univalent X (Fig. 9). Two types of metaphase II cells, with and without the X chromosome were encountered (Fig. 10).

C. limbatus **Candeze** 2n=21 (10+X0 male), N.F.a = 34

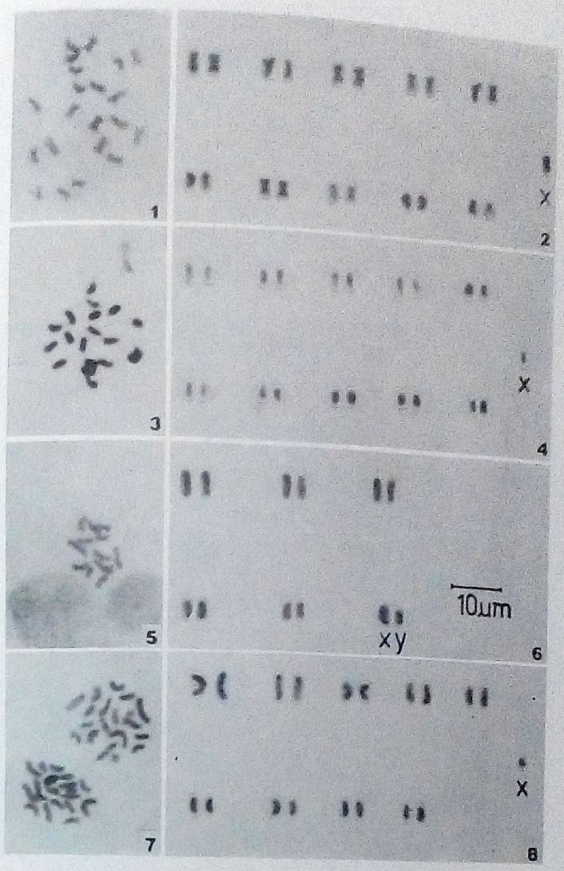
The spermatogonial metaphase revealed a diploid set of 21 chromosomes (Fig. 3). The autosomes decrease gradually (Fig. 4) and the X chromosome occupies fifth position in order of size (Table 2). Metaphase I revealed 10 dumb-bell shaped autosomal bivalents and the unpaired X chromosome (Fig. 11). Two types of metaphase II plates were observed, one without X (Fig. 12) and the other with X chromosome (Fig. 13). At metaphase II, morphology of chromosomes was obvious due to separation of chromatids, 7 autosomes and the X were metacentric while 3 autosomes were acrocentric.

***M. kumaunensis* Vats & Chauhan** 2n=12 (5+XY male), N.F.a = 20

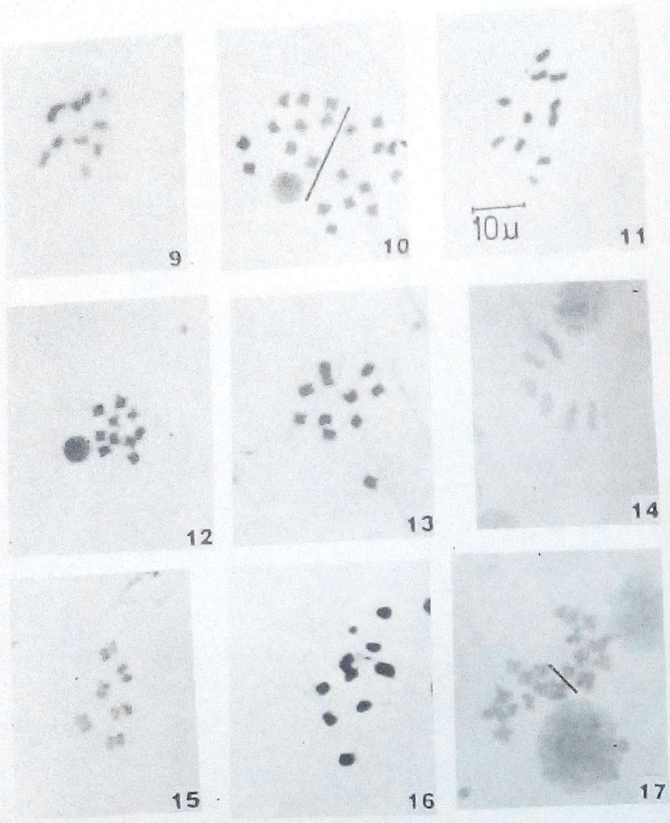
The diploid complement comprises 12 metacentric chromosomes (Figs. 5, 6). Metaphase I plate was constituted by 5 rod-shaped autosomal bivalents and the XY (Fig. 14). Two types of metaphase II plates, with X (Flg. 15) and with y in addition to 5 autosomes, were observed. Morphology of the chromosomes observed earlier was confirmed at this stage owing to chromatid separation.

***M. tenebrosus* Erichson** 2n=19 (9+X0 male), N.F.a = 32

Spermatogonial metaphase revealed 2n=19 (Fig. 7). The karyotype is composed of 5 pairs of metacentric/submetacentric (1-4,7) and 4 pairs of acrocentric (5,6,8,9) autosomes and an acrocentric X chromosome (Fig. 8). Chromosomes depict gradual decrease in size, the X chromosome being the smallest element of the diploid complement (Table 2). Metaphase I is composed of 9 autosomal



Figs. 1-8 : 1-2 *Cardiophorus haridwarensis*. 1. gonial metaphase, 2, karyotype, 3,4. *C. limbatus*. 3, gonial metaphase, 4, karyotype. 5,6. *Melanotus kumaunensis*. 5, gonial metaphase, 6, karyotype. 7,8. *M. tenebrosus*. 7, gonial metaphase, 8, karyotype.



Figs. 9-17 : 9,10. *C. haridwarensis*. 9, M I, 10, M II with and without X. 11-13. *C. limbatus*. 11, M I, 12, M II without X, 13, M II with X. 14,15. *M. kumaunensis*. 14, M I, 15, M II with X. 16,17. *M. tenebrosus*. 16, M I, 17, M II with and without X.

TABLE 2 : Data from the analysis of karyotypes of elaterids.

Species	Chromosome pairs in percentage length											Length (µm)		
	1	2	3	4	5	6	7	8	9	10	X	Y	TCL	X	Y
Cardiophorus haridwarensis	10.45	10.25	9.98	9.34	9.08	8.87	8.78	8.53	8.43	7.31	8.98	-	25.80	1.84	-
C. limbatus	12.12	11.15	9.69	9.24	8.85	8.79	8.59	7.79	7.40	7.34	9.04	-	25.95	2.33	-
Melanotus kumaunensis	18.16	16.45	15.77	12.61	11.65	-	-	-	-	-	14.67	10.69	28.15	3.84	2.79
M. tenebrosus	15.19	12.69	11.00	10.50	9.57	9.21	8.97	8.47	8.52	-	5.88	-	27.65	1.62	-

TCL, Total chromosome length.

bivalents, mostly dumb-bells, and a single X chromosome (Fig. 16). Occasionally, a ring bivalent was also observed. Metaphase II plates with and without the X chromosome were encountered (Fig. 17).

DISCUSSION

Cytologically, Elateridae is one of the most neglected groups. Till todate chromosomal data of only 89 species belonging to 24 genera of 4 subfamilies and 8 tribes are on record (Smith 1953, 1960, Manna & Lahiri 1972, Dasgupta 1977, Smith & Virkki 1978, Ferreira et al. 1984, Virkki & Denton 1987). This also includes 15 species of Indian origin. More than 70% of these species belong to only 2 subfamilies Pyrophorinae and Elaterinae. Pyrophorinae is known by only 39 species belonging to 10 genera and Elaterinae is known by 38 species from 11 genera. However, Cardiophorinae is known by 10 species all belonging to a single genus *Cardiophorus*. Even this meagre data reveal a wide spectrum

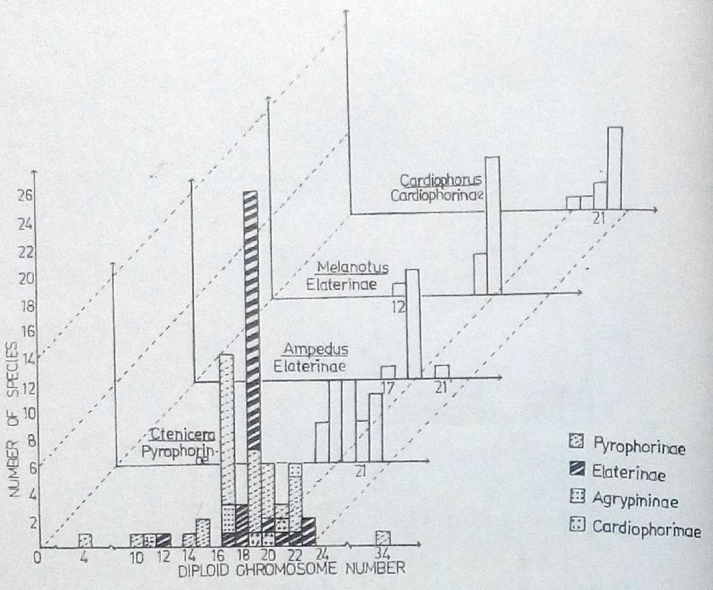


Fig. 18 : Histogram showing distribution of diploid number of chromosomes in family Elateridae and some well investigated genera.

of karyotypes, the diploid number of chromosomes varying from 2n=4 (1+XY), the lowest number recorded in Coleoptera, in *Chalcolepidius zonatus* (Ferriera et al. 1984) to 2n=34 in *Heteroderes macroderus* (Manna & Lahiri 1972), both belonging to subfamily Pyrophorinae.

The exact distribution of species and genera according to diploid chromosome complement in this family are shown in Fig 18. It reveals a conspicuous gap between 2n=4 and 2n=10 and again between 2n=23 and 2n=34. The common number possessed by 34 species is 2n=19 (9+X). However, it is evident from Figure 18 that this peak includes 26 species belonging to only 3 genera of Elaterinae. Obviously, the preponderance of 2n=19 is because of having more species of these 3 genera investigated cytologically and may not be a representative number of the family. During the present investigation, *Melanotus tenebrosus* revealed this number. 2n=20; 9+Xy_p, the typical condition in Coleoptera is possessed by only 7 species belonging to 5 genera.

The family Elateridae is characterized by the presence of simple male sex chromosome mechanisms. The common and most widespread sex mechanism is X0. It is possessed by 62 species, including 3 under the present investigation, followed by Xy_p which is represented by 19 species. The primitive tribe Pyrophorini depicts a peculiar sex trivalent Xpneo XneoYp in *Pyrophorus luminosus*. (Virkki et al. 1984). The structure and behaviour of this trivalent is reported to coincide with Xy_p (parachute bivalent) derivative Xpxneo XxneoYp found in Chrysomelids, Curoulionids and Tenebrionids (Smith & Virkki 1978). Neo XY and XY types are depicted by 2 species each. This includes XY depicted by *M. kumaunensis* under present investigation. In 4 species, the sex chromosome mechanism has not been recorded.

Occurrence of X0 and neoXY mechanisms indicate the involvement of loss of the y chromosome and X-autosome fusions in the evolution of karyotype in Elateridae. However, more data are required before any definite proposition can be made in this regard.

ACKNOWLEDGEMENTS

Our sincere thanks are due to the Kurukshetra University authorities for providing laboratory facilities and a fellowship to SV; to Dr. A.S. Yadav for help and to Dr. R.L. Chauhan for identification of the beetles.

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Errata

Karyomorphology of *Fimbristylis* from Punjab by Cheema Paramajeet et al. *J. Cytol. Genet.* **27** (2) : 163-173 (1992).

Page 163, 2nd line - *Read* 'Paramjeet Cheema'. Page 167, in headings of Table 1 - *Read* 'Species' 'Karyotypic analysis +++ '; 12th line - Read '2A' (*for* 3A); in the 2nd foot-note - *Read* '* Different'. Page 169 and 171, in legend to figures pertaining to *F.dichotoma* and *F. quinquangularis* - *Read* 'Pop. ii' (*for* Pop. i). Page 171, 21st line - Add *F. miliacea* after n=11.

Chromosomal inversions in *Anopheles stephensi* Liston- a malaria mosquito by K. Gayathri Devi & N.J. Shetty. *J. Cytol. Genet.* **27**(2) : 153-161 (1992).

Page 155, first para, 5th line - *Read* 'g' (*for* 'q'); third para, first and third lines - *Read* 'g' (*for* 'q'). Page 156, in Table 2, 12th and 13th lines - *Read* '+' (*for* 'x'). Page 157, in legend to figures - *Read* 'in' (*for* 'is'). Page 159, 2nd para, 5th line - *Read* '+/+'.

J. Cytol. Genet. **28**: 67-70 (1993)

KARYOLOGICAL INVESTIGATIONS ON FOUR SPECIES OF COCCINELLID BEETLES (POLYPHAGA : COLEOPTERA)

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SUMMARY

Chromosome studies were carried out on four species of lady-bird beetles belonging to subfamily Epilachninae. *Henosepilachna septima* (Dieke) possesses 2n=20 (9+X_yp) whereas *H. indica* (Mulsant), *H. ocellata* (Redt.) and *Epilachna atypica* (Dieke) are characterized by 2n=18 (8+X_yp). All the species, however, show X_yp male sex chromosome mechanism. Karyology of *H. indica*, *H. ocellata* and *E. atypica* is new addition to the coleopteran cytology. The details of karyotypes, behaviour of chromosomes during cell division and chiasma frequency have been described and discussed.

Key Words: Coleoptera, coccinellidae, karyotype, chiasma frequency.

INTRODUCTION

Coccinellids are effective natural enemies of crop pests, like aphids, coccids and mites, etc. These beetles are of great economic importance as they bring about biological control of several crop pests. About 5000 described species are known to belong to family Coccinellidae (Richards & Davies 1979). Coccinellids were the first among the karyologically investigated beetles (Stevens 1906). However, only 180 species are known cytologically (Yadav et al. 1991). Of these only 29 species belong to Indian fauna (Agarwal 1960, 1961, Manna & Lahiri 1972, Dasgupta 1977, Mittal et al. 1989, Yadav & Pillai 1974). Additional 3 species belonging to *Epilachna* have been recorded by Zamen (1969). During the present investigation, the karyotypic details, structure and the behaviour of chromosomes during mitosis and meiosis in 4 species of epilachnine coccinellids are presented.

MATERIALS AND METHODS

Field collected individuals were sacrificed for these investigations. A classified list of species, locality, period of collection, number of individuals utilized, and other cytological features are presented in Table 1. Adult males were utilized for making karyological preparations from the testes following the technique of Yadav & Lyapunova (1983).

OBSERVATIONS

***Henosepilachna septima* (Dieke) 2n=20 (9+Xyp)**

Spermatogonial metaphase exhibited the diploid number of 20 chromosomes (Fig.1). The karyotype comprised 9 pairs of autosomes and X and y sex chromosomes. Autosome pairs 1, 5,6,7 and 9 are metacentric and autosome pairs 2,3,4 and 8 are submetacentric. The X is a metacentric of the size of fourth pair of autosomes whereas y is spherical but has appreciable size (Fig.2).

TABLE 1 : Locality, period of collection and other cytological details of coccinellid beetles.

Species	Locality	Period of collection	No. of individuals sacrificed	$\bar{X}$	T.C.
Henosepilachna septima	Kalimpong (W.B.)	May 1991	8	-	
H. indica	- do -	- do -	5	14.32	0.92
H. ocellata	- do -	- do -	4	12.48	0.67
Epilachna atypica	- do -	- do -	3	12.92	-.90

$\bar{X}$ Mean chiasma frequency per nucleus at M I; T.C., Terminalization coefficient at M I.

M I plate carried 9 autosomal bivalents, both rings and rods and the sex bivalent Xy_p. The first division being reductional, 2 types of cells are observed one with y chromosome and the other with X chromosome in addition to 9 autosomes. (Fig.5).

***H. indica* (Muls.)** 2n=18 (8+Xyp)

The diploid complement comprised 18 chromosomes at spermatogonial metaphase (Fig.3). The karyotype is constituted by 8 pairs of autosomes and X and y sex chromosomes (Fig.4). Autosome pairs 1,5,6,7 and 8 are metacentric and 2,3 and 4 are submetacentric. X is submetacentric of the size of third pair of autosomes whereas, y is dot-shaped and is the smallest element of the karyotype.

Metaphase I revealed 8 rod and ring shaped autosomal bivlents and Xy_p (Figs. 6, 7a). Two types of metaphase II plates, one with X and the other with y chromosome in addition to 8 autosomes, were encountered (Fig. 7b). Morphology of the chromosomes was clear at this stage due to chromatid separation.

***H. ocellata* (Redt.)** 2n=18 (8+Xyp)

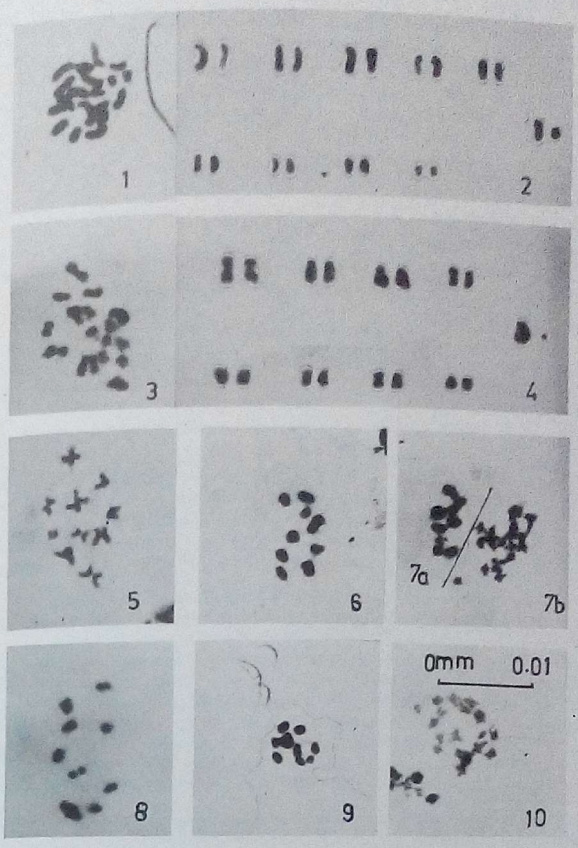
Diploid number of chromosomes 18 has been derived from metaphase I which comprised 9 bivalents; 8 autosomal bivalents and the Xy_p. (Fig. 8). The autosomal bivalents were either dumb-bell shaped or single rods.

***Epilachna atypica* (Dieke)** 2n=18 (8+Xyp)

Metaphase I depicted 8 rod and ring shaped autosomal bivalents and the Xyp (Fig. 9). The first meiotic division resulted 2 types of metaphase II plates (Fig. 10), one with X and the other with y in addition to 8 autosomes. The diploid number was derived from these 2 stages.

DISCUSSION

Coccinellidae is a middle-sized family consisting of 5000 taxonomically known species. However, cytologically, it is known by only 180 species of 48 genera belonging to 13 tribes and two



Figs. 1-10 : 1-2, *5. Henosepilachna septima*. 1, Spermatogonial metaphase, 2, Karyotype, 5, M II with X. 3,4,6-8. *H.indica*. 3, Spermatogonial metaphase, 4,Karyotype, 6,M I, 7a,M I with Xy_p, 7b, M II with X and y, 8, M I. 9,10. *Epilachna atypica*. 9, M I, 10, M II with X and y.

subfamilies (Agarwal 1960, 1961, Dasgupta 1977, Manna & Lahiri 1972, Mittal et al. 1989, Smith 1953, 1960, Takenouchi 1976, Yadav & Pillai 1974, Yadav et al. 1991). The diploid number of chromosomes varies from 12-26. Coccinellidae is characterized by the possession of typical polyphagan modal number 2n=20. During the present investigation *Henosepilachna septima* showed this number whereas, *H. indica*, *H. ocellata* and *Epilachna atypica* deviated from the modal number and exhibited diploid number of 18 chromosomes. This deviation as such is also depicted by the subfamily Epilachninae on the whole since out of 26 cytologically known species only 11 species possess 2n=20 whereas, 16 species possess 2n=18. The present observations on *H. septima* are in full agreement with the previous report by Kacker (1973) who described it as *E. septima*.

Coccinellid beetles are also characterized by a simple male sex chromosome mechanism, predominantly Xy_p - depicted by 85 species including the species under present investigation. The other most prevalent sex chromosome mechanism is neo-XY, possessed by 65 species. Besides these, other sex chromosome mechanisms like Xy, Xyr, Xyy, XXy, X+y, XO and XXXX are also known in species of Coccinellidae.

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CYTOTAXONOMIC STUDIES IN SOME SOUTH INDIAN SPECIES OF *ABUTILON*

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SUMMARY

Six species of *Abutilon* have been investigated in the present work. The wild species, *A. crispum* (2n = 14), *A. hirtum* (2n = 42), *A. indicum* (2n = 42), *A. persicum* (2n = 42) and *A. neilgherrense* (2n = 42) have the base number x = 7, while cultivated taxon, *A. striatum* with 2n = 16 reveals a different base number of x = 8. The chromosome number n =21 is newly reported in *A. neilgherrense* and in *A. hirtum* in the present work. Karyomorphological details have been worked out in all the species except in *A. neilgherrense*. Meiotic studies in *A. indicum*, *A. hirtum*, *A. persicum* and *A. neilgherrense* reveal normal behaviour with the formation of regular bivalents. The interrelationships of karyotypes and the role of polyploidy in the evolution of the genus *Abutilon* have been discussed.

Key Words: Cytotaxonomy, *Abutilon*.

INTRODUCTION

Abutilon is a diverse genus in respect of habit, leaf shape, hairs, stipules, flower colour, inflorescence, fruit and ecological adaptations. Chromosome numbers recorded so far in about 28 species is inadequate. However, it has pointed out the dibasic nature of the genus. But the karyomorphological details are lacking in the earlier reports. Further, South Indian populations have not been cytologically examined. Eight species are available in South India, of which 6 of them have been investigated in the present work and the data drawn from their chromosome number, karyomorphology coupled with exomorphic characters give additional information regarding their evolutionary interrelationships.

MATERIALS AND METHODS

The taxa for the present investigation were collected from many places in South India and maintained in the Botanical garden of Bangalore University. The root tips taken from the young plants were pretreated with 8-hydroxyquinoline and then were stained with basic fuchsin. For meiotic studies anther squashes were made in acetocarmine stain (1.5%). The analysis of the karyotypes was made by grouping the chromosomes into 3 main categories: (1) medium chromosomes (2.5 - 3.5 µm), (2) short chromosomes (1 - 2.5 µm) and (3) very short chromosomes (less than 1 µm). These are further classified based on the position of the centromeres into B^m and B^{sm} (medium chromosomes with nearly median and submedian centromeres respectively), C^m, C^{sm}, C^{st} and C^{ss} (short chromosomes with median, submedian, subterminal and SAT-chromosomes respectively), D^m and D^{sm} (very short types with median and submedian centromeres).

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OBSERVATIONS

Abutilon indicum (L.) Sweet.

This taxon has revealed 2n = 42 chromosomes. There are 6 pairs of medium chromosomes and 15 pairs of short chromosomes (Figs. 5, 15). The longest chromosome in the complement measures about 2.75 µm while that of the shortest chromosome is about 1.05 µm. The total length of the diploid complement is 60 µm. The karyotypic formula for this species is $2n = 42 = 6B^{m} + 6B^{sm} + 12C^{m} + 18C^{sm}$.

Meiosis is normal. Twenty one bivalents are observed at metaphase I (Fig. 6).

A. hirtum Don.

This taxon also has 2n = 42 chromosomes consisting of 6 pairs of medium sized chromosomes and 15 pairs of short type (Figs. 4, 14). The difference in the chromosome length ranges from 1.00 µm (shortest) to 2.80 µm (longest). The total chromosome length of the diploid complement is 68.3 µm. The karyotypic formula for this species is $2n = 42 = 6B^{m} + 6B^{sm} + 10C^{m} + 18C^{sm} + 2C^{st}$.

Meiotic behaviour is normal with regular formation of 21 bivalents at metaphase I (Fig. 3).

A. persicum (Burm.) Merr.

The somatic chromosome number is 42 in this species. There are only short and very short chromosome types, of which 17 pairs are short chromosomes and 4 pairs of very short type (Figs. 7, 16). The difference in the chromosome length ranges from 0.90 µm to 2.20 µm with a total length of 60.88 µm in the complement. The karyotypic formula is $2n = 42 = 6C^{m} + 28C^{sm} + 4C^{ss} + 2D^{m} + 2D^{sm}$.

In meiosis 21 bivalents are observed at metaphase I (Fig. 8).

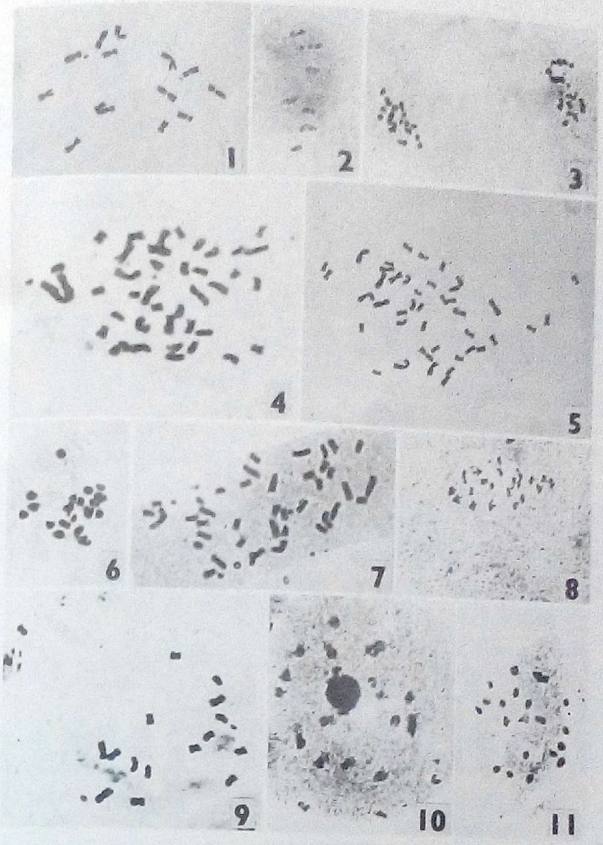
A. crispum Don.

Diploid number of 2n = 14 is determined in this species (Figs. 1,12). It has one pair of medium and 6 pairs of short chromosomes. The chromosome length of the shortest is about 1.45 µm while that of the longest is 2.68 µm. A total chromosome length of 30.08 µm is recorded and the chromosome types in the complement are $2B^{m} + 4C^{m} + 6C^{sm} + 2C^{ss}$.

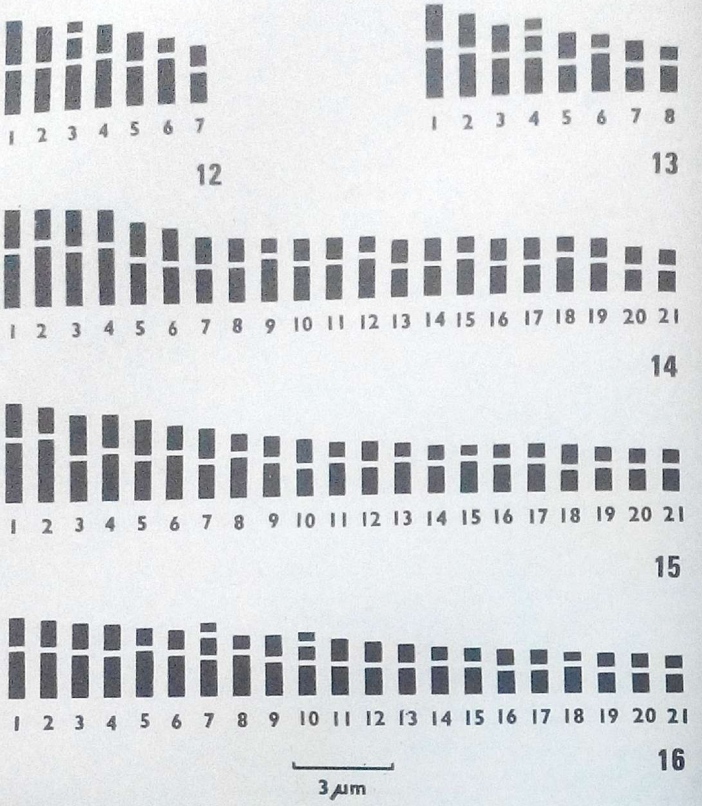
Meiosis is normal. Seven bivalents are observed at metaphase I (Fig.2).

A. striatum Dickson ex Lindl.

This species has the chromosome number of 2n = 16 (Figs. 9, 13) with medium and short chromosome types. The longest chromosome in the complement measures about 2.51 µm and that of the shortest is about 1.14 µm. The total length of the chromosomes is 27.20 µm. The karyotype consists of $2B^{m} + 8C^{m} + 4C^{sm} + 2C^{ss}$.



Figs. 1-11: 1,2, *Abutilon crispum*. 1. Somatic metaphase chromosomes, 2. Metaphase I with 7 bivalents. 3,4, *A. hirtum*. 3. Metaphase II showing 21 chromosomes at each pole, 4. Somatic metaphase chromosomes showing 2n = 42. 5,6, *A. indicum*. 5. Somatic metaphase complement with 2n = 42, 6. Metaphase I showing 21 bivalents. 7,8, *A. persicum*. 7. Somatic metaphase chromosomes showing 2n = 42, 8. Metaphase I with 21 bivalents. 9. *A. striatum*. Somatic metaphase chromosomes. 10,11, *A. nailgherrense*. Diakinesis and metaphase I respectively showing 21 bivalents.



Figs. 12-16: Idiograms of the karyotypes in *A. crispum*, *A. striatum*, *A. hirtum*, *A. indicum* and *A. persicum* respectively.

***A. neilgherrense* Munro ex Wt.**

Meiotic divisions reveal n = 21 chromosomes. The behaviour is normal with the formation of 21 bivalents at diakinesis and metaphase I (Figs. 10, 11).

DISCUSSION

Of the 6 species examined, the chromosome numbers of *A. hirtum* (2n = 42) and *A. neilgherrense* (n = 21) are reported here for the first time (Krishnappa & Munirajappa 1980). The present work also determines 2n = 42 in *A. indicum* and *A. persicum*, 2n = 14 in *A. crispum* and 2n = 16 in *A. striatum*. It is surprising that a single earlier publication (Roy & Sinha 1961) has given a different chromosome number of 2n = 32 in *A. indicum*, while all other earlier reports (Skovsted 1941, Subramanyam & Kamble 1966, Hazra & Sharma 1971, Sareen et al. 1974) and the present work reveal only 2n = 42 in this taxon. Similarly, the present findings and the earlier report of Gajapathy (1962) revealing 2n = 42 contradict the chromosome number of n = 16 reported by Sanjappa (1979) in *A. persicum*.

Although the chromosome number 2n = 16 is known in several species (Kuhn 1930, McClintock 1953, Krapovickas 1957, Niimoto 1966), 2n = 42 has been found to be a dominant number in the genus *Abutilon*. Thus, the species of *Abutilon* fall under 2 distinct base numbers namely, x = 7 and x = 8. In the 7 basic series, *A. crispum* is at the diploid level with 2n = 14 whereas the other 4 taxa are at the hexaploid level with 2n = 42. Among 8 basic series only *A. striatum* is investigated here, which is at the diploid level with 2n = 16.

Apart from the above, karyomorphological details in the 5 taxa have been furnished here for the first time. In general, all species have medium to short chromosomes. Chromosomes which could be regarded as long are altogether absent. Majority of their chromosomes have submedian primary constrictions. The somatic metaphase chromosomes fall within the range of 1.0 - 2.8 µm in length. The total chromosome length in their respective complements is approximately equal and proportional to their chromosome number. Hence, the diminution of chromosome size in polyploids is not significant.

However, the taxa are distinct both in exomorphic characters, chromosome markers and relative length of the chromosomes. *A. indicum* and *A. hirtum* are more closely related both occurring on plains in black cotton soils with moderate rain fall having cordate leaves, solitary flowers and the number of carpels ranging from 10 - 20, yet they differ in respect of pedicel length, hairs, flower colour and seed characters. Cytologically the karyotype of *A. hirtum* has one pair of chromosomes with subterminal primary constrictions, whereas such a pair is absent in *A. indicum*. Similarly, *A. persicum* (2n = 42) differs from the above two hexaploid species in possessing only five carpels and flowers in small clusters of terminal racemes. The karyotype is also unique showing very short chromosomes in its somatic complement. It has 2 pairs of SAT-chromosomes which are lacking in the other hexaploid taxa. Further, it is ecologically restricted occurring only in the hilly regions of Agumbe, Bisle, Kemmangundi, Kodaikanal in association with evergreen forest. Another hexaploid taxon, *A. neilgherrense* (n = 21) is distinct in possessing 2-10 flowered cyme and 10 carpels. Its ecological adaptation also appears to be different as it is found restricted to high altitudes (1950 m) of Nilgiri Hills.

The diploid species, *A. crinipum* (2n=14) deviates from hexaploid species in several characters. It is a diffuse annual herb and the size of the flower, leaf, seed etc., are relatively small when compared to those of hexaploids. It occurs at the foot hills of Ootacamund and Kodaikanal. This taxon has its own characteristic karyotype with one pair of SAT-chromosomes and more or less equal number of chromosomes with median and submedian primary constrictions. The other diploid species, *A. striatum*, on the other hand, is a cultivated species and altogether has a different chromosome number of 2n = 16. Its exomorphic characters are also of different nature.

The foregoing discussion reveals that the wild taxa examined here not only share many karyological and exomorphic features in common but also appear to have been evolved from a common stalk with the base number of x = 7. In these taxa with x=7 polyploidy has played a major role in speciation. All the 4 hexaploid taxa studied here are shrubby perennials and exhibit gigantism. On the other hand, the species with x = 8 so far recorded apparently remain at the diploid level. The occurrence of 2 base numbers (x = 7,8) also suggest the role of aneuploidy in the evolution of species in *Abutilon*.

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