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CYTOLOGY OF A TRIPLOID TEA

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SUMMARY

Cytological investigations of a triploid tea clone, UPASI-3 (SUNDARAM) were undertaken in the present study. The somatic chromosome number of this clone was found to be 45 (3x=45). Mitosis was regular. Meiosis was found to be highly irregular and these irregularities were encountered from the pachytene stage up to telophase II. Trivalents, bivalents and univalents of varying number were observed during meiosis I. The occurrence of trivalents up to 14 in number clearly implies the homology between the three genomes. Irregular disjunction of component chromosomes at anaphase I, irregular second division, laggards, cytomixis, supernumerary division, diads, triads, pentads etc. account for the high percentage of sterility. These cytological features together with data from morphology, leaf anatomy and photosynthetic pigments offer evidence for the autotriploid nature of UPASI-3 clone and substantiate its origin from *Camellia assamica*.

Key Words: *Camellia assamica*, autotriploid, UPASI-3, sterility.

INTRODUCTION

Tea is known as the queen of beverage plants. It is one of the most important plantation crops of the world and is a source of beverage leaves and therapeutic drugs such as caffeine and theophylline. Cytological investigations by Morinaga et al. (1929), Subbarao (1938), Longley & Tourjee (1959), Barua (1963), Bezbaruah (1971), Cherian & Stephen (1981) have established the diploid chromosome number of *Camellia sinensis* (China tea) and *Camellia assamica* (Indian tea) as 2n=30. A few natural and induced polyploids (Karasawa 1935, Simura 1935, Janakiammal 1952, Bezbaruah 1971, 1975, Kondo 1975, 1978) and aneuploids (Kondo 1975, Ackerman 1973) have also been reported. The present study was on the cytology of a triploid clone of a high-yielding Indian tea, (*C.assamica*) named Sundaram or UPASI-3. The mother plant of this clone was originally selected from the tea plantation of Brooklands Estate, Coonoor in the Nilgiris in 1961 by Venkataramani, as it showed luxuriant growth and high quality of leaves and was given the code No. B/5/63 (Venkataramani 1969). The clones raised from this mother plant have been subsequently named Sundaram or UPASI-3. Preliminary studies by Jayasurya & Govindarajulu (1973) have shown it to be an autotriploid. However, they have not provided the details of the cytological constitution of this clone and hence this study was undertaken.

MATERIALS AND METHODS

The material used in the study was samples of a triploid clone of *Camellia assamica*, named Sundaram or UPASI-3. The materials were collected mainly from the breeding fields of United Planters Association of South India (UPASI), Tea Research Institute, Valpari in Tamilnadu, UPASI, Tea Research Sub Station, Vandiperiyar and Merchiston Tea Estate, Ponmudi both in Kerala. For mitotic study, root tips from the nursery stocks as well as stem tips were used.

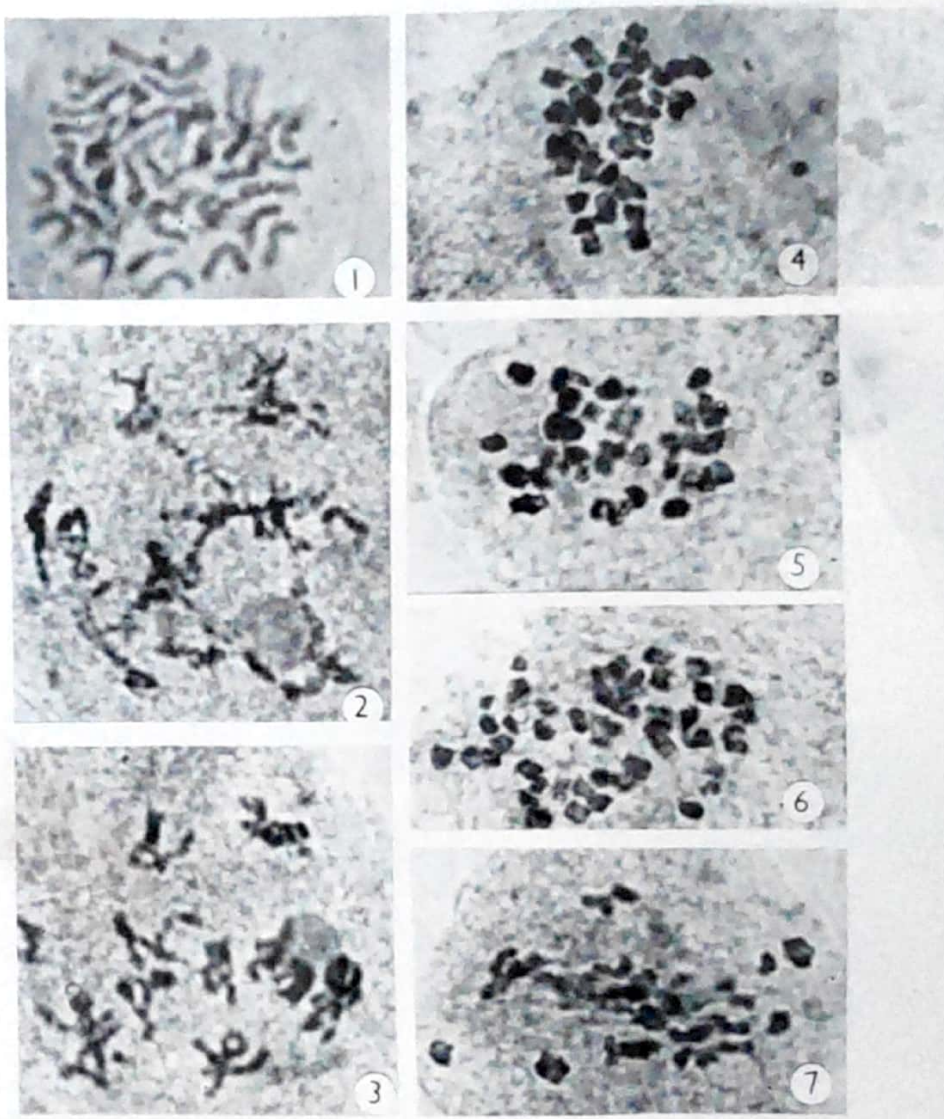
Root tips were collected at different intervals between 6 and 9.30 a.m., pretreated in hydroxyquinoline at 4° C for 2 h and then fixed in modified Carnoy's fluid (95% ethyl alcohol, chloroform, glacial acetic acid in 6:3:1 ratio) for about 24 h and squashed in 2% acetocarmine. Stem tips were likewise collected between 6 and 9.30 a.m. and pretreated in p-dichlorobenzene solution (saturated in distilled water) for 3 h at 4° C, fixed in Carnoy's fluid for 12 h as described by Bezbaruah (1968) and Cherian & Stephen (1981), hydrolysed in 1N HCl and stained in 2% acetocarmine. For meiotic study, young flower buds of intermediate size were collected between 6 and 8.30 a.m., fixed on Farmer's fluid (95% ethyl alcohol and glacial acetic acid in 3:1 ratio), for 24 h and squashed in 2% acetocarmine solution. Photomicrographs were made from fresh cytological preparations.

Pollen sterility was determined by using mature flower buds just before anthesis. Pollen grains were dusted on glass slides and stained with 1:1 mixture of glycerol and 2% acetocarmine solution by gentle warming. Fully stained and regular pollen grains were counted as fertile and others as sterile. A comparison between the diploid and triploid *C. assamica* was made in morphology, anatomy and analysis of photosynthetic pigments by spectrophotometry using Shimadzu UV visible spctrophotometer (UV-2100 S) using 80% acetone extracts of tea leaves according to the equations of Mac Kinney (Sirnoval 1967). Leaf anatomy of *C.sinensis* was also studied.

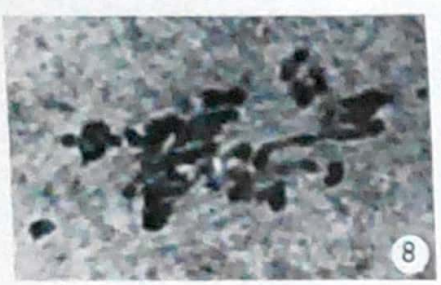
OBSERVATIONS

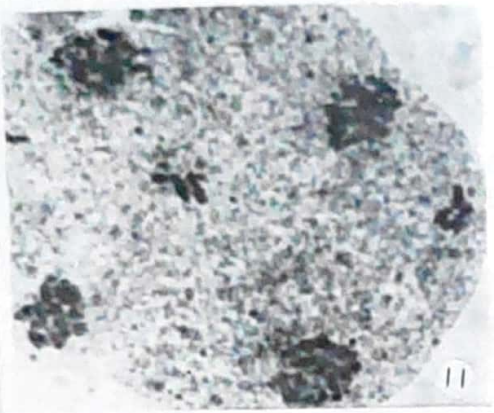
Acetocarmine squash preparations of meristamatic shoot tip cells in the mitotic metaphase showed 45 chromosomes (3x=45) as in Fig.1. Mitosis was regular. Acetocarmine preparations of pollen mother cells (PMCs) showed prevalence of trivalents at pachytene, diplotene, diakinesis and metaphase I stages. The variation in the number of trivalents, bivalents and univalents among different PMCs was scored and summerised in Table 1.

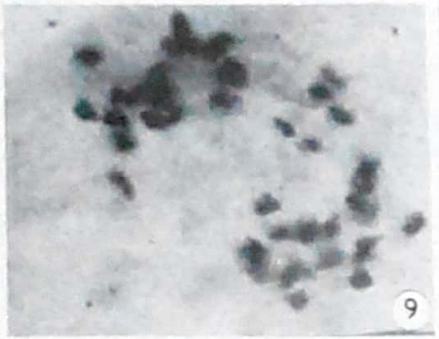
The formation of trivalents could be traced from pachytene stage onwards. Fig.2 shows a PMC in diplotene stage showing several trivalents, bivalents and univalents. Some new trivalent configurations as shown in Fig. 3 were also encountered. Metaphase I preparations also showed varying number of trivalents, bivalents and univalents (Figs. 4-6). Anaphase I was characterised by irregular disjunction of the homologues in trivalents (Figs. 7, 8). Fig.9 shows a PMC in late telophase I with bivalents and univalents lagging in the equatorial region of the cell. However, some PMCs are normal. Fig. 10 shows late anaphase II with 2 lagging chromosomes and unequal distribution of chromosomes. Telophase II with irregularities especially the formation of two additional chromosome groups is represented in Fig. 11. A PMC with 8 daughter nuclei with some laggards in the cytoplasm is represented in Fig.12. Fig.13 shows a tetrad in which two daughter nuclei are larger and two are smaller. Pollen sterility in the triploid was higher (97.72%) as compared to that of diploid tea (17%).

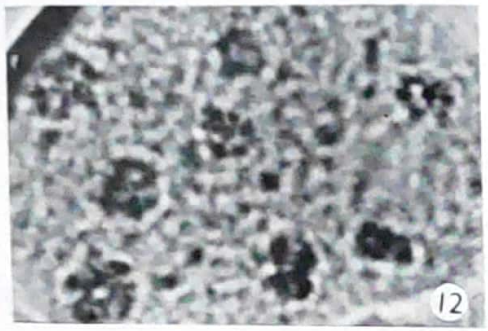


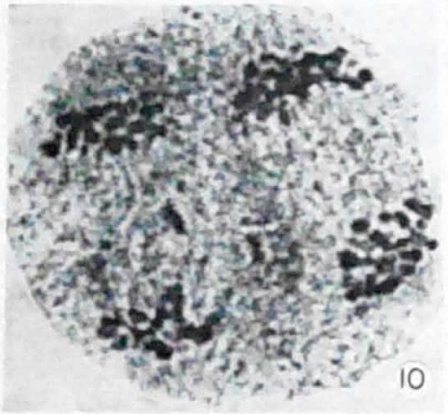
Figs. 1-7: Cytology of a triploid clone of *C.assamica*, UPASI-3 (Sundaram). 1. A shoot tip cell at mitotic metaphase. 2. A PMC at diplotene. 3. A PMC at diakinesis. 4-6. PMCs at metaphase I. 7. Irregular disjunction of chromosomes at anaphase I. (all x 1500)

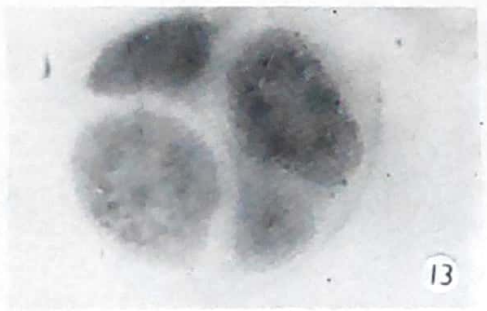












Figs. 8-13: Cytology of a triploid clone of *C. assamica*, UPASI-3 (Sundaram) 8. Anaphase I. 9. Late prophase II. 10. Late anaphase II with irregularities in chromosome segregation. 11. Telophase II. 12. Supernumerary division resulting in 8 daughter nuclei; a few laggards are also seen. 13. A tetrad stage showing 4 unequal microspores. (all x 1200)

TABLE 1: Frequency of PMCs at diakinesis and metaphase I with different constitutions of trivalents (III), bivalents (II) and univalents (I).

Cells with						Cells with stickiness and			
14_{III} 3_{I}	13_{III} 2_{II} 2_{I}	13_{III} 1_{II} 4_{I}	12_{III} 3_{II} 3_{I}	10_{III} 5_{II} 5_{I}	9_{III} 6_{II} 6_{I}	III and II of varying No.	3_{I}	4_{I}	Varying No. of univalents
3%	13%	10%	4%	7%	4%	42%	9%	3%	5%

TABLE 2: Estimation of photosynthetic pigments by spectrophotometry.

Sample	Absorbance (nm)			Pigments	Quantity of pigments
	663	645	480		
Triploid (UPASI-3)	3.52	1.50		chl-a	0.04
				chl-b	0.02
			2.63	carotenoids	1.28
Diploid (*C. assamica*)	2.72			chl-a	0.03
		1.10		chl-b	0.01
			1.94	carotenoids	0.93

Inspite of the higher degree of pollen sterility some fruits were found to develop. However, at maturity there was nothing inside the seed. Sections of the maturing fruits showed that the embryo developed up to the heart-shaped stage and then aborted.

Morphologically, this triploid clone showed an increase in leaf size, colour, internodal length, flower size, number of petals etc. Leaf anatomy showed a single layer of palisade cells but the cells were larger than those in diploid *C.assamica*, in contrast to *C.sinensis* where the palisade was double-layered and composed of smaller cells. Guard cells were bigger in size as compared to those of *C.assamica*. The subsidiary cells showed a marked similarity with *C.assamica* but differ from those of *C.sinensis*. The quantity of photosynthetic pigments in the triploid was much higher than that of the diploid variety as represented in Table 2.

DISCUSSION

The basic chromosome number of the genus *Camellia* has been established as x=15 (Morinaga et al. 1929, Janakiammal 1952, Barua 1963, Longley & Tourjee 1959, Kondo 1978, Cherian & Stephen 1981). In the present study, the material showed 2n(3x)=45 in stem tip and root tip cells. This is in agreement with the report of Karasawa (1935), Janakiammal (1952), Bezbaruah (1975), Kondo (1975) on the chromosome counts of triploid tea growing in their localities and that of Jayasurya & Govindarajulu (1973) on the triploid clone presently investigated.

The most frequent formation of 13 trivalents and occasional occurrence of 14 trivalents (Table 1) and the total counts of individual chromosomes in mitotic metaphase as 45, excludes the possible origin of this clone by trisomy of a few chromosomes and confirms that it is an

autotriploid of *C.assamica* in conformity with the findings of Jayasurya & Govindarajulu (1973) who have also reported a maximum of 14 trivalents in PMCs. In addition to the classical trivalent configurations described by Belling & Blakeslee (1924), Riley (1948) Sybenga (1972, 1975), several new trivalent configurations were observed presently due to variations in chiasma formation and terminalisation.

Difficulties encountered in the disjunction of constituent chromosomes of trivalents at anaphase I caused unequal distribution of chromosomes to either poles. In some cells, trivalents were found to lag and in some others 2 chromosomes move to one pole and one to the opposite pole. Laggards were also reported in diploid hybrid between *C.sinensis* and *C.assamica* by Cherian & Stephen (1981) and in tetraploid clone by Kondo (1978). Most of the irregularities in the first division of meiosis have been reported in the autotriploids of other plants (Riley 1948, Sybenga 1975). Cytoplasmic fusion between adjacent PMCs resulting syncytia observed in this triploid, was similar to the situation reported by Kondo & Ackerman (1978) in the amphidiploid clone of "Fragrant Pink Improved" (B 60345).

Second division of meiosis in PMCs was also abnormal. Mostly, hyperhaploid number of chromosomes were seen as in Fig.10. In a few cells, clumping and stickiness of chromosomes causing non-disjunction of daughter chromosomes were encountered. Disposition of a pair of telophase II nuclei at right angles to another is a new phenomenon found in some PMCs. The occurrence of 8 daughter nuclei as shown in Fig.12, may be explained to be the result of micronuclei formation by the aggregation of lagging chromosomes as seen in Fig.11. There is also possibility of a third division by which 4 daughter nuclei get duplicated. Size of these daughter nuclei is smaller than the normal ones and hence, the first explanation appears more appropriate. Micronuclei formation has been reported by Kondo (1978) in tetraploid tea. Nevertheless, supernumerary division in PMCs has been reported in *Zea mays* (Beadle, 1931), *Impatiens sultani* (Tara & Namboodiri 1974), and in diploid hybrid tea (Cherian & Stephen, 1981). The occurence of triads, tetrads and pentads of microspores formed is the reflection of meiotic irregularities in this triploid clone of *C.assamica*. The aberrant meiosis in this autotriploid clone is responsible for high incidence of pollen sterility. Fertile pollen grains are those which receive at least a haploid or diploid set of chromosomes (King 1933). This is a matter of chance or due to positive adaptation to overcome meiotic irregularities at least in a few PMCs. Reports of such positive adaptations in autopolyploids have been reviewed by several authors (Stebbins 1940, Riley 1948). The embryo does not develop beyond heart shaped stage in this triploid and hence nothing is found inside the seeds at maturity. Data from morphology, anatomy and pigment analysis of this triploid UPASI-3 show characteristics of polyploids as compared to its diploid progenitor, *C.assamica*.

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STUDIES ON BIOSYSTEMATICS AND CLASSIFICATION IN THE GENUS *ALYSICARPUS*

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SUMMARY

Karyotype analysis, nuclear DNA estimations, study of chromosome pairing, attempts at hybridization, comparison of the storage proteins, albumins and globulins on the basis of their electrophoretic mobility as well as a comparison of the isoperoxidases were made in *A. hamosus, A.monilifer, A.vaginalis, A.vaginalis* var. *nummularifolius, A.bupleurifolius, A.longifolius, A.pubescens, A.rugosus* var. *heyneannus, A. rugosus* var. *styracifolius, A.tetragonolobus* and *A. belgaumensis*. These studies have shown that *A.belgaumensis* has a higher chromosome number, n=11 as compared to the rest of the species which have n=8. 4C nuclear DNA value in this species is also observed to be higher but the per chromosome DNA content is comparable to those in the members of subgenus *Microcalycinae*. Attempts of hybridizations between A. *belgaumensis* and other species of *Alysicarpus* were not successful. Similarity indices for storage proteins and isoperoxidases also indicated *A.belgaumensis* as a distinct species. A comparison of storage proteins in *A.belgaumensis* and 3 species of *Desmodium* indicated *A.belgaumensis* as showing more affinity towards members of *Alysicarpus* than *Desmodium*. In case of meiotic chromosome pairing *A.belgaumensis* showed 3 quadrivalents indicating its origin from the taxa having x=8 as the number. Thus the present study indicated the existence of 3 subgenera under the genus *Alysicarpus*.

Key Words : *Alyscarpus*, biosystematics, phylogeny, DNA, PAGE.

INTRODUCTION

Alysicarpus Neck. belongs to the tribe *Desmodiae* of *Fabaceae* and consists of species with a potential for development into fodder crops for improvement of grasslands and for use as cover crops in the plantations. It has 35 species distributed in Africa, Asia, Australia, Polynesia and Tropical America (Hutchinson 1964). Baker (1876) divided the genus into 2 groups viz., *Microcalycinae*, characterized by the calyx not longer than the first joint of the pod and

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Macrocalycinae where the calyx is much longer than the first joint of the pod and its teeth imbricate in the fruiting stage. Danzell (1851) working on plants of western India described *A.parviflorus* and named some of the collections from the same region as *A.rotundifolius*. Baker (1876) transferred these 2 species of *Alysicarpus* to *Desmodium* as *D.parviflorum* and *D.rotundifolium* and did not assign any reason for this transfer. Prain (1897) states that "these two species do not accord well with the generic definition of *Desmodium* and are more conveniently referred to as *Alysicarpus* in which they were originally placed by Danzell" and excluded *A.parviflorus* (*D.parviflorum*) and *A.rotundifolius* (*D.rotundifolium*) from the *Desmodium* complex. He suggested that these 2 species and 22 other closely related species *A.belgaumensis* and *A.racemosus* be placed in a group intermediate between the 2 genera.

Four species of *Alysicarpus*, *A.belgaumensis A.parviflorus*, *A.racemosus* and *A.rotundifolius* stand apart as they have intermediate characters between *Alysicarpus* and *Desmodium*. Prain (1897) having realized the above situation created a new subgenus *Desmodiastrum* under the genus *Alysicarpus* to accommodate the above 4 species. Prior to the establishment of the genera *Alysicarpus* and *Desmodium* most of the species of these 2 and other related genera were treated under the genus *Hedysarum*. During the segregation of the different species of *Heydysarum*, 3 species were included in *Alysicarpus* and 33 species under *Desmodium*. Since then, several species were added to both *Alysicarpus* and *Desmodium*.

Dutt et al. (1979) on the basis of chemical studies established that the genus *Alysicarpus* is a natural and homogeneous group except *A.belgaumensis* which is characterized by the conspicuous absence of flavones and many phenolic acids. They observed that the taximetric study in the genus *Alysicarpus* by Bhalla & Dakwala (1978) supports their findings and suggested the retention of *A. belgaumensis* in *Alysicarpus*, considering it as a third line of evolution along with *Microcalycinae*, and *Macrocalycinae* which otherwise extends an apparent support towards Prain's (1897) treatment of the genus.

Earlier karyomorphological studies (Ohashi 1971, Sanjappa 1977, 1982) have indicated the independent generic status of *Alysicarpus* and *Desmodium* having the most common base numbers of x=8 and x=11 respectively. Sanjappa (1977) reported n=11 for *A.parviflorus* (referred to as *D.parviflorum*) and *A.rotundifolius* (referred to as *D.rotundifolium*) which led him to suggest their inclusion in *Desmodium* rather than in *Alysicarpus*. Sanjappa & Sathyananda (1979) reported n=8 for *A.racemosus*. John et al. (1986) reported 2n=22 for *A.belgaumensis*. These chromosome number reports alone cannot be of much significance in generic delimitation because distinct genera having chromosome number n=11 were once placed under *Desmodium* but now separated. In addition, Sanjappa (1977) reported x=10 as one of the base numbers in *Desmodium* which is not uncommon in *Alysicarpus* (Meige 1960, Gadella & Kliphuis 1964, Hsu 1968).

Recently, Pramanik & Thothathri (1986) opined that the subgenus *Desmodiastrum* be raised to the status of a genus so that it would act as a connecting link between the genera *Alysicarpus* and *Desmodium* though the data in support of such a treatment are meager.

The objectives of the present investigation were to collect cytogenetical and biochemical data to elucidate the interspecific and intervarietal relationships as well as the systematic position of the species initially treated as belonging to a separate subgenus *Desmodiastrum* under the genus *Alysicarpus*. The present investigation is also an attempt to answer some of the questions regarding the classification within the genus *Alysicarpus* and deals with *A.hamosus* Edgew., *A.monilifer* Edgew., *A.vaginalis* DC., *A.vaginalis* DC. var. *nummularifolius* (*Microcalycinae*), *A.bupleurifolius* DC., *A.longifolius* Wt & Arn., *A.pubescens* Law., *A.rugosus* DC.var. *heyneanus*, *A.rugosus* DC., var. *styracifolius*., *A.tetragonolobus* Edgew. (*Macrocalycinae*) and *A.belgaumensis* Wight. (*Desmodiastrum*).

MATERIALS AND METHODS

For meiotic studies, flower buds were collected in the forenoon and fixed in 1:3 acetic-alcohol for 24 h, thereafter, preserved in 70% alcohol. Squash preparations were made in 2% acetocarmine. Feulgen-microdensitometry using a Vicker's M 86 microdensitometer was employed for DNA estimations. For crossing the species, the flowers were emasculated at least 3 h prior to anthesis and bagged, pollinated at the time of anthesis and pod set checked after one week. Seed storage proteins were extracted, fractionated and lyophilized (Kartha & Sethi 1957, Osborne 1925). Laemmli's (1970) method was followed for SDS-PAGE and for native PAGE, SDS was omitted from the gel and the electrophoresis buffer. To study the zymogram for isoperoxidases standard procedures for the extraction, electrophoresis and staining (Schrauwen 1966) were followed. Jaccard's similarity indices were determined to compare the protein profiles and the zymograms (Hadacoa et al. 1980).

OBSERVATIONS

A.belgaumensis has the highest amount of nuclear DNA content (4C = 13.19 pg). In *Microcalycinae* the DNA contents ranged between 9.23 pg (*A.vaginalis*) and 9.92 pg (*A.vaginalis* var. *nummularifolius*). In *Macrocalycinae* it ranged between 11.06 pg (*A.bupleurifolius*) and 12.48 pg (*A.rugosus* var. *heyneannus*. When per chromosome DNA amount was calculated it was comparable to those in the members of *Microcalycinae* (0.3 pg), whereas the members of *Macrocalycinae* have higher per chromosome values (0.37 pg). Meiotic chromosomal pairing in the species of *Alysicarpus* except *A.belgaumensis* showed only bivalent pairing. In *A.belgaumensis* a variety of configurations were noticed viz., 11 bivalents (40%), 9 bivalents and 1 quadrivalent (40%), 7 bivalents and 2 quadrivalents (14%) or 5 bivalents and 3 quadrivalents (6%). Only 16% of all bivalents entered into multivalent pairing and the rest 84% remained as bivalents. There was no pod-set when *A.belgaumensis* was crossed with other species of *Alysicarpus*.

In case of Jaccard's similarity indices (SI) for the storage albumins and globulins, *A.belgaumensis* showed distinctness and more slanting towards *Microcalycinae*. For isoperoxidases also the species showed distinctness. One isozyme species was common to all the species of *Alysicarpus* studied. *A.monilifer*, *A.vaginalis* var. *nummularifolius* and *A.bupleurifolius* showed closer relationships in sharing all common isozyme bands (SI = 100%). Similarly, *A.longifolius*, *A.rugosus* var. *heyneanus* and *A.rugosus* var. *styracifolius* shared 3 common bands (SI = 100%) and *A.pubescens* and *A.tetragonolobus* also shared 3 bands (SI = 100%). *A.belgaumensis* showed 2 common bands with *A.hamosus* and *A.vaginalis*, while it shares only one band with rest of the species. With species of *Desmodium* the similarity indices *A.belgaumensis* showed was less than that it showed with members of *Alysicarpus* (18.18, 16.67 and 10 for native albumins, 3.33, 16 and

0 for SDS- albumins, 10, 2.56 and 5.56 for native globulins and 9.3, 4.17 and 9.3 for SDS globulins with *D.heterocarpum, D.laxiflorum* and *D.triquitum* respectively).

DISCUSSION

The classification within the genus is based on the pod morphology. Though the pod character forms the basis of classification within the genus, the morphology of the pod is quite distinct in the species and varieties. The main delimiting character between *Alysicarpus* and *Desmodium* is the turgid or flat pod character. In this respect, *A.hamosus* (Babu 1974) and *A.belgaumensis* show an intermediate character between turgid and flat pod morphology.

Cytological studies have shown that x=8 is the most common base number in *Alysicarpus* and x=11 is the most common base number is *Desmodium*. In *Microcalycinae* x=7 and x=10 (Hsu 1968, Kappali & Patil 1987) and in *Macrocalycinae* x=10 (Meige 1960, Gadella & Kliphuis 1964) are also known as base numbers. In species under *Desmodiastrum* x=11 is the most common base number (Sanjappa 1984, John et al. 1986), x=8 is also reported (Sanjappa 1979). Thus the chromosome numbers known so far indicate that x=7, 8,10 and 11 are the base numbers in *Alysicarpus*. In *Desmodium* though x=11 is the most common base number, x=10 also is known (Sanjappa 1977, 1984). These chromosome numbers alone cannot be parameters for generic delimitations because there are distinct genera having chromosome number x=11 which were once under *Desmodium* but now being separated on the basis of other characters. The common base numbers in *Alysicarpus* and *Desmodium* can at the most considered as indicative of close affinity between the two genera.

Observations on the chromosome numbers and karyotypic details point towards 3 lines of evolution in the genus *Alysicarpus*. (i) *Microcalycinae*: Base numbers x=7, 8 or 10 where 8 is the most common base number ; lower length of the chromosome pairs and hence lower total length of the chromosome complements; lower degree of asymmetry of the karyotypes. (ii) *Macrocalycinae*: Base numbers x=8 or 10 where 8 is the most common base number; higher individual chromosome pair lengths and higher total length of the chromosome complement; higher karyotype asymmetry. (iii) *Desmodiastrum*: x=11 is the most common base number, x=8 also is known; lower length of the individual chromosome pairs but higher total length of the chromosome complement due to the addition of three chromosome pairs, probably due to aneuploidy; higher karyotype asymmetry. Thus, the observations on the karyotypic details are in agreement with Prain's (1897) classification of the genus.

The 4C nuclear DNA contents in the species and varieties of *Alysicarpus* also indicate 3 lines of evolution in the genus.

(i) *Microcalycinae*: 4C nuclear DNA content lower and hence the per chromosome DNA content also lower. (ii) *Macrocalycinae*: 4C nuclear DNA content higher and the per chromosome DNA content also higher. (iii) *Desmodiastrum*: 4C nuclear DNA content higher but the per chromosome DNA content lower.

The clustering of the 4C nuclear DNA contents in the members of different subgenera indicates them as natural groups. The higher 4C nuclear DNA value in *A.belgaumensis* points towards its possible origin through aneuploidy. Its similarity with the members of *Microcalycinae* in case of per chromosome DNA contents points towards its possible origin from some member of *Microcalycinae* or both *A. belgaumensis* and the members of *Microcalycinae* as originated from the same ancestor/s.

The observation of only bivalent pairing at diakinesis in species and varieties having chromosome number n=8 and regular 8:8 segregation prove them to be regular diploids. This indicates the homology between the parental genomes and the species and varieties having chromosome number n=8 as diploids. In *A.belgaumensis* the observation of maximum of 3 quadrivalents further supports the view that this species is a derived aneuploid since this species has 3 additional pairs of chromosomes than the diploids. The observation of higher frequencies of 11 bivalents and 9 bivalents and 1 quadrivalent pairing indicate that probably one of the additional pairs of chromosomes is a duplication. The lower frequencies of 7 bivalents and 2 quadrivalents and 5 bivalents and 3 quadrivalents associations indicate these 2 additional pairs of chromosomes as possibly duplications with structural alterations or alien chromosomes with partial homology with some of the chromosomes of the diploid complement. In *A.belgaumensis* also the chromosome segregation at anaphase I and anaphase II is normal. This indicates the species as a stable aneuploid.

The higher percentage of pollen fertility in *A.belgaumensis* indicates this species as a stable aneuploid. Experiments on cross pollination between the species and varieties proved them as cross incompatible. This indicates the existence of cross breeding barrier/s between the species and even between the varieties. The occurrence of common bands in the native albumin, SDS-albumin and SDS-glubulin profiles of the species and varieties indicate the homogeneity of the genus. The species and varieties under *Microcalycinae* showing higher similarity indices between them than with the members of *Macrocalycinae* or *A.belgaumensis* proved the subgenus *Microcalycinae* as a natural group. Similarly, the member of *Macrocalycinae* showing higher similarity indices between them than with the members of *Microcalycinae* or *A. belgaumensis* proved the subgenus *Macrocalycinae* as a natural group. The study of protein profiles again points towards 3 lines of evolution.

To study the differences in case of a homologous protein peroxidase isozymes were analysed and compared on the basis of mobility of the major bands. The occurrence of a band peculiar only to *A.belgaumensis (Desmodiastrum)* further indicates the distinctness of this species from the other two subgenera. From the isozyme profiles of peroxidases, in *Microcalycinae*, *A.monilifer* and *A.vaginalis* var. *nummularifolius* appears to be closely related, in *Macrocalycinae, A. longifolius, A. rugosus*, var. *heyneannus* and *A. rugosus* var. *styracifolius* appear to form one group and *A.pubescens* and *A.tetragonolobus* appear to form another group. *A.bupleurifolius* shows distinctness from the rest of the species under *Microcalycinae*. *A.belgaumensis* shows more closeness towards *A.hamosus* than to other species under *Microcalycinae* or *Macrocalycinae*.

Thus there appears to be 3 different lines of evolution in the genus (i) *Microcalycinae*, (ii) *Macrocalycinae* and (iii) *Desmodiastrum*. Either they originated from the same ancestral stock or *Microcalycinae* represent the primitive group from which other 2 subgenera originated. In *Microcalycinae* there are 3 groups (i) *A.hamosus*, (ii) *A.vaginalis* and (iii) *A.monilifer* and *A.vaginalis* var. *nummularifolius*. In *Macrocalycinae* also there are 3 groups (i) *A.bupleurifolius*, (ii) *A.longifolius*, *A.rugosus* var. *heyneannus* and *A.rugosus* var.*styracifolius* and (iii) *A.pubescens* and *A.tetragonolobus*. The observations made in the present investigation extend support to Prain's (1897) classification of the genus.

In case of *A.belgaumensis* the observation of higher similarity indices with members of *Microcalycinae* as compared to the very low similarity indices observed with the species of *Desmodium* analyzed does not agree with the consideration of this species as belonging to *Desmodium* (Baker 1876, Sanjappa 1977, 1982) or Pramanik & Thothathri's (1986) consideration of the subgenus *Desmodiastrum* as a separate genus.

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CHROMOSOME POLYMORPHISM IN EASTERN HIMALAYAN MAIZE

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SUMMARY

Antiquity and diversity of maize grown by tribals of eastern Himalayan region is impressive. The chromosomal polymorphism in 45 native maize races of this region has been presented and discussed. The presence of heterochromatic block, satellites, B-chromosome, abnormal chromosome 10 and some new knob forming positions have been observed.

Key Words: Maize, chromosome, knob, polymorphism.

INTRODUCTION

Eastern Himalayan Region of India stretches from 22°N to 29.3°N latitude and 88.05° E to 37.24° E longitude. The altitude of the region ranges from 97 m in the plains to as high as 5000 m above M.S.L. in the hills. The region comprises 7 states of northeast (Assam, Arunchal Pradesh, Nagaland, Manipur, Mizoram, Meghalaya and Tripura), Sikkim as well as districts of Darjeeling, Coochbehar and Jalpaiguri in West Bengal. The wide variety of maize ecotypes is cultivated by aborigines in small isolated pockets in the region since many centuries (Stonor & Anderson 1949, Singh 1977). The antiquity and diversity of maize in the region has attracted the attention of many evolutionists (Dhawan 1964, Sachan & Sarkar 1982, Kumar & Sachan 1991). The present study deals with the chromosomal polymorphism in the maize of this area.

MATERIALS AND METHODS

The 45 local races of maize grown in different geographical areas of eastern Himalayan region were available in Division of Genetics, Indian Agricultural Research Institute (IARI) New Delhi. These maize strains belonged to Nagaland (10), Tripura (10), Meghalaya (10), Sikkim (10), Arunachal Pradesh (2) and Assam (3). The strains were grown at the Research farm of IARI, New Delhi in the kharif season of 1988. The young emerging tassels were fixed in ethanol acetic acid (3:1) and stored at 4±1°C. Pachytene analysis of pollen mother cells (PMCs) were done by normal acetocarmine squash method. Tassels of at least 5 plants were analysed for each maize strain. Knob position of each individual chromosome were identified following the standard pachytene karyotype of maize (Longley & Kato 1965, Neuffer et al. 1968). Mean relative lengths of all 10 chromosomes and their arm ratio were measured in 12 representative strains to have an idea about the differences in their length. The PMCs were also studied at diakinesis for the presence or absence of B-chromosomes and at anaphase and telophase for tracing the course of B-chromosomes and for other cytological observations. The presence or

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TABLE 1: Mean relative length and S.E. of pachytene chromosomes in northeastern Himalayan maize.

Collection Nos.	Chromosomes									
	I	II	III	IV	V	VI	VII	VIII	IX	X
S-10	16.81 ± 0.23	13.91 ± 0.41	12.95 ± 0.21	11.58 ± 0.24	10.84 ± 0.44	7.93 ± 0.20	7.18 ± 0.24	7.24 ± 0.32	6.93 ± 0.29	4.67 ± 0.17
S-21	18.03 ± 0.63	12.81 ± 0.74	12.47 ± 0.20	10.15 ± 0.25	9.12 ± 0.38	8.71 ± 0.47	7.99 ± 0.25	8.21 ± 0.32	7.69 ± 0.26	5.02 ± 0.41
S-24	17.41 ± 0.33	12.82 ± 0.25	11.95 ± 0.72	10.87 ± 0.32	9.97 ± 0.34	8.67 ± 0.37	7.59 ± 0.42	7.40 ± 0.30	6.92 ± 0.55	6.26 ± 0.29
S38	16.37 ± 0.48	13.64 ± 0.35	13.52 ± 0.63	12.05 ± 0.35	10.17 ± 0.38	8.52 ± 0.36	8.38 ± 0.42	7.71 ± 0.40	5.40 ± 0.51	4.28 ± 0.30
T-7	13.97 ± 0.40	12.78 ± 0.42	12.34 ± 0.59	11.06 ± 0.52	11.19 ± 0.36	9.86 ± 0.32	9.43 ± 0.33	7.78 ± 0.40	5.98 ± 0.16	5.53 ± 0.29
T-18	14.48 ± 0.62	12.86 ± 0.48	12.49 ± 0.52	11.20 ± 0.37	10.26 ± 0.45	8.76 ± 0.27	8.25 ± 0.54	8.37 ± 0.33	7.26 ± 0.24	5.96 ± 0.25
T-21	15.74 ± 0.73	12.95 ± 0.54	12.59 ± 0.15	10.94 ± 0.49	9.87 ± 0.17	8.89 ± 0.37	8.52 ± 0.32	7.89 ± 0.25	6.86 ± 0.22	5.83 ±0.21
A-1	15.80 ± 0.26	13.46 ± 0.34	13.17 ± 0.43	11.07 ± 0.72	10.92 ± 0.36	8.44 ± 0.34	7.96 ± 0.31	8.12 ± 0.31	7.15 ± 0.16	5.78 ± 0.19
M-15	15.55 ± 0.40	13.15 ± 0.62	12.68 ± 0.47	11.03 ± 0.78	10.24 ± 0.26	8.67 ± 0.43	8.24 ± 0.38	7.66 ± 0.21	7.34 ± 0.31	5.42 ± 0.41
N-23	14.34 ± 0.64	12.75 ± 0.53	11.91 ± 0.40	11.15 ± 0.52	10.29 ± 0.23	9.80 ± 0.33	9.14 ± 0.45	8.83 ± 0.38	7.13 ± 0.21	4.80 ± 0.27
N-26	15.12 ± 0.60	13.69 ± 0.17	13.38 ± 0.28	11.87 ± 0.43	10.15 ± 0.54	8.80 ± 0.25	8.45 ± 0.50	7.83 ± 0.48	6.11 ± 0.50	4.76 ± 0.13
N-35	13.80 ± 0.25	12.90 ± 0.60	12.15 ± 0.57	10.68 ± 0.34	10.32 ± 0.16	9.23 ± 0.28	9.07 ± 0.42	8.52 ± 0.26	7.48 ± 0.45	5.55 ± 0.18

TABLE 2: Arm ratio and S.E. of the pachytene chromosomes in northeastern Himalayan maize.

Collection No.s	Chrosomes									
	I	II	III	IV	V	VI	VII	VIII	IX	X
S-10	1.27 ± 0.02	1.48 ± 0.09	2.15 ± 0.12	1.67 ± 0.02	1.22 ± 0.05	5.98 ± 0.30	2.80 ± 0.14	3.13 ± 0.14	2.02 ± 0.02	2.62 ± 0.14
S-21	1.15 ± 0.09	1.32 ± 0.05	2.17 ± 0.05	1.67 ± 0.06	1.18 ± 0.06	6.48 ± 0.18	2.53 ± 0.07	3.07 ± 0.02	2.07 ± 0.05	2.48 ± 0.07
S-24	1.14 ± 0.03	1.25 ± 0.03	2.21 ± 0.06	1.96 ± 0.03	1.22 ± 0.06	6.74 ± 0.18	2.73 ± 0.06	3.22 ± 0.06	2.14 ± 0.04	2.57 ± 0.05
S-38	1.20 ± 0.01	1.16 ± 0.03	2.10 ± 0.07	1.89 ± 0.07	1.12 ± 0.01	6.89 ± 0.07	2.70 ± 0.07	3.29 ± 0.07	2.11 ± 0.04	2.55 ± 0.04
T-7	1.26 ± 0.01	1.19 ± 0.03	2.05 ± 0.04	1.77 ± 0.04	1.08 ± 0.03	6.32 ± 0.12	2.50 ± 0.12	2.96 ± 0.03	2.10 ± 0.07	2.55 ± .03
T-18	1.22 ± 0.02	1.21 ± 0.01	1.98 ± 0.01	1.97 ± 0.01	1.09 ± 0.03	6.42 ± 0.12	2.50 ± 0.05	3.08 ± 0.04	2.19 ± 0.01	2.52 ± 0.02
T-21	1.29 ± 0.01	1.23 ± 0.03	2.05 ± 0.04	1.97 ± 0.02	1.17 ± 0.05	7.00 ± 0.05	2.62 ± 0.06	3.14 ± 0.03	2.12 ± 0.02	2.53 ± 0.06
A-1	1.30 ± 0.04	1.45 ± 0.06	2.03 ± 0.05	1.82 ± 0.05	1.06 ± 0.03	6.30 ± 0.19	2.52 ± 0.05	3.15 ± 0.03	2.10 ± 0.02	2.58 ± 0.12
M-15	1.17 ± 0.02	1.19 ± 0.12	1.70 ± 0.05	2.04 ± 0.03	1.14 ± 0.03	5.79 ± 0.15	2.55 ± 0.06	3.07 ± 0.02	2.00 ± 0.09	2.62 ± 0.03
N-23	1.29 ± 0.07	1.15 ± 0.04	1.99 ± 0.02	2.10 ± 0.07	1.07 ± 0.01	6.70 ± 0.14	2.64 ± 0.03	3.17 ± 0.02	2.11 ± 0.05	2.60 ± 0.07
N-26	1.33 ± 0.02	1.23 ± 0.04	2.07 ± 0.04	1.96 ± 0.02	1.05 ± 0.02	6.60 ± 0.04	2.45 ± 0.05	3.03 ± 0.03	2.10 ± 0.05	2.63 ± 0.05
N-35	1.18 ± 0.05	1.42 ± 0.02	2.05 ± 0.04	2.06 ± 0.15	1.07 ± 0.03	6.55 ± 0.05	2.65 ± 0.03	3.10 ± 0.07	2.02 ± 0.02	2.49 ± 0.07

absence of satellites on the short arm of sixth chromosome, heterochromatic blocks on the long arm of second chromosomes and abnormal chromosome 10 (K 10) was also recorded in all strains included in the present study.

RESULTS AND DISCUSSION

Data related to total length of all 10 chromosomes of a strain was transformed into relative length to avoid any discrepancy due to differential condensation. The relative length and arm ratio of all 10 chromosomes of 12 representative maize strains have been presented in Tables 2 and 3. Marked differences were observed in the relative lengths of different chromosomes in different strains. The relative length of chromosomes was smaller in case of T-7 (Tripura) and N-35 (Nagaland). However, arm ratio of different chromosomes did not exhibit any such significant difference.

The presence of abnormal chromosome 10 (K10) in the northeastern Himalayas (NEH), region was rare, being restricted to Tripura and Nagaland strains only in very low frequency. The maize strains having K10 were N-37 (Nagaland) and T-17 (Tripura). However, B-chromosome was observed in maize strains from Sikkim, Tripura, Nagaland and Assam (Table 4) in a much higher frequency. B-chromosomes were found in 9 out of 45 collections studied. Collections from Assam (AS-59, AS-60) and Sikkim (S-21, S-24 and S- 29) had 1 or 2 B-chromosomes Nagaland collections (N-24, N-29 and N-44) had 1-3 B-chromosomes and the lone Tripura collection (T-17) possessed 2 B-chromosomes.

The presence of satellite on short arm of sixth chromosome was highest in maize strains of Tripura and Assam (Table 4) in comparison to those of Sikkim and Nagaland (40.0 and 42.0% respectively). Satellites were absent in both the strains from Arunchal Pradesh. Heterochromatic block near centromere on the long arm of chromosome 2 was observed with a very high frequency

TABLE 3: Summary of knob composition in northeastern Himalayan maize.

Region	No. of coll.	Mean knob No.	Range	Total knob position	Common knob position	New knob	Rare knob positions
Arunachal Pradesh	2	8.5	7-10	11	3L, 4L, 6S, 6Lb, 8La, 9ST	-	-
Assam	10	6.7	6-8	9	2La, 4L, 6S, 6Lb, 7L, 8la, 9ST	-	5L, 7ST
Sikkim	10	5.8	4-9	18	2La, 4L, 6S, 8La, 9ST	-	2S, 4S, 7ST, 8Lb
Meghalaya	10	5.9	4-8	16	2La, 4L, 6S, 6Lb, 7L, 8La, 9ST	2LT	1Sa, 1La, 5L, 6La, 8Lb.
Nagaland	10	7.1	5-9	18	2La, 4L, 6S, 6Lb, 81a, 9ST	K10, 9Lb	1Sa, 2S, 10La
Tripura	10	7.3	4-11	20	2La, 4L, 6S, 6lb, 8La, 9ST	K10, 1Lb, 2Lb	1Sa, 2S, 8Lb, 9La

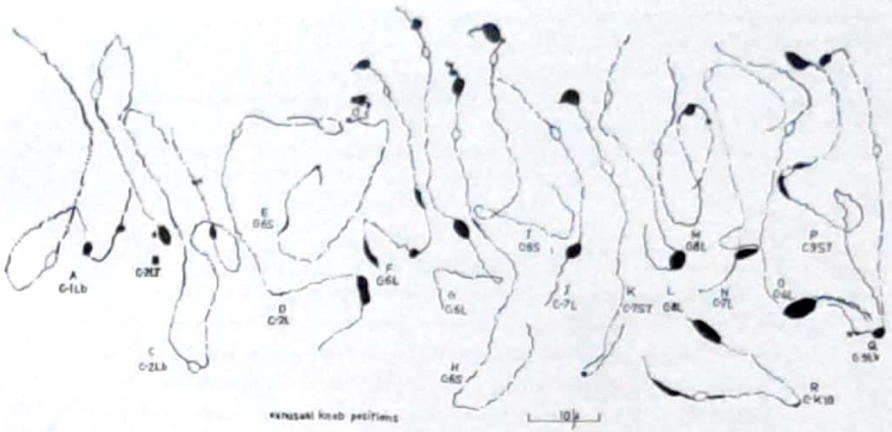

Fig. 1: Unusual positions and sizes of heterochromatic blocks /knobs in northeastern Himalayan maize. *A. 1Lb knob in T-7. *B. Large 2LT knob in M-240. *C. 2Lb knob in T-17. D. Very large 2La knob in S.P. Nepal. E. Satellites in tandem near 6S knob in N-44. F. Large heteerochromatic block terminating long arm of chromosome 6 in N- 35. G. Large 6Lb knob in S- 38. H. Twin 6S knob in S-55. I. Unusually large 6S knob in S- 21. J. Large size 7L knob in S- 27. K. Unusal shape and size of 7ST knob in A- 2. L. Very large 8La knob in S- 25. M. 8Lb knob n AS- 312. N. Very large 7L knob in N- 26. O. Unusually large sized 4L knob in T-20. P. Large 9ST knob in K-2. *Q. 9Lb knob in N- 52. R. K10 knob in N- 37. (* unusual new knob positions in Fig. 1-A,B,C & Q).

in the entire region (Table 4). It was present in all the strains studied from Meghalaya, Assam and Arunchal Pradesh, although in Tripura collections it was comparatively less frequent (76.9%). The Presence of knob in different geographical regions varied to a considerable extent. The average knob number, range, total knob positions and common knobs in different regions has been shown in Table 5. Most common knob forming positions in this area are 6 S, 9 ST, 2 La and 4 L. Presence of some new knob positions, viz., 1 Lb, 2 Lb, 2 Lt and 9 Lb, have been observed for the first time in different maize strains. Interestingly, all these new knob positions have been recorded on long arms of different chromosomes namely, chromosomes 1,2 and 9. All these knobs are small except the medium-sized knob at 2 LT. Two such new knobs at 1 Lb are present in 2 different maize strains from Tripura, namely, T-7 and T-17 respectively, whereas 2 LT knob was observed in M-240 collection from Meghalaya and 9 Lb knob in N-52 from Nagaland. The presence of these new knob positions in different regions of NEH suggests their adaptive value. They also indicate the uniqueness of NEH maize. Some unusual new knob positions at 7 L, 8 S, 8 L and 10 La positions in Himalayan primitives had been reported earlier also (Gupta & Jain 1971). In a series of reports on interspecific hybrids in maize, it has been observed (a) transposition of knobs from terminal to internal positions, (b) knob amplification and (c) interconversion of knob from heterochromatic condition to euchromatic condition and vice versa (Eubanks 1986, 1987, 1988). It has been suggested that knob DNA may change in alternate form of heterochromatin and euchromatin during the period of genomic shock. Origin of knob at 2 LT position in one of Meghalaya strains can be

explained as inversion or terminal deficiency but presence of 1 Lb, 2 Lb, and 9 Lb positions cannot be explained on the basis of deletion or unequal crossing over as suggested by earlier workers (Jotshi 1982, Pereira & Sachan 1984). Hence, the origin of new knob positions in NEH maize can be explained as genome's response to unusual climatic conditions after its introduction in NEH in prehistoric times. At the same time, presence of these new knob positions along with usual ones on the same arm of the chromosome suggests their adaptive value. Polymorphism and presence of new and unusually large knobs in some of the maize strains grown in this region have been shown in Fig. 1.

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INDUCED MUTANTS IN YELLOW SARSON

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SUMMARY

A wide range of macromutations were induced in 2 sub-varieties (TM-17, TM-21) of yellow sarson, Barassica campestris L.var. Sarson Prain by gamma rays, diethyl sulphate (dES), maleic hydrazide (MH) and a combination treatment of gamma rays and diethyl suplhate (dES). The different categories of macromutants isolated in both the sub-varieties presented significant advantageous traits like large pods with large seeds and certain seed type mutants like bold-seed mutant and tiny-seed type mutant. A comparative study of the mutants resulting due to different mutagenic treatments showed that the mutation frequency was random for different characters and varied within the treatments.

Key Words: Mutants, yellow sarson, mutagens.

INTRODUCTION

Among the various oil yielding crops grown in India, mustard occupies a prominent position. It is the major rabi oil seed crop of the country. The mustard plant especially yellow sarson is not popular in Andhra Pradesh and so attempts have not been made to select improved varieties that can adapt well to the local climatic conditions, to produce maximum yield. Hence, 2 sub-varieties of *Brassica campestris* L.var. Sarson Prain viz., TM-17 and TM-21 were selected for the present study. The present study has been directed to induce genetic variability by means of mutagen treatments to determine mutagenic efficiency and to screen the population for advantageous morphological mutants with desirable attributes.

MATERIALS AND METHODS

The seeds of the 2 sub-varieties of yellow sarson were treated with the physical mutagen: gamma rays with 3 different doses of 20 kR, 40 kR and 60 kR and chemical mutagens: diethyl sulphate (dES) and maleic hydrazide (MH) with 3 different concentrations of 0.5%, 1% and 1.5% and a combination treatment of gamma rays and dES. 250 seeds each were subjected to gamma rays and presoaked in distilled water for 4 h prior to sowing. Similarly, 250 seeds each were presoaked for 4 h in distilled water, prior to a 3 h treatment with the respective chemical mutagens and later washed thoroughly in distilled water before sowing in the field. The seeds pertaining to each treatment were sown in the randomized block design field (RBD) with 2 replications and grown to maturity. Plants were screened and a number of variants could be visually detected in the M_1 generation and isolated in the M_2 generation as mutants. A wide range of macromutations were observed in all the treatments (Tables 1, 2). Several mutants showed differences in pod characters and seed size,

RESULTS AND DISCUSSION

The frequency of various pod type mutants and seed type mutants produced are given in Tables 1 and 2 (Figs.1-3). The pod type mutants with the highest frequency were isolated from the combination treatments of 40 kR + 1% dES in TM - 17 and 600 kR + 1% dES in TM - 21.

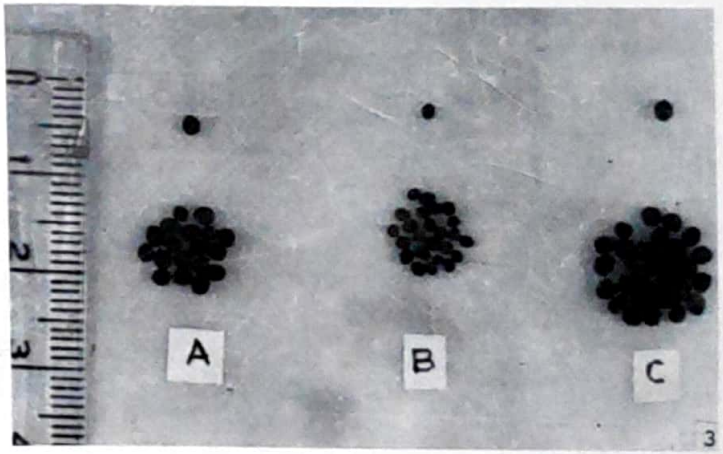
The highest frequency of bold seed type and small seed type mutants was recorded from 1% dES treatment and 40 kR gamma irradiation in case of TM - 17. In case of TM - 21, the bold seed type mutants and small seed type mutants were recorded from 1.5% MH and 40 kR + 1% dES combination treatment. The highest number of mutants were recorded from combination treatment of 40 kR + 1% dES in TM - 17 and 60 kR + 1% dES in TM - 21.

The overall mutation frequency ranged from 1.6% to 4.67% in TM - 17 and 1% to 5.32% in TM - 21. However, with different doses and concentrations of the mutagens used, combination treatments of gamma rays and diethyl sulphate (dES) induced the highest frequency in both the varieties, this was followed by dES, gamma rays and MH in TM - 17 whereas in TM - 21, it was followed by gamma rays, MH and dES.

All the pod type mutants and seed type mutants of TM - 17 and TM - 21 were further field tested (M_3) to assess their true breeding nature. All the mutants discussed here were found to breed true.

TABLE 1: Frequency and spectrum of macro-mutants in M_2 population in yellow sarson sub-var. TM-17.

Treatment	Population	Giant sized pod mutant	Abnormal shape pod mutant	Bold seed type mutant	Small seed type mutant	Total No. of mutants	% of mutants
Control	416	-	-	-	-	-	-
Gamma rays							
20 kR	395	1	1	2	4	8	2.02
40 kR	360	1	2	2	9	14	3.88
60 kR	325	2	1	3	1	7	2.15
Diethyl sulphate							
0.5%	390	1	1	1	4	7	1.79
1.0%	372	1	2	10	2	15	4.03
1.5%	341	2	3	2	3	10	2.93
Maleic hydrazide							
0.5%	396	3	1	1	3	8	2.02
1.0%	368	1	1	2	2	6	1.63
1.5%	350	2	2	4	4	12	3.42
Combination							
20 kR + 1% dES	353	2	1	3	3	9	2.54
40 kR + 1% dES	342	7	8	1	-	16	4.67
60 kR + 1% dES	336	4	4	1	1	10	2.97



Figs. 1-3: 1. Giant-sized pod mutant. 2. Blunt tip pod mutant. 3. (A) Control. (B) Small seed type mutant. (C) Bold seed type mutant.

Several reports were made by many workers claiming to have isolated advantageous as well as inferior mutants by utilizing physical and chemical mutagens on mustard (Jacob 1956, 1957, Mikaelson et al. 1968, Gustafsson et al. 1971, Zafar & Haq 1971, Fowler & Stefanson 1972, Kumar

TABLE 2: Frequency and spectrum of macro -mutants in M_2 population in yellow sarson sub-var. TM - 21.

Treatment	Population	Giantsized pod mutant	Abnormal shape pod mutant	Bold seed type mutant	Small seed type mutant	Total No. of mutants	% of mutants
Control	408	-	-	-	-	-	-
Gamma rays							
20 kR	382	2	3	4	1	10	2.61
40 kR	361	2	2	5	1	10	2.77
60 kR	332	3	3	4	2	12	3.61
Diethyl sulphate							
0.5%	392	2	1	3	2	8	2.04
1.0%	376	2	-	3	2	7	1.86
1.5%	339	1	1	2	1	5	1.47
Maleic hydrazide							
0.5%	398	1	2	1	-	4	1.00
1.0%	371	4	3	2	1	10	2.69
1.5%	352	1	1	10	3	15	4.26
Combination							
20 kR + 1% dES	362	1	2	4	4	11	3.03
40 kR + 1% dES	345	2	1	2	12	17	4.92
60 kR + 1% dES	338	7	8	2	1	18	5.32

& Das 1978, Badwal & Chaurasia 1979, Gomez Compocaesar 1980, Verma & Rai 1980a 1980b, Das & Rahman 1988).

However, reports concerning the successful production of varied viable mutants in mustard have also been published indicating a trend similar to the present study. They were the pressed pod mutant (Rai 1957), coloured pod type mutant (Nayar 1968), bold-seeded mutant (Singh & Srivastava 1975), and seed coat colour mutant (Varma & Rai 1980b).

The advantageous mutants recorded in the present study could be further characterized by biochemical techniques and used in breeding programmes to evolve new improved varieties.

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GENETICS OF CERTAIN SILK YIELD TRAITS IN *ANTHERAEA MYLITTA* - A DIALLEL ANALYSIS

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SUMMARY

Diallel analysis involving 8 x 8 (8 diverse genotypes and their 28 non-reciprocal crosses) of tasar silkworm *A. mylitta* D. was conducted for estimating the gene effects and the diallel analysis was carried out by Griffing method for 4 yield component traits. Both additive and non-additive genetic variances were found to be significant for different traits. Recurrent selection procedure for maximum exploitation of the prevalent gene action is proposed for evolving high yielding tasar silkworm varieties.

Key Words: *Antheraea mylitta*, diallel analysis, gene action, selection.

INTRODUCTION

The knowledge of gene action is essential for efficient breeding procedure leading to rapid improvement. Limited information on gene action in *A.mylitta* is available (Siddiqui et al. 1988). Hence, an experiment was conducted with 8 genotypes using 8 x 8 diallel for estimating the nature and magnitude of gene action.

MATERIALS AND METHODS

Nonreciprocal crosses in all possible combinations were made among 8 inbreed lines of *A.mylitta* viz. R_{57}, GE_1, GE_2, GE_3, $Nagri_1$, $Nagri_2$, ymg and Laria 8 at Central Tasar Research and Training Institute, Piskanagri, Ranchi. The distinguishing features of the inbreeds are presented in Table 1.

Eight parents and 28 hybrids were reared with 5 replication of each cross and parent in randomized block design layout. Larvae from each disease free laying were considered for one replication. Reciprocal crosses were not made in the present investigation because Jolly et al. (1969, 1972) and Bardaiyar et al. (1976) reported non-significant reciprocal differences in *A.mylitta*. Observations were recorded randomly for equal number of samples from each replication for absolute silk yield, cocoon weight, cocoon shell weight and silk ratio. Computation was made on the mean values of each replication.

Analysis of variance of the data was done by the method detailed by Panse & Sukhatme (1961). The genetic analysis of the diallel data was done by the procedure described by Jinks (1954) and Hayman (1954 a,b). Heritability was carried out according to the method of Mather & Jinks (1971).

TABLE 1: Parental inbreeds and their main features.

Parent	Inbreed lines	Main features
1	R-57	Derived from sal based ecorace *Raily*, green larvae, grey cocoon, bivoltine, higher shell weight, high yield.
2	GE-1	Green larvae, grey and yellow cocoon, bivoltine, higher shell weight, higher fecundity, moderate yielder. A selection from *Daba* ecorace.
3	GE-2	Green larvae, grey and yellow cocoon, bivoltine, higher shell weight, moderate yielder. A selection from Daba.
4	GF-3	Green larvae, grey and yellow cocoon, bivoltine, higher shelll weight, higher yielder, derived from *Daba* ecorace.
5	$NAGRI_1$	Green larvae, grey cocoon, bivoltine, higher shell weight, higher yielder. A selection from (*Sukinda* x *Dada*) x (*Laria* x *Daba)*
6	$NAGRI_2$	Green larvae, grey cocoon, bivoltine, higher shell weight, higher yielder. A selection from (*Sukinda* x *Dada*) x (*Laria* x *Daba)*
7	YMG	Yellow larvae, grey and yellow cocoon, bivoltine, moderate shell weight, low yielder. A selection from *Daba*
8	LARIA-8	Derived from a sal based ecorace Laria, higher yielder with high shell weight, bivoltine in nature.

RESULTS AND DISCUSSION

The analysis of variance (Table 2) showed significant differences between the genotype and the progenies for all characters indicating the diversity of the chosen genetic components of the parent. Component analysis was done to observe the contribution of additive and non-additive gene effects (Table 3).

The additive component : The estimates of additive genetic variance (D) were highly significant for all the 4 traits.

The dominance component : The estimates of dominance components H^1 and H^2 were significant at 1% level for all the traits. The overall dominance effect of heterozygous loci (h^2) was also significant.

TABLE 2: ANOVA for 4 yield characters in 8 x 8 diallel set of crosses of *A. mylitta*.

Source	D.F.	Absolute silk yield (g)	Cocoon weight (g)	Shell weight (g)	Silk ratio (%)
Replication	3	667.30**	0.05	0.01	0.41
Treatment	35	27951.61**	1.01**	0.75**	28.33*
Parent	7	21652.68**	0.51**	1.06**	48.52**
F_1	27	27964.19**	0.79**	0.63**	21.44**
Parent F_1	1	71893.49**	10.25**	0.08**	73.08**
Error	105	2962.34	0.03	0.03	1.61

** Significant at P = 0.01

TABLE 3: Genetic parameters for absolute silk yield and its components in *A. mylitta*.

Components	Absolute silk yield (g)	Cocoon weight (g)	Shell weight (g)	Silk ratio (%)
D	4640.10*	0.11*	0.25**	11.73**
F	349.49	-0.14	-0.03	1.37
H_1	18387.05*	0.42**	0.15**	5.50**
H_2	16158.14**	0.34**	0.13**	4.51**
h^2	8511.00**	1.25**	0.25**	8.81**
E	766.31	0.00	0.00	0.39

* Significant at P = 0.05; ** Significant at P = 0.01.

The component F : This is measure of covariance between additive and dominance effects and can either be positive if u (the proportion of dominant or positive genes) is greater than v (the proportion of recessive or negative genes) or negative if u<v. In the present investigation, F was positive for absolute silk yield and silk ratio indicating the excess of dominant positive genes whereas F was negative for cocoon weight and shell weight indicating that these traits were carrying recessive and negative genes.

Ratio of the genetic components : The ratio and difference of the components have been presented in Table 4.

The estimates of degree of dominance H1 were higher than for cocoon weight and absolute silk yield indicating over dominance for these traits. However, partial dominance was observed for shell weight and silk ratio as the value of H^1 is less than D.

The ratio $H^2/4H$ denoted the mean product of Ui and Vi averaged over all the parents of a diallel set of crosses. When u and v are symmetrically distributed i.e., v = u = 0.5, the ratio will give

TABLE 4: Proportions and differences in the genetic components.

	Absolute silk yield (g)	Cocoon weight (g)	Shell weight (g)	Silk ratio (%)
(H_1/D)	6519.52	1.88	0.77	0.68
$H_2/4H_1$	0.21	0.20	0.21	0.20
$\frac{(4\,DH_1) + F}{(4\,DH_1) - F}$	1.03	0.52	0.84	1.21
h^2/H_2	0.52	3.67	1.91	1.95
H_1-H_2	2228.91	0.07	0.02	0.99
Heritability: Broad sense	0.81	0.62	0.79	0.78
Narrow sense	0.40	0.57	0.43	0.10
Genetic advance in % of mean	43.19	4.49	21.85	4.68

the value of $H^2/H^1 = 0.25$. In the present study all the traits exhibited asymmetrical distribution of positive and negative homozygous loci as the value is less than 0.25.

The ratio [(4DH1) + F]/[(4DH1) - F], which gives the relative value of dominant and recessive genes, for absolute silk yield was about 1 (1.0385) indicating the symmetry in gene distribution amongst the parents. While other traits showed asymmetry in gene distribution as the value was different from the unity. This further confirms the conclusion drawn in the above paragraph regarding asymmetry of the dominant and recessive genes for the traits under investigation. The difference H1-H2 for all the traits, was far from zero supporting the findings which indicate asymmetrical distribution of the positive and negative genes in the parents.

The ratio h^2/H^2, which is an approximate measure of sets of genes showing dominance was higher than 1 for cocoon weight, shell weight and silk ratio indicating that these traits were under the control of 2-3 genes. Contrary to this, the value of h^2/H^2 was less than 1 for absolute silk yield which indicates that this trait is under the control of at least one set of genes.

Heritability : Based on genetic components, broad and narrow sense heritabilities were calculated. Broad sense heritabilities ranged from 0.6126 to 0.8101 being highest for absolute silk yield (0.81) followed by shell weight (0.97), silk ratio (0.78) and lowest for cocoon weight (0.62). The estimates of narrow sense heritabilities in respect of all traits viz., absolute silk yield, cocoon weight, shell weight and silk ratio were 0.40, 0.57, 0.43 and 0.10 respectively.

Genetic gain : Genetic gain was worked out on the basis of narrow sense heritability. The genetic gain was moderate 49.13% and 21.85% for absolute silk yield and shell weight respectively while low gain was recorded for cocoon weight and silk ratio.

Broad and narrow sense heritabilities were high for absolute silk yield and shell weight and the predicted genetic gain was moderate for these characters. High heritability was thus associated with moderate genetic gain indicting that the additive gene effects contributed a major part in the expression of these traits. Broad sense heritability was too high for cocoon weight and silk ratio but narrow sense heritability were moderate for cocoon weight and low for silk ratio. Similarly genetic gain was found to be low for cocoon weight and silk ratio. Thus narrow sense heritability and genetic gain could suggest rather limited progress in obtaining better segregants in selected families.

In any crop improvement programme, a superior homozygous line is essential to exploit only the additive or fixable gene effects. Hence, knowledge of relative magnitude of the genetic variance controlling the different traits is of great importance. In the present investigation, both additive and non-additive genetic variances are prevalent for silk yield and its traits viz., cocoon weight, shell weight and silk ratio as D, H1 and H2 were found to be positive and significant. Dominance component H1 was higher than D for absolute silk yield and cocoon weight indicating the degree of dominance to be in the range of over dominance. On the contrary shell weight and silk ratio showed partial dominance.

The proportion H1/D was more than 1 for absolute silk yield and cocoon weight indicating that non-additive components were higher for these traits. Similarly, magnitude of additive components was higher as compared to non-additive components for shell weight and silk ratio as (H1/D) was less than 1.

The choice of breeding methodology depends upon the nature and magnitude of gene action. For full exploitation of the additive gene effects intercrossing of the requisite segregants is desirable. The presence of a significantly high proportion of dominance effects stress the need for maintaining certain degree of heterozygosity. Under such a situation where additive and non-additive gene effects are important some form of recurrent selection programme should be adopted for greater improvement.

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KARYOTYPIC STUDIES IN SOME SPECIES OF *TRIFOLIUM*

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SUMMARY

Chromosome numbers were reported for *Trifolium glanduliferum* Boiss., *T. gracilentum* Tor. & Gray, *T. micranthum* Viv., *T. bilineatum* Fresen. and *T. caucasicum* Tausch. Karyotypic studies were made in all except *T. caucasicum*. All the species were found to be diploids with 2n=16 except *T. caucasicum* which was a hexaploid with 2n=48. Mostly, in all species of *Trifolium* chromosomes with median and nearly median or submedian centromeres were observed.

Key Words: *Trifolium*, hexaploidy, karyotype.

INTRODUCTION

The genus *Trifolium* is a member of the subfamily Papilionatae of the family Leguminosae. There are about 248 known species of *Trifolium* distributed throughout the world of which only 20 species are cultivated. Some species of *Trifolium* viz., *T. repens, T. pratense, T. incarnatum* and *T. hybridum* are highly valued as forage crops. The chromosome numbers in some *Trifolium* species have been reported (Wexelson 1928, Pritchard 1962, 1969, Britten 1963, Anderson et al.1972, Giri et al. 1981, Taylor et al. 1993). Chromosome morphology of *Trifolium* species has also been studied (Pritchard 1967, Chen & Gibson 1971, Giri et al. 1981).

The main objectives of the present work were to determine the chromosome numbers and to study the karyotypes of some species of *Trifolium*.

MATERIALS AND METHODS

Seeds of *Trifolium glanduliferum* Boiss., *T. gracilentum* Tor. & Gray, *T. micranthum* Viv., *T. bilineatum* Fresen. and *T. caucasicum* Tausch. were received from the Department of agronomy, University of Kentucky, U.S.A. Source and origin of seeds are shown in Table 1. For mitotic studies the root tips of 1-2 cm long were pretreated in 8-hydroxyquinoline for 5 h at 18°C. Pretreatment was done at 11.30 A.M. These root tips were fixed in acetic-alcohol (1:3). The root tips were then stained in a mixture of 1N HC1 and orcein (1:9) and kept in this stain for 24 hours and squashed in 45% acetic acid. Chromosome number count was made. Camera lucida drawings were made at 10x100 magnification.

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TABLE 1: Accession number, origin and somatic chromosome numbers of *Trifolium* species.

Species	Kentucky No.	USDA Plant Intro. No.	Source of origin	Sat chrom.	Chrom. No. (2n)
T. bilineatum	S-226-1	516754	Ethiopia	2	16*
T. caucasicum	S-150-2	516276	USSR	ND	48*
T. glanduliferum	S-129-1	294666	Turkey	2	16*
T. gracilentum	S-135-3	516319	USA	2	16*
T. micranthum	S-153-3	249756	Greece	ND	16

* Reported for the first time; ND Not determined.

Chromosome counts were made in all 5 species but the karyotypes have been analysed only in 4 of them (*T. glanduliferum, T. gracilentum, T. micranthum* and *T. bilineatum*). The chromosome categorization was made as suggested by Adhikary (1974).

RESULTS AND DISCUSSION

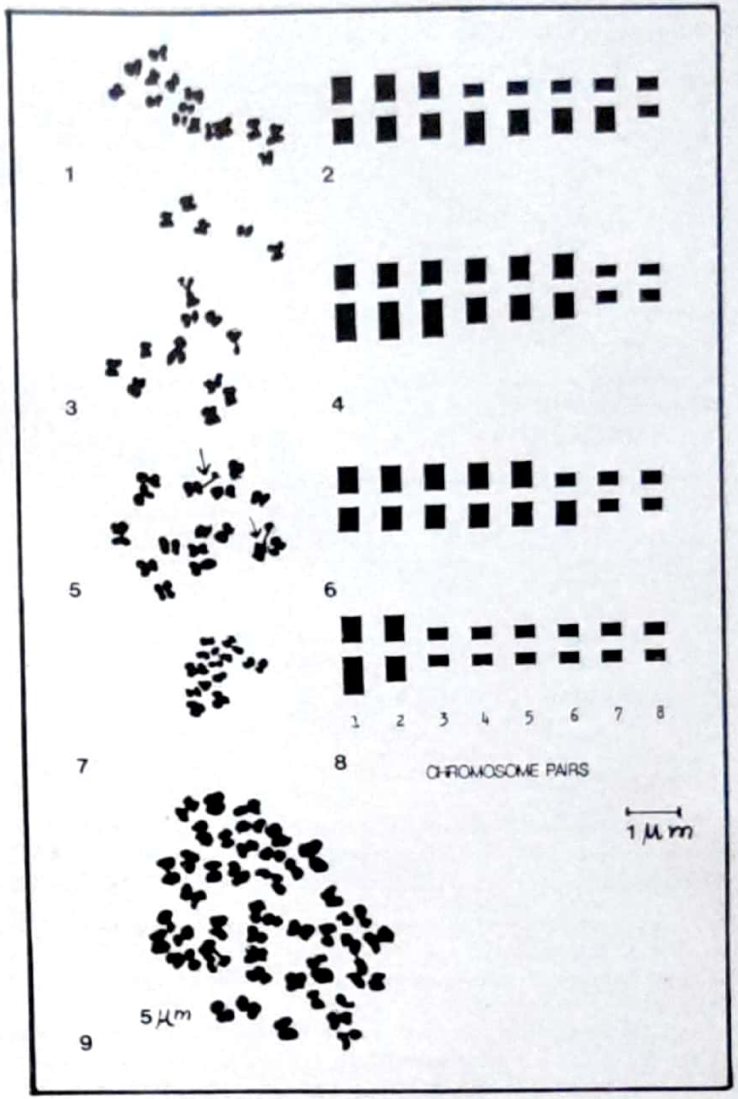
Chromosome numbers of *T. glanduliferum, T. gracilentum, T. bilineatum* and *T. micranthum* were found to be 2n=16 (Figs. 1-8). However, *T. caucasicum* has the chromosome number 2n=48 (Fig. 9). These are the first reports of chromosome numbers of the species except for *T. micranthum*. Kliphuis (1962) had determined the chromosome number of *T. micranthum* as 2n=16.

In *T. bilineatum* (Sect. *Lotoidea)* 2 types of chromosomes with median and nearly submedian centromeres were observed (Fig. 6). A pair of satellited chromosomes was also observed. The total length of chromosome ranged from 0.62 to 1.24 μm. The total chromatin length was 16.74 μm (Table 2).

In *T. gracilentum* (Sect. *Lotoidea)* 2 types of chromosomes with median and nearly median centromeres were observed (Fig.4). The chromosome length ranged from 0.62 to 1.53 μm. A pair of satellited chromosomes was also seen. Total chromatin length was 19.10 μm (Table 3).

TABLE 2: Karyotypic details of somatic chromosomes of *T. bilineatum*.

Chrom. pairs	Long arm (μm)	Short arm (μm)	Total length (μm)	Arm ratio (S/L)	centromere
1	0.62	0.62	1.24	1.00	m
2	0.62	0.62	1.24	1.00	m
3	0.62	0.62	1.24	1.00	m
4	0.62	0.62	1.24	1.00	m
5	0.62	0.62	1.24	1.00	m
6	0.62	0.31	0.93	0.50	nsm
7	0.31	0.31	0.62	1.00	m
8	0.31	0.31	0.62	1.00	m



Figs. 1-9: Somatic chromosomes and idiograms of *Trifolium* species. 1,2. *T. glanduliferum*, 3,4. *T. gracilentum*, 5,6. *T. bilineatum*, 7,8. *T. micranthum*, 9. *T. caucasicum*.

TABLE 3: Karyotypic details of somatic chromosomes of *T. gracilentum*

Chrom. pairs	Long arm (μm)	Short arm (μm)	Total length (μm)	Arm ratio (S/L)	Centromere
1	0.91	0.62	1.53	0.68	nm
2	0.91	0.62	1.53	0.68	nm
3	0.91	0.62	1.53	0.68	nm
4	0.62	0.62	1.24	1.00	m
5	0.62	0.62	1.24	1.00	m
6	0.62	0.62	1.24	1.00	m
7	0.31	0.31	0.62	1.00	m
8	0.31	0.31	0.62	1.00	m

In *T. glanduliferum* (Sect. *Paramesus*) 2 types of chromosomes with median and nearly submedian centromeres were observed (Fig. 2). Total length of chromosomes ranged from 0.62 to 1.24 μm. A pair of satellites was seen. Total chromatin length was 16.48 μm (Table 4).

TABLE 4: Karyotypic details of somatic chromosomes of *T. glanduliferum*

Chromo. pairs	Long arm (μm)	Short arm (μm)	Total length (μm)	Arm ratio (S/L)	Centromere
1	0.62	0.62	1.24	1.00	m
2	0.62	0.62	1.24	1.00	m
3	0.62	0.62	1.24	1.00	m
4	0.80	0.31	1.11	0.38	nsm
5	0.62	0.31	0.93	0.50	nsm
6	0.62	0.31	0.93	0.50	nsm
7	0.62	0.31	0.93	0.50	nsm
8	0.31	0.31	0.62	1.00	m

In *T. micranthum* (Sect. *Chronosemium*) 2 types of chromosomes with median and nearly median centromeres were observed (Fig. 7). Satellited chromosomes were not seen. The total length of the chromosome ranged from 0.62 to 1.53 μm. Total chromatin length was 12.98 μm (Table 5).

In *T. caucasicum* (Sect. *Trifolium*) the somatic chromosome number is 2n=48 (Fig.9) It is an hexaploid with the base number of x=8. Karyotypes of the above species of *Trifolium* were analyzed for the first time. The somatic chromosome number of *T. micranthum* (as *T. filliforme*) with 2n=16 was in agreement with the previous report of Karpechenko (1925). In all species examined here, the chromosomes with median and nearly median or submedian centromere were observed. The total chromatin length was maximum in *T. gracilentum* (19.10 μm). In the karyotype of *Trifolium* there is not as much difference in the type of chromosome than in other species. These can be considered as more primitive. In some cases the chromosomes are nearly median types and

TABLE 5: Karyotypic details of somatic chromosomes of *T. micranthum*.

Chrom. pairs	Long arm (μm)	Short arm (μm)	Total length (μm)	Arm ratio (S/L)	Centromere
1	0.91	0.62	1.53	0.64	nm
2	0.62	0.62	1.24	1.00	nm
3	0.31	0.31	0.62	1.00	m
4	0.31	0.31	0.62	1.00	m
5	0.31	0.31	0.62	1.00	m
6	0.31	0.31	0.62	1.00	m
7	0.31	0.31	0.62	1.00	m
8	0.31	0.31	0.62	1.00	m

with a pair of satellited chromosomes. Specialized ones have smaller chromosomes (Swanson 1965). However, *T. micranthum* has more number of median and short types of chromosomes.

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EFFECT OF MONOCROTOPHOS ON ROOT TIP CELLS OF *ALLIUM CEPA* AND *A. SATIVUM*

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SUMMARY

The cytogenetic and biochemical effects of organophosphorous systemic insecticide, monocrotophos were studied in *Allium cepa* and *A. sativum*. The analysis of root tip cells of treated plants revealed chromosomal aberrations. There was a direct relationship between the frequency of aberrations and insecticides. treatment. Besides, various biochemical parameters were analysed and found that monocrotophos interferes with many metabolic activities. The results indicate that monocrotophos has cytotoxic properties and even acts as a mutagen.

Key Words: Cytotoxic, biochemical, monocrotophos, *Allium cepa*, *A. sativum*.

INTRODUCTION

In an agricultural country like India, there is a widespread and liberal use of insecticides to increase crop production. Monocrotophos (dimethyl (E)-1-methyl-2-methyl carbomyl vinyl phosphate) is one of the widely used broad spectrum organophosphorous insecticide. It is popular insecticide because of its systemic and contact action against a wide range of pests including mites, sucking insects, leaf eating beetles, boll worms and other larvae. However, many of these compounds and their breakdown products are known alkylating agents of DNA (Bedford & Robinson 1972). Studies of various insecticides have shown that they do cause genetic damages (Amer & Ali 1986, Adam et al. 1990, Pratibha Devi et al. 1991). A continuous assessment of genetic damage from limited exposure to organophosphorous insecticides is essential (Degraeve et al. 1984). Not much report is available with regard to the effect of monocrotophos. The present work was undertaken with a view to assess the cytotoxic and biochemical effects of this insecticide on root tip cells of *Allium cepa* L. and *A. sativum* L.

MATERIALS AND METHODS

Allium cepa and *A. sativum* were chosen as the plant materials. The bulbs were grown in water. When the root tips reached a length of 2 cm, they were transferred to insecticide solution with different concentrations of 0.1%, 0.2% 0.3%, 0.4%, and 0.5%. After 8 h treatments, the root tips were excised, washed, fixed in Carnoy's solution and preserved in absolute alcohol, until further use. Squash preparations using acetocarmine stain were made to observe chromosomal abnormalities. Sunderland & Mcleish's (1961) method was followed for the estimation of nucleic acids. Total organic nitrogen and protein content were estimated using microkjeldhal's method (Anonymous 1965). Buffer soluble protein was estimated using Lowry et al. (1951) method. More & Stein's (1949) method was used to estimate total free amino acid.

RESULTS AND DISCUSSION

There was a great reduction in mitotic index as the concentration of insecticide increased (Table 1). This may be due to the inhibition of DNA synthesis (Schneiderman et al. 1971). Monocrotophos produced various types of mitotic abnormalities such as clumping, stickiness, C-metaphase, fragments, laggards and bridges (Table 1). In *A. cepa* highest frequency of laggards (3.54%) and bridges (2.18%) at anaphase were seen in 0.5% concentration. In *A.sativum*, highest frequency of laggards (2.42%) at anaphase were seen in 0.5% concentration. In *A.cepa*, there was a steep rise in the total percentage of abnormal cells (24.85%) at 0.5% concentration. Alkylating

TABLE 1: Chromosomal abnormalities at various concentrations of monocrotophos in *Allium cepa* and *A. sativum*.

Species	Concentration	No. of cells observed	Mitotic index (%)	Percentage of cells showing abnormality
Allium cepa	0.0	5215	25.83	0.37
	0.1	8227	14.77	4.44
	0.2	10245	11.59	7.50
	0.3	12310	8.17	11.93
	0.4	16415	5.78	13.40
	0.5	18718	4.58	24.85
A. sativum	0.0	4325	28.09	0.25
	0.1	4668	23.50	2.64
	0.2	5417	18.00	7.39
	0.3	7819	10.58	10.64
	0.4	9785	7.31	12.73
	0.5	10116	6.54	16.34

agents are known to cause chromosomal breakage by binding to DNA regions rich in G-C pairs (Cummins 1969). Anaphase bridges are formed due to unequal exchange of chromosomes (Pronoti & Raghuvanshi 1986). Many workers have reported similar results in other materials (Kaur & Grover 1985, Padmaja et al. 1986). Of the 2 species studied greater amount of abnormality was recorded in *A.cepa* than in *A.sativum*.

With regard to biochemical parameters, in general, there was a dose dependent relationship. As the concentration increased there was a decrease in DNA, RNA, buffer soluble protein and amino acid (Table 2). The maximum reduction was seen in 0.5% concentration in both the species. As the DNA and RNA decrease, the protein and amino acid contents also decrease indicating a direct relationship between the two. Ignacimuthu & Babu (1988) pointed out that the decrease in mean DNA content per cell is directly proportional to the decrease in the rate of RNA turn over. Similar observations have been made by Santakumari & Stephen (1988). The reduction in buffer soluble protein and total free amino acid indicates that monocrotophos interferes with nitrogen metabolism as observed by Rost & Reynold (1985) in *Pisum sativum*.

TABLE 2: Biochemical effects at different concentrations of monocrotophos in *Allium cepa* and *A. sativum*.

Species	Concentration (%)	DNA (mg/g fresh wt)	RNA (mg/g fresh wt)	Buffer soluble protein (mg/g fresh wt)	Amino acid (mg/g fresh wt)
		Mean ± S.D	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Allium cepa	0.0	262.81 ± 1.06	586.50± 1.50	185.33 ± 0.74	28.60 ± 0.28
	0.1	231.81 ± 1.19	568.13 ± 0.88	176.00 ± 0.65	15.53 ± 0.34
	0.2	217.56 ± 1.19	495.94 ± 1.19	153.33±1.15	13.87 ± 0.50
	0.3	214.24 ± 0.24	483.001 ± 2.00	131.07 ± 3.04	8.97 ± 0.37
	0.4	172.04 ± 0.88	169.50 ± 1.75	63.07±0.02	6.00 ± 0.33
	0.5	107.52 ± 0.65	316.50±2.50	32.67±1.36	4.27 ± 0.38
A. sativum	0.0	392.36 ± 2.12	757.50±1.50	205.07 ± 2.18	32.20 ± 0.16
	0.1	373.38±2.38	715.25±2.75	196.53 ±2.78	20.87 ± 0.09
	0.2	355.38±3.25	693.20 ± 3.95	190.67 ± 2.13	16.03 ± 0.21
	0.3	39.01 ± 0.24	614.50±0.75	149.87 ± 5.56	15.20 ± 0.16
	0.4	199.94 ± 0.19	481.63±0.88	133.33± 1.15	9.40 ± 0.16
	0.5	135.50 ± 2.38	352.50±1.50	120.80 ± 0.86	8.27 ± 0.25

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CYTOLOGICAL INVESTIGATIONS ON *VERBENA HYBRIDA*

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SUMMARY

Meiotic screening of 4 cultivars of *Verbena hybrida* Hort. viz., Purple White Eye (n=5), Pinkish Scarlet (n=5), Shade of Rose (n=5) and Dwarf Sparkle (n=5) and karyotype analysis of 2 cultivars, Purple White Eye (2n=10) and Pinkish Scarlet (2n=10) have been performed. Both the cultivars showed asymmetric karyotypes. The possible cytogenetic basis for the variation in the colour of the florets of the cultivars is discussed.

Key Words : *Verbena*, karyotype, meiosis

INTRODUCTION

Verbena comprises a set of lovely half-hardy ornamentals cultivated chiefly for their long peduncled, brightly coloured inflorscence (Bailey 1928). *V. hybrida* Hort. the 'florist Verbena', is a summer bedding species with many cultivars. It is extensively grown as a garden ornamental in this part of the country. Here, an account of the possible cytogenetic basis for the wide spectrum of colour variations noted in the florets of 4 cultivars of *V. hybrida* is given.

MATERIALS AND METHODS

The root tips after pre-treatment with 8-hydroxyquinoline and the flower buds were fixed in Carnoy's fluid (1:1:3 chloracetic- ethanol). Smears of anthers and squashes of the root tips were made in 2% acetocarmine. Pollen fertility is determined by the stainability of pollen in 1:1 acetocarmine-glycerine mixture. In each case an average of 25 random readings were taken.

RESULTS AND DISCUSSION

V. hybrida var. Purple White Eye

The violet-flowered variety showed 5 bivalents at diakinesis (Fig.1). The chromosome number is 2n=10 (Fig.2). The karyotype is asymmetric. The length of the chromosomes varies from 2.01 μm to 4.33 μm (Table 1). Pollen sterility is estimated to be 60%.

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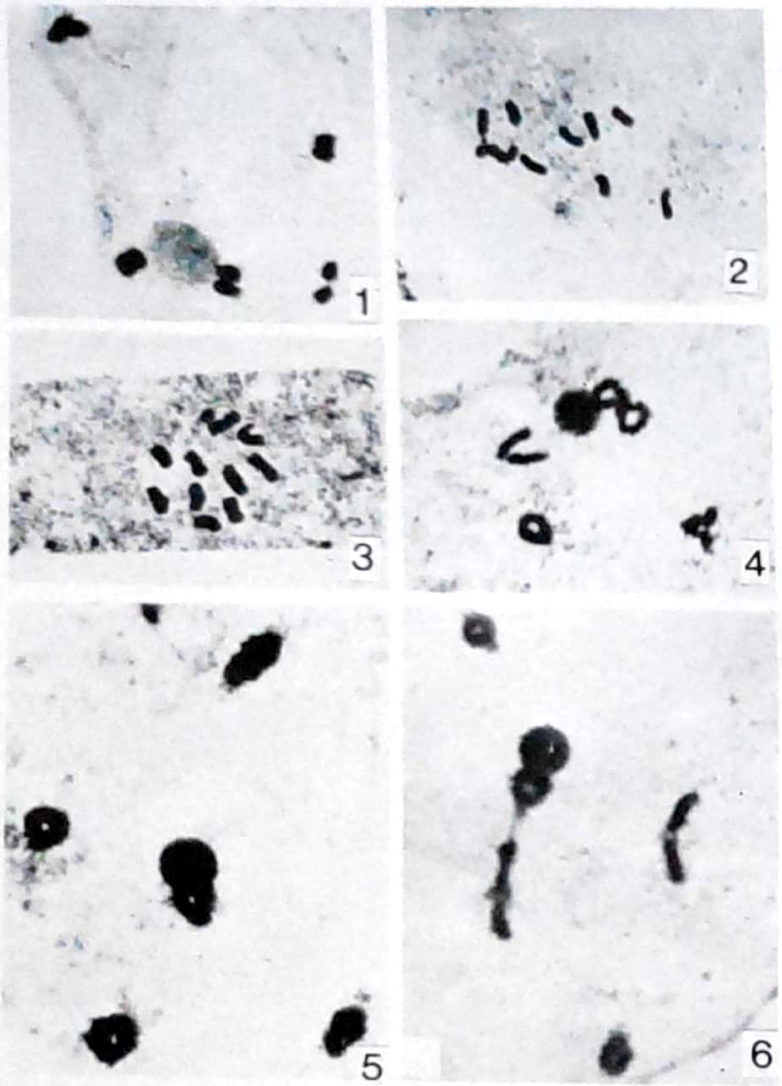

Figs. 1-6: Chromosome numbers of *V. hybrida* (all x 1200) 1 & 2. Var. Purple White Eye. 1. Diakinesis (n=5). 2. Somatic chromosomes. 3 & 4. Var. Pinkish Scarlet. 3. Somatic chromosomes. 4. Diakinesis (n=5). 5. Var. Shade of Rose. Diakinesis (n=5). 6. Var. Dwarf Sparkle. Diakinesis (n=5).

TABLE 1: Details of diploid set of chromosomes in *V. hybrida* var. Purple White Eye.

Chromosome pair	Chromosome length (in μm)		Total (in μm)	Arm ratio	Centromere
	Short arm	Long arm			
1	2.00	2.33	4.33	1.16	sm
2	2.00	2.33	4.33	1.16	sm
3	1.00	2.67	3.67	2.67	st
4	1.33	1.67	3.00	1.25	st
5	1.00	1.01	2.01	1.01	m

***V. hybrida* var. Pinkish Scarlet**

This reddish-flowered variety at diakinesis showed one ring-shaped and 2 rod-shaped bivalents and one 8-shaped tetravalent (Fig.3). The somatic chromosome number is 2n = 10 (Fig.4). The karyotype is heterogeneous. The chromosomes ranged in length from 3 μm to 4.33 μm (Table 2). Pollen sterility is 61.7%.

TABLE 2: Details of diploid chromosome set in *V. hybrida* var. Pinkish Scarlet.

Chrmosome pair	Chromosome length (in μm)		Total (in μm)	Arm ratio	Centromere
	Short arm	Long arm			
1	2.00	2.33	4.33	1.16	sm
2	2.00	2.33	4.33	2.00	st
3	1.30	1.67	3.00	1.26	m
4	1.00	2.00	3.00	2.00	st
5	1.00	2.00	3.00	1.16	sm

***V. hybrida* var. Shade of Rose**

The florets are pink. At diakinesis, 5 ring-shaped bivalents were noticed (Fig. 5).

***V. hybrida* var. Dwarf Sparkle**

The inflorescence is deep magenta in colour. The pollen mother cells showed 2 rod-and 3 ring-shaped bivalents at diakinesis (Fig.6). Pollen sterility is 58.6%.

In 'Moss Verbena' the extra chromosomes of hypotriploids and trisomics induce larger flower size and profuse flowering (Arora 1977, 1978). The significant morphological changes have been associated with trisomy in a number of *Datura* species (Blakeslee 1922), *Nicotiana* (Rick & Barton 1954), *Corchorus* (Iyer 1968). However, genetic dissimilarity in a set of species belonging to a particular group may or may not involve any change in the number of chromosomes. Generally, such qualitative differences between the genomes of the different cultivars of the same species can be ascribed to many factors of which translocation and inversion predominate. But, here the wide spectrum of variations noticed in the flower colour of the 4 cultivars of *V. hybrida* having same ploidy status (2n=10) is attributed to the base changes in the genetic material which accounts for

their ancestral hybrid origin. Such a genetic diversity steadily boosts up the effect of factors promoting recombination.

The 2 varieties of *V. hybrida* showed heterogeneous karyotypes. The karyological differences between the 2 cultivars worked out here reveal that a series of cryptic changes might have occurred in the karyotypes in the long course of evolution, which finally resulted in the alteration of the visible appearance of the chromosomes. This is evidenced by the formation of rod-shaped and interestingly 8-shaped multivalents at diakinesis in *V. hybrida* var. Pinkish Scarlet.

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STUDIES ON OVARIAN POLYTENE CHROMOSOMES OF *ANOPHELES SUBPICTUS* AND *A. VAGUS*

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SUMMARY

The ovarian polytene chromosomes of *Anopheles subpictus* and *A. vagus* are described. The distinct and well-banded chromosomes prepared from the semigravid females revealed a considerable homology in the banding pattern of the X chromosomes. However, such a similarity has not been observed in respect of the autosomes. In addition, the chromosome complement of *A. vagus* is characterised by the presence of a few asynaptic regions.

Key Words : *Anopheles subpictus, A. vagus,* Polytene chromosomes.

INTRODUCTION

Anopheles subpictus and *A. vagus* (Diptera : Culicidae) belong to series *Pseudomyzomia* of the subgenus *Cellia*. They occur sympatrically and widely distributed in the oriental region. However, morphologically, they differ only in minor features. The high density of *A. subpictus* in villages in India poses a grave vector problem. Atmoesdjone & Dennis (1977) found this species naturally infected with the filarial worm, *Wuchereria bancrofti* in Flores, Indonesia (White 1979). But *A. vagus* plays no major part in transmitting the disease. In view of these, a comparative cytological studies of these 2 species are of interest. The polytene chromosomes of *A. subpictus* obtained from salivary gland cells (Narang et al. 1973, Seetharam & Chowdaiah 1974) and their karyotypes were described (Aslamkhan & Baker 1969, Avirachan et al. 1969, Narang et al. 1972). However, in *A. vagus*, barring some preliminary reports (Kitzmiller 1976), no detailed cytological studies have been made. Since the discovery of a well defined polytene chromosomes in the ovarian nurse cells of mosquitoes has proved itself as a major advance in the mosquito cytogenetic studies (Coluzzi & Kitzmiller 1975), an attempt has been made here to study the chromosomes of *A. subpictus* and *A. vagus*.

MATERIALS AND METHODS

The material utilized in the present study comprises the wild caught strains of *A. subpictus* and *A. vagus*. The specimens were collected from the cowsheds of a village lakshmipura near Bangalore City. Since the efforts made to colonize these species in the laboratory proved futile, field collected semigravid females were utilized for the study. The

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ovaries dissected from the females collected early in the morning yielded results. Slides were prepared according to the standard method for anophelines by French et al. (1962). All observations and photomicography were carried out with a Ziess phase contrast microscope. The photomaps were made according to the method followed by Stalker (1964).

OBSERVATIONS

The polytene chromosome complements prepared from the adult ovarian nurse cells of *A. subpictus* (Fig.1) and *A. vagus* (Fig.2) reveal that each complement consists of 3 pairs of chromosomes : a short X, and 2 pairs of longer autosomes. The X chromosomes generally isolated in squash preparations, while the remaining 4 autosomal arms showed a tendency to associate in pairs in agreement with the nomenclature (2R with 2L and 3R with 3L). These chromosomes easily correspond with the subtelocentric X and submetacentric autosomes of metaphase chromosome complements described by Avirachan et al. (1969). Because of the several similarities in banding pattern with the previously described (Kitzmiller, 1976) members of the subgenus *Cellia*, the same numbering system has been employed for the present polytene chromosome photomaps (Figs 3,4). The X chromosome contains zones 1-6; 2R zones 7-19, 2L zones 20-28; 3R zones 29-37 and 3L zones 38-46. Lettered subdivisions of the numbered zones are entirely arbitrary.

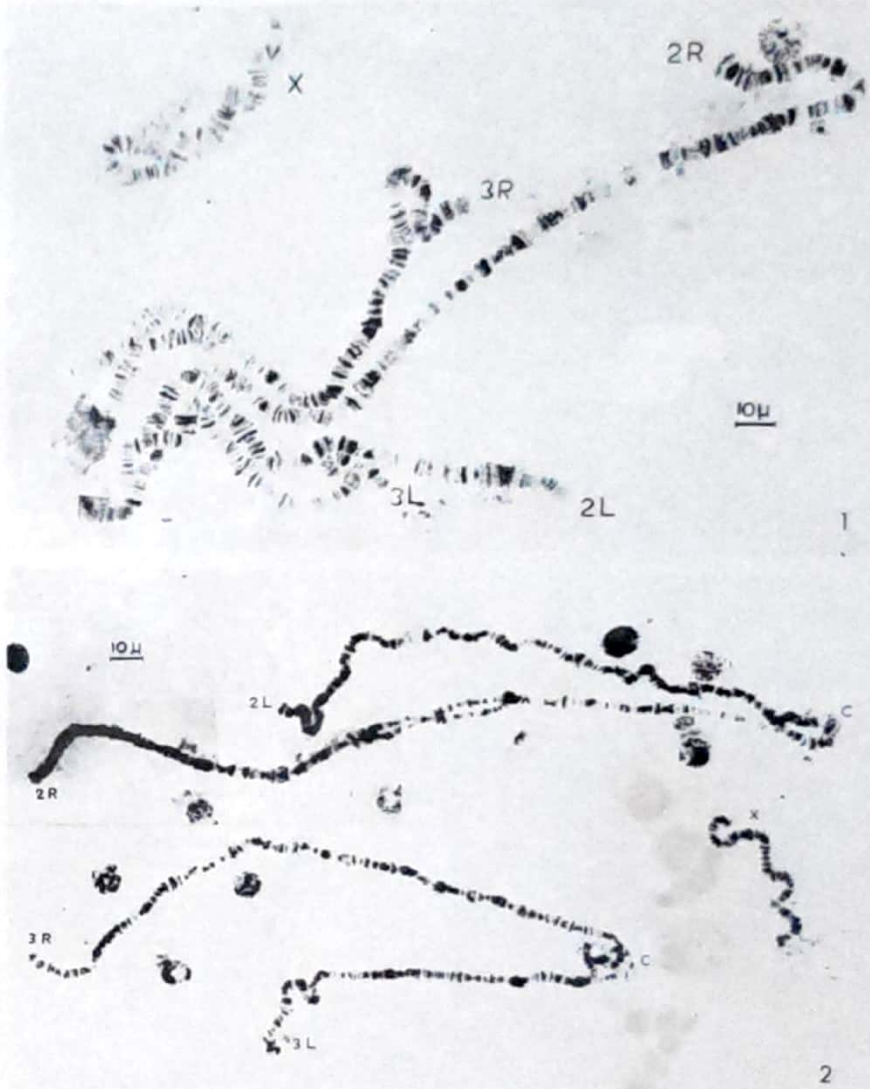
Diagnostic features of the chromosomes of *A. subpictus*

X- chromosome

The X chromosome is the shortest element in the complement measuring about 80 μm in length and may be easily recognized by its size alone. The presence of light bands followed by a pair of thickly stained dark bands marks the free end region 1A. Another pair of dark bands, seen at 2A, followed by a small puff represents the region 2BC. A series of dark bands, present in zone 3 as a whole is diagnostic. A characteristic puff with weakly staining dotted bands is typical of 4C and 5B. Two pairs of 4 extremely thick dark bands occurring in 5C constitute a landmark. The centromeric end of the chromosome is typically expanded into a broad bulb (6B, 6C) with dotted bands forming another good landmark of the X chromosome.

Chromosome 2 (right arm)

This is the longest chromosomal arm in the complement and measuring 311 μm. It may be recognized by one or by a combination of the following areas. The free end of the arm is usually semiflared with 3 light dotted bands and 3 slightly curved thick bands in the region 7A, followed immediately by a fairly enlarged, diffused puff in 7B which characterises the free end. Each of the regions 8B and 9AB contains a series of dark bands, is easily recognised in the region 10A. The middle portion of the arm contains 2 thick bands in 13B, a series of curved thin bands at 13C, followed by 3 thick bands in 14A and 4 bands in 14B, appearing usually intensely stained, so that the entire region gives the impression of being darkly stained. A fairly enlarged puff, starting with 3 bands in 16B, contains one dark dotted, one thick and a few highly stained dotted bands, forms a good landmark. A series of dark bands is usually seen in each of the regions 17BC, 18A and 18C. The tip of the centromeric end often seems semiflared with broken dark bands.



Figs. 1&2 : Ovarian polytene chromosome complement of *Anopheles subpictus* and *A. vagus*. 1. *A. subpictus*. 2. *A. vagus*.

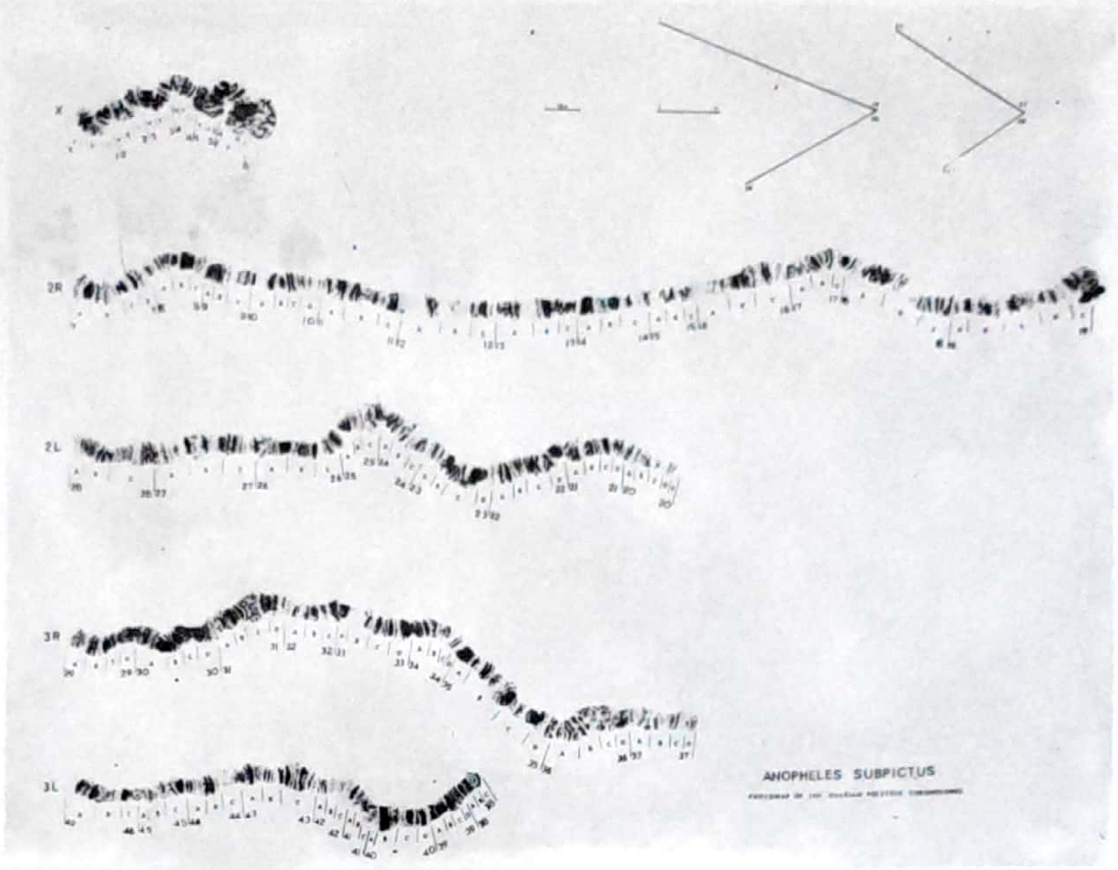


Fig. 3: Photomap of the ovarian polytene chromosomes of *Anopheles subpictus*.

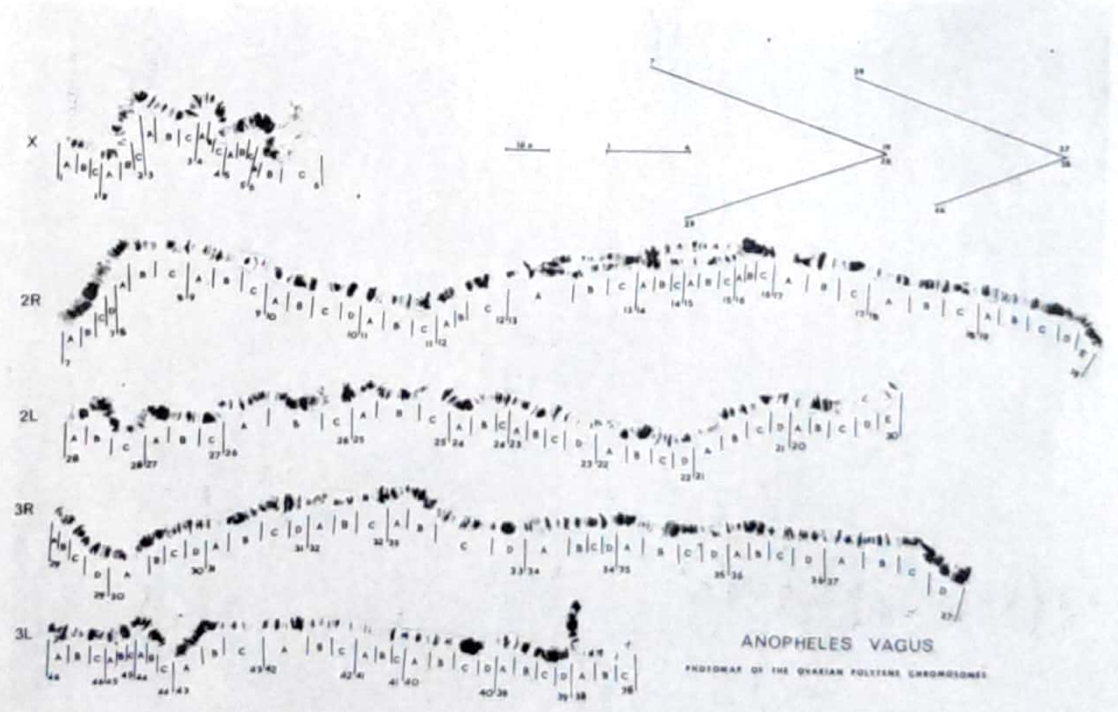


Fig. 4: Photomap of the ovarian polytene chromosomes of *Anopheles vagus*.

Chromosome 2 (left arm)

This arm measures 191 μm. The free end of the arm is easily identified with its fan-shaped expansion followed by a series of lightly stained dotted bands and 2 thick dark bands in 28B. There are 2 typical puffs present at the junction of the zone 28 and 27, appearing dumb-bell shaped with a thin, lightly stained bands and forming a good landmark. A thick, dark band marks the constriction between 27A and 27B. A group of 5 moderately stained dark bands, followed by light bands characterises the region 27C. Another group of thick dark bands present at the constriction between 26A and 26B is recognizable. Three typical puffs, each being located in regions 25C, 24B and 23C, are easily recognized. The region 22A is marked with 2 thick bands and each of the regions 21B and 21C is marked with a single thick dark band. The remainder of the arm is relatively more lightly stained and variable, especially near the centromere.

Chromosome 3 (right arm)

The free end of this arm usually consists of lightly stained dotted dark bands at 29A followed by 2 thick dark bands in 29C. A typical puff with 2 thick dark and 2 dotted dark bands is seen in the region 30B. The best recognized area is an enlarged puff with 2 thick, dark bands and lightly stained dotted bands in the region 31C and it forms a good landmark. There are 2 groups of dark bands on each at the constriction between 32C and 33A and between 33D and 34A. These are diagnostic. Another group of dark bands marks the region 35A. Two dark bands in 35B followed immediately by a typical puff. A series of lightly stained and evenly spaced bands in zones 36 and 37 as a whole is characteristic for the centromeric end. The length of the arm measures 198 μm.

Chromosome 3 (left arm)

The left arm is the shortest arm and measuring 128 μm. The free end of this arm can be recognized by its fan-shaped lighter area which is preceded by 3 pairs of diagnostic bands in region 46A. The middle pair is lighter than the other 2. The region 46C is marked with 2 dark bands. There are 5 thick, dark bands present at the junction of 45C and 44A and 2 dark bands in 44B are consistent. A small puff with 2 thick dark bands in the region 43A and another fairly enlarged puff with 4 thick, dark and light dotted bands in the region 43C form a good landmark for the middle portion of the arm. There are 2 very thick dark bands easily recognized in the region 40B. The remainder of the arm contains relatively darkly staining bands which are variable especially towards the centromere.

Diagnostic feature of the chromosomes of *A. vagus*

X-chromosome

It is the shortest element in the complement and measuring about 87 μm. The most striking feature of X chromosome is the similarity of banding pattern with that of *A. subpictus*. This chromosome is immediately recognized by the broad, expanded area in the region 6. The whole region usually is asynaptic and contains dotted bands. This area is subject to a much distortion and

the bands are often broken. The other landmarks of the chromosome are the presence of 2 pairs of dark bands in 5C, 3 dark bands at 1A and also at 2A, appearing usually heavily stained. A series of dark bands alternating with thin bands as diagnostic for zone 3. Region 4C - 5A usually consistent of a light puff with finely stained bands.

Chromosome 2 (right arm)

This is the longest autosomal arm in the complement and measures 260 μm. The free end of this arm has typically semiflared tip followed immediately by a thickly stained dark band in 7A. A diffused puff with 3 dark bands marks the region 7BC. Another series of dark bands between 8A and 8B and again from 10A - 10B is easily recognized. A pair of thick, dark bands present in each of the regions 10D and 11A is diagnostic. A typical puff with dotted and thin dark bands marks 11C. A series of 3 pairs of dark bands is consistent in the region 12. A single dark band in 13A at times appears asynaptic and this extends upto 13C. Again, another asynaptic area, beginning at 14B and ending in 16A followed immediately by a series of thickly stained dark bands at 16BC, is the most diagnostic. The heavily stained dark bands are constant for the regions 17B and 17C. The remaining arm consists of relatively darkly stained bands which are variable especially near the centromere.

Chromosome 2 (left arm)

The free end of this arm is easily recognized by a fan-shaped lighter area followed by 2 thick dark bands in the region 28A. The presence of a series of dark bands marks the regions 28B, 27C and 26B. A group of moderately stained dark bands in region 27C is characteristic. The middle portion of the arm is recognized by a series of thick dark bands which begins from 25C and ends in 24A. A fairly enlarged puff with 3 dark bands alternating with light bands characterizes the region 23C. There are 3 dark bands present in each of zones 22B and 20B which are diagnostic towards the centromeric end. The extreme end of this arm consists of a relatively lightly stained area. This arm measures about 215μm.

Chromosome 3 (right arm)

The free end of this arm is easily recognizable by its lightly stained bands followed by a small puff with thin bands in the region 29AB. A series of dark bands beginning from 29C and ending in 29D is diagnostic. The region 30B is marked with thick dark bands and the region 31C has a typical puff. There is a small puff followed by 3 thick dark bands occurring in the regions 32C to 33A. Thick and thin dark bands in 33B are consistent. A group of 4 dark bands present in 33D and also in 35A is consistent and characterizes the regions. A series of dark bands appearing from 36A and ending in 36B is also diagnostic with the other bands, sometimes varying in their staining intensity. Most of the remaining part of the arm consists of dark bands alternating with light ones. The arm measures abut 215μm.

Chromosome 3 (left arm)

The lightly stained free end of this arm is followed by 2 extremely thick dark bands at 46C. A small puff at 45B is characteristic towards the free end. The zone 44 as a whole, consisting of a

series of dark bands beginning with 44A and ending in 43B except 44C, is diagnostic. Three extremely thick dark bands mark the region 42A. A group of thickly stained dark bands is present in the region 40CD forming a significant landmark. Another series of dark bands, beginning from 36D, alternating with thin bands often appears variable in their staining intensity. The bands present in the zone 38 as a whole, usually, are asynaptic and are followed immediately by a series of thickly stained dark bands characterizing the centromeric end. This arm measures 157µm.

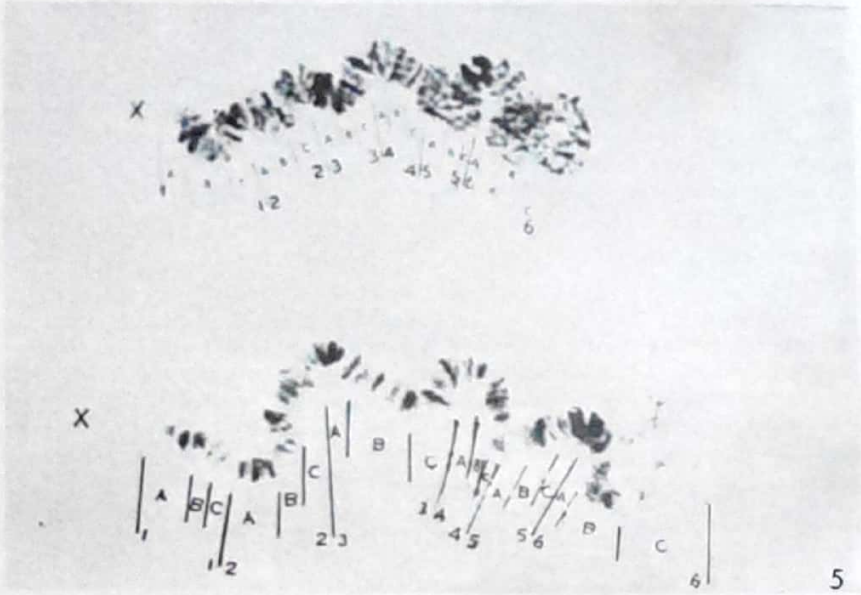


Fig. 5: Photomap of the ovarian polytene X chromosome of *Anopheles subpictus* and *A. vagus*.

DISCUSSION

The present study of adult ovarian nurse cell chromosomes has provided diagnostic characters of adult females and would be help in their ecological and epidemiological investigations. The data obtained in the present chromosomal studies are compared with the respective homologous chromosomes of *A. subpictus* and *A. vagus* to assess their relationships based on similarities of banding pattern. The comparison of the chromosomes clearly reveal homologies in most of the chromosomal arms, probably because of the close morphological resemblances and the sympatric occurrence of the 2 taxa. The polytene X chromosomes (Fig.5) in contrast to the opinion of Kitzmiller (1977) presents similar banding pattern in both the species.

The only difference observed in the X chromosome was broad, expanded centromeric end of the zone 6 and was usually asynaptic in *A. vagus*. The homology of the banding pattern and puffing pattern of autosomes between the 2 species were observed in the regions 7C, 8A, 11A, 12C, 14A, 16B, 19A and a puff in 7B of the chromosomes 3, right arm. The major differences observed in this arm are the presence of asynaptic regions between 13A-13C and 14B-16A of *A. vagus*. The chromosome 2, left arm shows the identical banding pattern in the region 28B, 27C, 24A, 24C, 21B and a puff in 23C. The chromosome 3, right arm shows the similarities in the banding pattern of region 29C, 30B , 23C-33A, 34D and the puffs of region 31C, 35B, 35D. The identical banding pattern of chromosome 3, left arm is shown in the region 40B, 40C of *A. vagus* with 40B and 39C of *A. subpictus*. This arm of *A. vagus* also shows a characteristic asynaptic region near the centromeric end. The remaining regions of autosomes reveal some differences.

This comparative study indicates that the X chromosomes of the 2 species appears remarkably similar, but the autosomes exhibit some differences as well as similarities. Similarity of the banding pattern provides support for their close relationship. However, similarity of the banding pattern alone does not necessarily indicate their genetic affinity. This can only confirmed by hybridization, fertility and the degree of synapsis of F1 chromosomes. The similarity of the banding pattern reflects their morphological and taxonomic status and leads us to presume that these 2 species probably are derived, one from the other, or are separated recently from some common ancestors.

The other interesting feature frequently noted in the chromosomes of *A. vagus* is the localization of the asynaptic regions in the homologous paired chromosomes. It is presumably due to the existence of some genetic differences between the homologous sections rather than to the pressure applied during squashing. Sometimes we can suspect that these chromosomes are truly homologous or perhaps contain an inversion or translocation. A further study is necessary to confirm this postulate.

ACKNOWLEDGEMENTS

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RADIATION INDUCED INVERSIONS AND TRANSLOCATIONS IN *VICIA FABA*

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SUMMARY

Inversion and translocation heterozygotes were induced by using gamma rays in *Vicia faba*. Inversion heterozygotes were characterized by the presence of bridge-fragment configurations at anaphase I and II. Translocation heterozygotes had in most of the PMCs, ring/chain of 4 chromosomes and 4 bivalents. Both the heterozygotes were highly sterile. It is expected that the mutants when established, would have altered karyotypes, useful for other experimental studies on *V. faba*.

Key Words : *Vicia faba*, inversion, translocation, gamma rays.

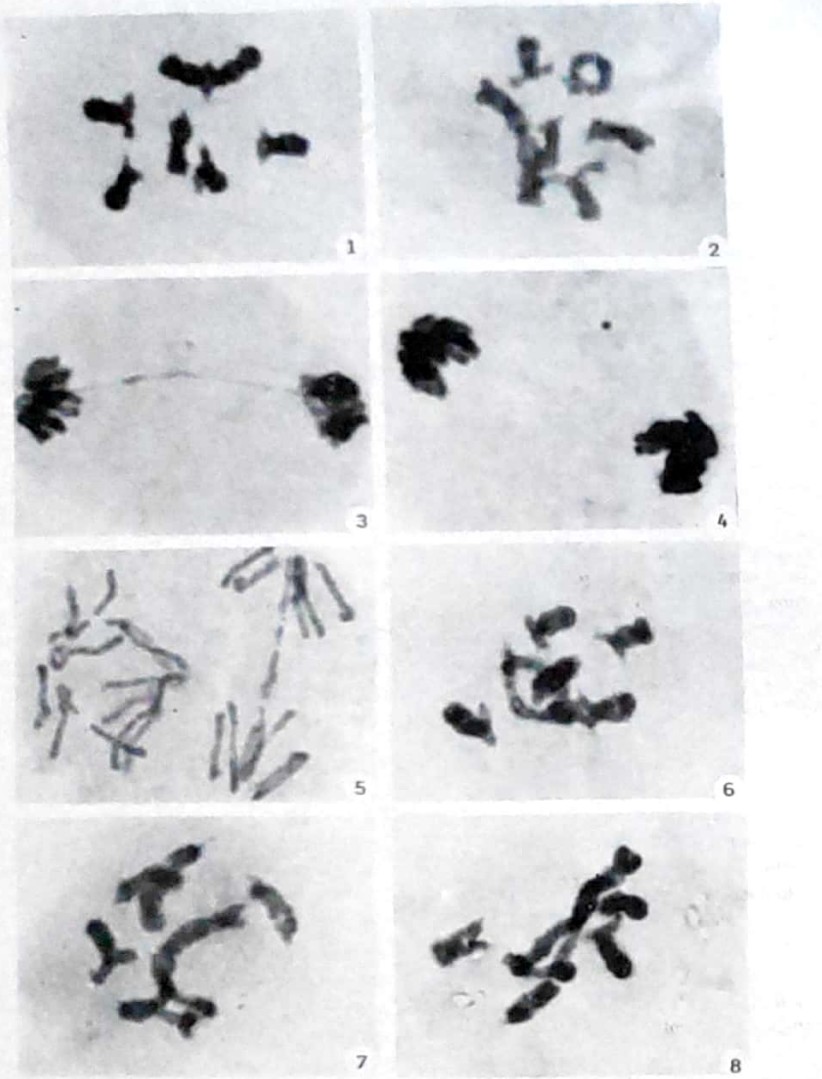
INTRODUCTION

Though *Vicia faba* L. (Leguminosae) is one of the most favourable materials for the study of induced chromosomal aberrations at mitosis, chromosomal mutants are rare. There have been limited studies on induced translocation and inversion heterozygosity in *V. faba* (Michaelis & Rieger 1959, Sjödin 1971a, b). The usefulness of these lines cannot be overemphasized. Besides their use in genetic mapping, translocations if established, may alter the normal karyotype of the species too. Inversions also, under certain conditions might change the karyotype by repatterning of chromosome segments. The altered karyotype in *V. faba* is of immense value in the study of induced chromosomal aberrations at mitosis as it facilitates the recognition and differentiation of ST (subtelocentric) chromosome involved in the aberration which otherwise are difficult to pinpoint.

As we are interested in the study of induced chromosomal aberrations at mitosis, we have been trying to establish some lines of *V. faba* with new karyotypes. This is expected to be achieved by treating the seeds with gamma rays, analysing the PMCs for translocation or inversion, if detected, selfing the plant and then raising the progeny. During the last 2 winters, we have analysed about 100 plants of M_1 and M_2 generations. Here, we describe 2 inversion and translocation heterozygotes each, detected in the population raised after treating the seeds with gamma rays.

MATERIAL AND METHODS

Dry seeds (6% moisture) of *V. faba* were irradiated by 10 and 20 kR doses of gamma rays at the B.A.R.C., Bombay. The control and irradiated seeds were sown in the pots. For meiotic analysis, young flower buds were fixed in 1:3 (acetic acid:absolute alcohol) mixture for at least 24 h. Anthers were squashed in 1% acetocarmine. Pollen stainability was estimated by staining the pollen grains in acetocarmine. Photomicrographs were taken from temporary preparations using the JENAVAL research microscope with attached photomicrographic equipment EXPOMET.



Figs. 1-8: Meiotic stages in *Vicia faba* (x 1000). 1. Control MI 6II. 2-5. Inversion heterozygote. 2. Metaphse I showing 6 bivalents note loop like structure in one bivalent. 3. Anaphase I note a bridge and a fragment. 4. Anaphase I note fragment. 5. Anaphase II note bridge-fragment. 6-8. Translocation heterozygote. Metaphase I note a ring of 4 chromosomes and 4 bivalents in all.

OBSERVATIONS

Control : Control plants always formed bivalents (2x = 12) at diakinesis and metaphase I in all the 25 PMCs observed (Table 2, Fig. 1). There was always a characteristic bivalent of a larger dimension and 5 small bivalents of almost equal size in each PMC at metaphase I. The chiasma frequency was 20.4 per cell and terminalization coefficient was 0.31. Anaphase I distribution of chromosomes was normal (6:6). Pollen stainability was more than 95%. On an average, there were 5-7 seeds per pod and 50-60 seeds per plant. Seeds were brown and black in colour.

Inversion heterozygotes : Two plants (I_1, I_2) exhibiting inversion heterozygosity were isolated from the population raised from irradiated seeds. One plant each was from 10 and 20 kR dose. 50 PMCs analysed at diakinesis and metaphase I had 6 bivalents in each. However, in some of them, typical inversion loop was observed and in some preparations as depicted in Figure 2, the absence of crossing over in the loop was evident. It may be made clear here that these configurations did not give definite clue to the presence of inversion heterozygosity.

At anaphase I and II, 100 PMCs analysed in both the plants I_1, and I_2, were found to have various configurations indicate of the presence of and a fragment heterozygosity (Table 1). At anaphase I, a bridge and a fragment (BF) was observed in 18% of PMCs of I_1 and 12% PMCs of I_2 (Fig.3). Loop and fragment (LF) was observed in 7% PMCs of I_1 and 4% PMCs I_2 of (Fig. 4), although in all the PMCs loop was not clearly visible due to superimposition. Two loops and 2 fragments (LLFF) were observed in 2% of cells of I_1 only. At anaphase II also BF configuration was observed in 21% of I_1 and 10% of I_2 PMCs (Fig.5). Two bridges and fragments (BBFF) at different poles were recorded in 4% of cells of I_1 and 2% of cells of I_2. BFF and FF configurations were also recorded in some PMCs (Table 1). The pollen stainability was only 30-40% and only one seed was collected from one of the plants.

Translocation heterozygotes : One plant each (T_1, T_2) was isolated from the population raised from 10 and 20 kR irradiated seeds. The T_1 was characterized by the presence of a ring/chain of 4 chromosomes and 4 bivalents in 88% of its PMCs (Table 2, Figs. 6-8). The shape, size and configuration of ring of 4 clearly indicated the involvement of the biggest (M) pair and one of the 5 smaller (ST) pairs of the karyotype in the interchange complex. Chain of 4 was found in only one cell. The anaphase I was highly abnormal. Of the 50 cells analysed laggards were present in 8% and bridge without a fragment in 12% of cells. Pollen stainability was 60% and seed set was very poor (10-15 plant). The colour of the seed was greenish-yellow and they seemed to be inviable.

The T_2 was also characterized by the presence of a ring/chain of 4 chromosomes in 80% of its PMCs (Table 2). But in this case the biggest pair was not involved, instead 2 of the 5 ST pairs of chromosomes were involved in the interchange complex. The anaphase I was abnormal in this plant also. Of the 50 cells recorded, 8 had bridges and 7 had laggards. Pollen stainability was 70%. Seed set was poor (6-8/plant) and the colour of the seed was greenish-yellow. A few seeds kept for germination failed to do so.

TABLE 1: Frequency of different configurations at meiosis in inversion heterozygotes in *Vicia faba*.

Genotypes	No. of cells analysed	Anaphase I			Anaphase II			
		BF (%)	LF (%)	LLFF (%)	BF (%)	BBFF (%)	BFF (%)	FF (%)
Control	25	-	-	-	-	-	-	-
I_1	100	18	7	2	21	4	8	9
I_2	100	12	4	-	10	2	5	3

TABLE 2: Chromosoal associations in control and translocation heterozygotes in *Vicia faba*.

Genotypes	No. of cells analysed	Diakinesis/metaphase I							
		Ring of 4		Chain of 4		Ring + Chain of 4			No. of bivalents Mean
		No.	Mean	No.	Mean	No.	Mean	%	
Control	25	-	-	-	-	-	-	-	6.00
T_1	25	21	0.84	1	0.04	22	0.88	88	4.24
T_2	25	18	0.72	2	0.08	20	0.80	80	4.40

DISCUSSION

Since the radiation studies were started in our laboratory, various doses of gamma rays (5, 10, 20, 30, 40, 50 kR) have been used, however, our past experience showed that while the lower doses did not give any significant result, in higher doses (40, 50 kR) seeds failed to germinate. Therefore, only 2 doses 10 and 20 kR were used for the present investigation. The production of only 2 plants each of inversion and translocation heterozygotes out of a population of about 100 plants showed that the induction of stable chromosomal changes is not of a common occurrence. It should be noted here that the same dose produced about 50% of cells with aberrant chromosomes observed at mitotic metaphase. This indicated that most of the cells with abnormal chromosomes are eliminated or the aberrations are repaired and only the cells with normal complement enter into the germinal tissue.

Relatively, a low frequency of induced inversions and translocations in *V. faba* has been recorded by Sjödin (1971 a,b) also. The possible reason for such a behaviour could be that the short arm is too small to participate in any of the aberrations requiring substantial portion of chromosome arm. The rarity of induced inversion can be judged from the fact that till Sjödin's (1971b) report there was only one such record of Michaelis & Rieger (1959).

Both the inversion heterozygotes isolated in the present investigation had bridge-fragment configuration at anaphase I and II, indicating paracentric nature of inversion. As evident from Table 1, various possible types of configurations resulted from different numbers and positions of cross over inside and outside the inversion loop. The presence of one bridge - fragment at anaphase I was due to one crossover inside the inversion loop. In PMCs where only the fragment was visible it was presumed that this was due to overlapping of loop by other chromosomes at the poles and that the

original configuration was loop-fragment. That this conclusion was correct was evident from bridge-fragment configuration observed at anaphase II which could result only from one loop-fragment of anaphase I. The above configurations were the consequence of one crossover inside and one outside the inversion loop. On the basis of meiotic stages it was difficult to precisely pinpoint the chromosome in which inversion had taken place. However, mitotic complement could be helpful, provided the marker region was involved. This aspect is still under investigation.

It was also observed that in some cases though the inversion loop was formed, no crossing over took place (Fig. 2) and therefore, did not result in bridge-fragment configuration. In the present case, therefore, actual frequency of bridge-fragment configuration must have been much more than the observed one.

The 2 translocation heterozygotes produced characteristic configuration of ring/chain of 4 chromosomes and 4 bivalents at diakinesis/MI. However, in T_1 the chromosomes involved in interchange were the first metacentric (M) pair and one of the 5 smaller subtelocentric (ST) pairs, while in T_2 2 of the 5 smaller ST pairs were involved. This indicated random distribution of translocation breakpoints, although Sjödin's (1971a) studies have shown that first, second and fifth chromosome pairs displayed lower aberration frequency than the other 3 chromosome pairs.

In both T_1 and T_2, the ring of 4 chromosomes showed predominantly adjacent orientation as has been observed in other plants like *Crotalaria* (Verma & Raina 1990). As is known, this would produce unbalanced types of gametes, and this was reflected in the present case by high sterility exhibited by both the plants. There are certain conditions for predominant directed orientation like symmetry in chromosome size, centromere position, chiasma frequency and location, genotype, etc. (Burnham 1956, Sybenga 1972) but there are many exceptions and real understanding of basic principles is poor (Rickards 1983). It is also established now that each translocation is unique to some degree and must be considered accordingly (Ford & Clegg 1969, Jacobs et al. 1970). Therefore, it was not surprising to find many contrasts in the observations of Sjödin's and ours, though the material was the same.

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DIPLOIDIZATION IN AUTOTETRAPLOID *ERUCA SATIVA*

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SUMMARY

Induction of autotetraploidy has been achieved in *Eruca sativa* Mill. (2n = 22) by using 0.2% aqueous colchicine. The autotetraploids (2n = 4x = 44) were advanced to C_2 generation. A comparative morphocytogenetiçal analysis has been carried out on both generations of tetraploids along with their diploid parent. Autotetraploids exhibited the gigantic nature due to enhancement in their vegetative parameters. Meiotic diploidization was observed both in C_1 and C_2 generations of autotetraploids.

Key Words : Diploidization, *Eruca*, autotetraploid.

INTRODUCTION

Eruca sativa Mill., commonly known as 'taramira', is grown in northern India as a fodder plant. The oil extracted from the seeds, in is used chiefly for burning. Successful induction of polyploidy, using colchicine as a polyploidizing agent, is a well established phenomenon. This potential cytological technique is a handy tool in tampering with the genotypes and in widening the variation in gene pool The present study was undertaken to study the morphocytogenetical features of *Eruca sativa* autotetraploids in C_1 and C_2 generations along with their diploid parents and to explore the possibilities of developing high yielding polyploid strains.

MATERIAL AND METHODS

Seeds of Eruca sativa were sown in pots. For inducing polyploidy cotton-swab saturated with 0.2% aqueous colchicine solution was kept on growing meristem for 12 h. Seeds from C_1 generation autotetraploids were collected and sown in next season with their parental diploids. For meiotic studies the flower buds of adequate size from C_1 and C_2 autotetraploids and also from diploids were fixed in Carnoy's fluid II and preserved in 90% alcohol. Anthers were squashed in 2% acetocarmine to study the meiotic behaviour and pollen fertility. Data on morphocytogenetical parameters of diploid, C_1 and C_2 generation autotetraploids were statistically analysed to determine variation and impact of polyploidization on vigour and fertility.

RESULTS AND DISCUSSION

Eruca sativa autotetraploids exhibited luxuriant vegetative growth over their diploid parent. C_2 generation autotetraploids were more vigorous showing significant enhancement in most of the parameters (Table 1). Autotetraploids had delayed flowering which continued for longer duration. Gigantism, a common feature exhibited by all the synthesized autotetraploids, is due to increase in

TABLE1: Character means of 2x and 4x (C_1 and C_2 generations) in *Eruca sativa*.

Character	2x	4x	
		C_1	C_2
Plant height (cm)	87.8 ± 0.1	103.1 ± 0.6	113.4 ± 0.8
No. of branches	11.4 ± 0.4	22.0 ± 0.9	27.8 ± 0.1
No. of nodes	15.3 ± 0.7	28.0 ± 1.3	26.0 ± 0.1
Pods/Plant	101.0 ± 1.0	46.0 ± 0.8	63.2 ± 1.3
Pod length (cm)	3.7 ± 0.8	3.0 ± 0.1	3.2 ± 0.8
Seed/Pod	28.9 ± 0.5	16.8 ± 0.4	20.0 ± 1.8
Yield/pland (g)	6.4 ± 0.1	1.6 ± 0.4	3.8 ± 0.3
Pollen fertility (%)	96.4	33.0	78.6

cell size (Arya et al. 1988). Retention of gigantism by polyploids even in C_2 generation is viewed as an advantageous phenomenon. Morphological changes in autotetraploids appear directly proportional to the quantitative change in chromosomes.

Meiosis in diploids showed 11 bivalents at metaphase I and an equal anaphasic separation 11:11. Also, the meiosis of both C_1 and C_2 autotetraploids had mostly bivalents and a few univalents at metaphase I. There was an equal chromosomal disjunction of 22:22 at anaphase I. On an average, diploids had 4.8 ring and 6.2 rod bivalents per PMC. C_1 autotetraploids had 11.6 ring, 9.8 rod bivalents and 1.0 univalents per PMC, whereas C_2 autotetraploids had 11.7 ring 9.7 rod bivalents and 1.0 univalent. The mean chiasma frequency per chromosome was 0.7 in diploids, C_1 autotetraploids and C_2 autotetraploids (Table 2). Chiasma frequency in tetraploids, higher or almost equal to that in diploids has been reported by Levan (1937) and Ghosh et al. (1974).

Pollen fertility in diploids, C_1 and C_2 autotetraploids was 96.4%, 33.0% and 78.6% respectively. Poor pollen fertility in C_1 autotetraploids may be due to physiological or biochemical factors (s). Seed yield in C_2 autotetraploids got doubled in comparison to C_1 plants. Distinct improvement in pollen and seed fertility was noticed in C_2 autotetraploids. Similar results were

TABLE 2 Means of chromosomal associations and chiasmata per cell and per chromosome in diploid and C_1, C_2 autotetraploid *Eruca sativa*.

Chromosomal configurations	2x	4x	
		C_1	C2
Bivalents:			
Ring	4.8	11.6	11.7
Rod	6.2	9.8	9.7
Univalents	-	1.0	1.0
Chiasmata/cell	15.8	30.7	31.2
Chiasmata/chromosome	0.7	0.7	0.7

obtained by Gupta & Roy (1986). The autotetraploid *Eruca* may be more suitable for fodder due to gigantic nature of plants.

In case of autotetraploids, the basic genome is represented four times and hence it is generally expected that the chromosomal association in them should be in the form of all quadrivalents (Arya et al. 1988). But *Eruca sativa* autotetraploids had mostly bivalents which could be due to the diploidization and few univalents which could have been due to precocious separation of bivalents.

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ACROCENTRIC ASSOCIATION IN HUMAN CHROMOSOMES - AN INDICATOR OF PREDISPOSITION TO GENETIC DEFECTS

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SUMMARY

Screening of 2718 metaphases from 75 individuals (38 females and 37 males) consisting of women with 2 or more recurrent abortions/still births their respective spouses, individuals with congential malformations, diagnosed cases of cancer and normal population clearly indicates that acrocentric association (ACA), a known cytological feature that may lead to nondisjuctional and/or translocational events, is found even in normal individuals at a frequency we have designated as "baseline value". In our opinion, although there may not be any direct concordance between the degree of association and specific disease/symptom(s), there exists a pattern. The relatively higher frequency of ACA even in the known developmental defects (congential malformations) which are either polygenic or single gene disorders also indicate the significance of ACA in the destabilisation of genomic balance. We suggest that the frequency and behavioural pattern of acrocentric associations may be used as an indicator of predisposition to genetic defects.

Key Words: Acrocentric associations, chromosomes, cancer, congenital malformations, genetic predisposition.

INTRODUCTION

The characteristic pattern of, 'acrocentric associations' (ACA) was first reported by Ferguson-Smith & Handmaker (1961) and is now an established phenomenon. ACA has gained importance in cytogenetics because there are reports indicating concordance between variability and frequency of ACA with a number of clinical states including Down syndrome, increasing frequency of spontaneous abortions and predisposition to Turner syndrome and malignancy (Jackson-Cook & Brown 1987, Murthy et al, 1989). Some of this variability has been shown to follow familial patterns (Mikelsaar & Hsu 1979).

According to Ferguson-Smith & Handmaker (1961), acrocentric associations may be considered a preamble to nondisjunction and/or structural rearrangements. There are reports that

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about 40% of lethal trisomies in humans involve acrocentric NOR-bearing chromosomes (Boue & Boue 1977), and it has been indicated that these result from meiotic non-disjunction (Jacobs & Morton 1977). A number of researchers have attributed a high risk of meiotic nondisjunction involving D and G group chromosomes to a high frequency of ACA in parents of Down syndrome (Babu & Verma 1985, Hansson 1979). In addition, the involvement of chromosomes 13 and 14 in constitutional translocations in premalignant disorders (Pathak 1986), has prompted investigations on the behaviour of human acrocentrics.

Considering the reports that variations in size and number of satellited acrocentrics and the frequency of ACA occur in mitoses even in normal individuals (Henderson & Atwood 1976), we felt it necessary to evaluate the degree of variability in the frequency of ACA in a wide spectrum of age groups of normal individuals of both sexes and then to compare this value with that of in a wide range of referred cases (Table 1). The basic objective of this investigation was, however, to assess the possibility of using ACA frequency as a dosimeter in identification of high risk individuals.

MATERIALS AND METHODS

A total of 75 individuals (38 females & 37 males) aged between six months to 70 years, consisting of 4 groups of referred cases and normal individuals were evaluated (Table 1).

Peripheral blood lymphocyte cultures were set up in triplicate from each individual in medium RPMI 1640 (HiMedia, India) supplemented with 10% FCS (Sigma, USA), 40 µg/ml PHA (Sigma, USA), penicillin (100 IU/ml) and L-glutamine (0.29 mg/ml). Harvesting was done after 70 hrs. incubation at 37°C following the usual colchicine (18.18 µg/ml, 1hr., 37°C) - hypotonic (0.075M KCl, 8 ml, 15min, 37°C) - fixative (1:3 acetic methanol) schedule (Watt & Stephan 1986).

Air dried slides were initially stained in 8% buffered Giemsa (pH 6.8) and then GTG banded following modification of Seabright (1971). Ag-NOR staining was carried out following Howell and Black (1980).

A total of 2718 metaphases were screened for scoring of ACA. The criterion for scoring was the presence of intersatellite connection between the associated acrocentrics (Zang & Beck 1976). We have not discriminated between homologus and non-homologous associations.

The association index (AI) was calculated according to following formula (Hansson 1979):

$$AI = \frac{\text{number of associated chromosomes of a specific type}}{\text{total number of chromosomes in this group}}$$

For statistical evaluation, test for significance of the mean ACA between the referred cases and normal individuals was carried out using students 't' test and $\chi 2$ test was performed to verify the hypothesis whether the involvement of D and G group chromosomes in the association complexes fits into the expected 3:2 ratio or whether there is any significant deviation. This would indicate any bias in participation by any chromosome group in a disorder

OBSERVATIONS

Frequency of association

Group Id showed the highest frequency of ACA (42.38%) followed by group Ia (38.86%), Ib (27.96%) and Ic (20.97%). The same for normal individuals (group II) was 7.14%. We have considered this as the baseline value (Table 1). The range of association frequency has been shown in Fig. 1.

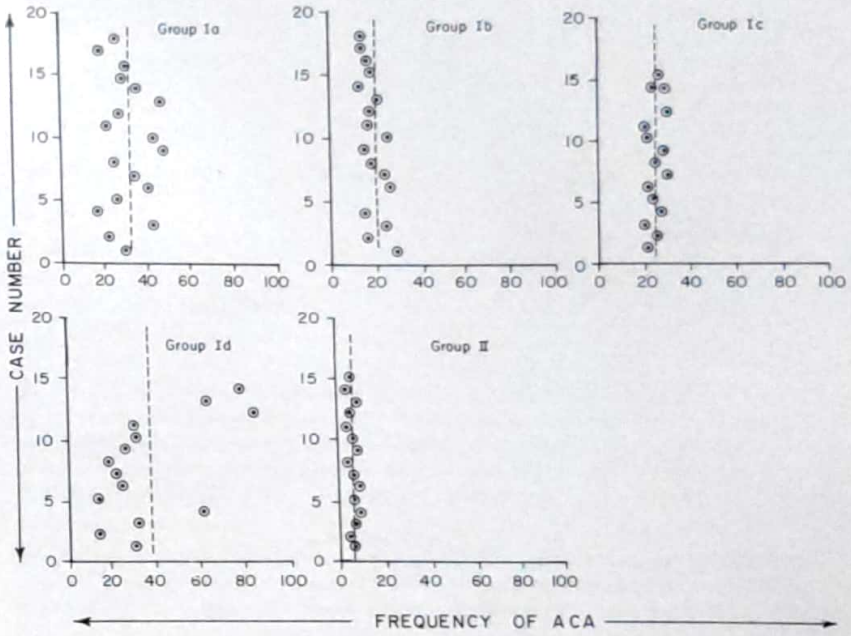

Fig. 1: Distribution in individual case (O) and the mean value (———) of ACA frequency in different groups.

Statistical analysis clearly indicated the presence of total ACA in highly significant (p greater than 0.0001) frequencies in all the referred groups compared to normal individuals excepting group Ic, where it was significant at 0.05% level.

TABLE 1: Details of the cases evaluated and distribution of ACA.

	Group description	No. of samples evaluated	No. of metaphases screened	ACA type (%)					Cells with (%)		
				Total	DD	DG	GG	Multi-ple	1 ACA	2 ACA	3 ACA
Ia	Women with two or more recurrent abortions/ still births	15	586	32.86	10.24	15.54	2.47	4.59	84.71	14.65	0.63
Ib	Their respective spouses	15	565	27.96	10.26	12.03	2.83	2.83	89.86	9.45	0.67
Ic	Individuals with congenital malformations (skeletal abnormalities: phocomelia, Klippelfeil syndrome, Talipes, Apert's syndrome, scoliosis)	15	596	20.97	5.87	8.05	2.34	4.69	92.17	7.83	--
Id	Individuals with diagnosed cancer (breast, ovary, cervix-uteri, rectum, urinary bladder, hairy cell leukemia, osteosarcoma).	15	361	42.38	11.08	-	2.77	6.92	79.66	19.49	0.85
II	Normal control	15	630	7.14	3.01	2.22	1.90	0.00	97.78	2.22	--

Types of association

With respect to the type of association (DD, DG, GG and multiple), DG association was found in maximum frequency with highest value in group Id (21.61%) and lowest in group (Ic (8.05%). The frequency of DD association was almost the same in group la (10.24%), Ib (10.26%) and group Id (11.08%). However, this frequency was relatively lower in group Ic (5.87%). GG association was present in a very low frequency ranging between 2.34-2.83% among the referred cases (Table 1).

Multiple associations (involving more than two acrocentrics) were present in all excepting group II (Table 1). The highest (6.92%) and lowest (2.83%) frequencies were in group Id and Ib respectively, while intermediate values were present in group la (4.59%) and group Ic (4.69%).

Number of associations per cell

When ACA containing cells were grouped on the basis of number of ACA per cell, a preponderance of cells with one ACA was observed in all the groups with the highest frequency in control group. Interestingly, there was a clear decline in the frequency of cells with one ACA in the referred cases with a concomitant increase in cells with two or more ACA (Table 1). Cells with two or more ACA were observed in higher frequency in cancer cases and in recurrently aborting women. The male partners (group Ib) showed only 7.83 percent cells with two ACA and no cell with more than two ACA.

Association index

Association Index (AI) was highest in group Id being 0.13 for D and 0.15 for G group chromosomes. The X^2 analysis clearly indicated that the values fitted well in the expected 3:2 ratio in cases of congenital malformations (group Ic), malignancy (group Id) and control population (group II). However, there was preponderance of participation of D group chromosomes in recurrently aborting women and their spouses (group Ia and Ib) (Table 2).

TABLE 2: Statistical analysis of the data on AI.

Group	Participation in number				Association index		χ2	Probability
	D group		G group		D	G		
	Obs	Exp	Obs	Exp				
Ia	217	93	186	124	0.11	0.06	12.50	Overdomina nce of 'D'
Ib	179	86	159	106	0.11	0.06	5.98	Dominance of 'D'
Ic	120	76	118	78	0.07	0.06	0.076	0.5 - 0.3
Id	293	207	300	200	0.13	0.15	0.352	0.7 - 0.5
II	52	33	51	34	0.02	0.02	0.012	0.9, accepted

DISCUSSION

The formation of association between human acrocentrics can be explained on the basis of their molecular nature. They share two types of repeated DNA sequences, satellite (Gosden et al. 1975) and rDNA (Evans et al. 1974). It has been demonstrated that multiple copies of rRNA genes are located at the stalk region of the ten acrocentrics which have been suggested to provide homology needed for crossover events between non-homologous chromosomes (Evans et al. 1974). This hypothesis was further extended by Kurnit et al. (1984), and Choo et al. (1988). It has also been suggested that various subfamilies of alphoid DNA sequences which are arranged in long arrays in tandemly repeated 170 bp monomeric or 340 bp dimeric units and shared by the NOR bearing chromosomes, provide further sequential homology (Kurnit et al. 1984, Willard & Waye 1987). Choo et al. (1988), proposed recombination and exchange between non-homologous chromosomes as the evolutionary process responsible for the 'homogenization' of the p TRA-2 subfamily of alphoid DNA on the different acrocentric chromosomes. According to them such a process appears to be more selective for chromosomes 13, 14 and 21. Sharing of alphoid DNA sequences by chromosomes 14 and 22 under stringent hybridisation conditions has also been reported (Jorgensen et al. 1987).

However, according to Cheung et al. (1990), rearrangement can occur between regions of no obvious homology, suggesting that homologous recombination may not be the only mechanism for acrocentric association.

It is worth mentioning here that we observed participation of chromosomes 13,14 and 21 in association complexes in 78% of the cases. The frequent involvement of these chromosomes in Robertsoian translocation is well documented (Dutrillaux et al. 1973).

An interesting picture emerges when our data, which is unbaised as we have covered a wide range of age groups in five categories, is taken in conjunction with the existing knowledge on acrocentric association in different symptomatic groups.

Data presented in Table 1 indicates that both male and female partners of recurrently aborting cases have significant level of ACA frequency. But when calculations were made after removing the four individuals belonging to group Ib who exhibited very high frequency of ACA (36-59%), the mean ACA frequency was reduced to 7.81%, almost at par with the baseline value. This seems to indicate that in the present series, the female parents contributed more in the spontaneous/recurrent abortions rather than the male parents. In an earlier study of 4088 spontaneous abortions, Hassold & Jacobs (1984) observed that 26.1% was due to the trisomies of different chromosomes, in which acrocentrics were significantly involved. The in vitro cytogenetic study of unfertilized oocytes also revealed frequent involvement of D and G group chromosomes in nondisjunctional events (Pellestor et al. 1991). It may also be pointed out here that human sperm karyotypes show a prevalence of structural aberrations over aneuploidies. Martin (1989), reported a mean frequency of 7% structural abnormalities whereas aneuploidies were 1.6% in a sample of 4347 sperm karyotypes.

According to Mamaev & Mamaeva (1990), the increased NOR activity of the acrocentrics in tumor cells should be considered the most important factor in oncognesis since it increases the synthesis of rRNA and allows the cell to use its proliferative advantage more completely rather than the other cellular elements. Since it is well documented that the degree of ACA is directly correlated with the DNA synthetic actity (Yan & Stanley 1988), our observation of the highest percentage of total ACA and multiple associations in group Id clearly reflects the physiology of the cell in malignancy. it may be mentioned here that the Ag-NOR proteins (nucleolin) syntheized by rDNA has been observed to play a significant role in nuclear exchanges and retention of proliferative state of the cell (Pession et al 1991). This protein also has significant role in disjunctional events. It may be of interest to point out that ACA has not only been observed in metaphase but also in interphase nuclei, using kinetochore specific antibody in a breast tumor cell line (Haaf & Schmid 1991).

Our data confirms the fact that ACA is found even in normal individuals at a frequency that has been designated as "*baseline value*", which however, may vary for different population groups. When the frequency exceeds to baseline value, it becomes detrimental to the cells or the individual(s) carrying it. We have observed statistically significant higher frequency of ACA in all the referred cases. The increase in the frequency may be due to internal and/or external factors or stimuli which trigger higher synthetic activity. Figure 1 clearly indicates that deviation of the ACA frequency was not significant from the mean value of respective groups. Slide-wise variation was also in the same range.

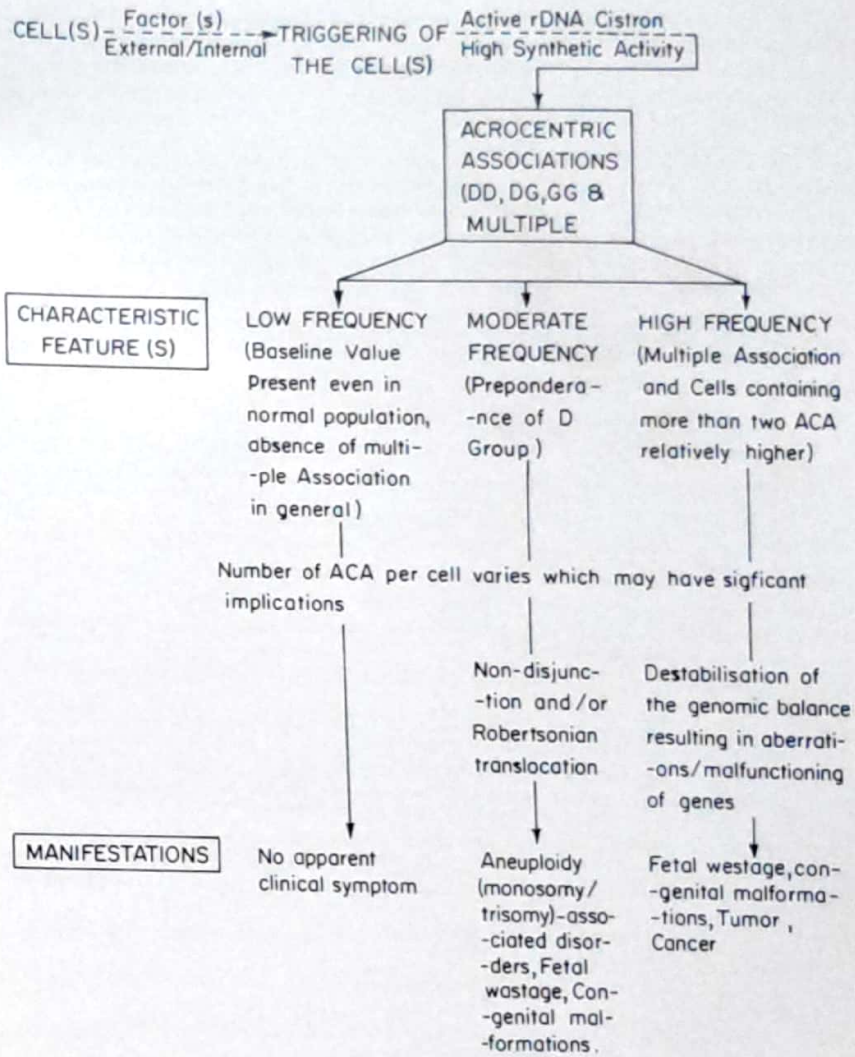

Fig. 2: Flow chart depicting possible involvement of ACA in clinical manifestations.

In our opinion, although there may not be any direct concordance between the degree of associations and specific disease/symptom(s), there exists a pattern, that has been summarised in Fig.2. The presence of cells with more ACAs may be significant in contributing to the destabilisation of the genomic balance which may lead to nondisjunction and/or structural rearrangements.

In addition, it seems that each behavioural pattern of human acrocentrics has its own importance in the manifestation of a disorder which may or may not directly involve acrocentrics. The relatively higher frequency of ACA even in the known developmental defects (group Ic), which are either polygenic or single gene disorders, also indicate the significance of this study. There are earlier reports indicating their association with cellular and organismal development (Denton et al. 1981, Yan & Stanley 1988).

We suggest that all cytogenetic laboratories should look for the ACA frequency. This would not require any extra effort except scoring. Such an approach would generate data to set the "*baseline value*" for different population groups.

We feel that this very simple approach if used as a 'preliminary' screening procedure, may facilitate identification of high risk individuals who can be further monitored for specific disease or defect.

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ON THE CYTOLOGY OF FIVE SPECIES OF COCCINELLIDAE (COLEOPTERA : POLYPHAGA)

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SUMMARY

Cytological investigations were carried out on 5 species of lady-bird beetles belonging to subfamily Coccinellinae, viz., *Pharoscymnus flexibilis* Muls., *P. horni* Weise, *Illeis bistigmosa* Mulsant, *I. cincta* Fabricius and *Coccinella transversalis* Fabricius. All the species, with the exception of P. *horni*, possess 2n=20. *P. horni*, however, has 2n=22. These beetles are characterized by a simple X_{Y_p} male sex chromosome mechanism. *P. horni* depicted the maximum mean chiasma frequency of 15.32 per nucleus. *P. flexibilis* and *C. transversalis* karyotypes show only biarmed chromosomes, whereas *I. bistigmosa* carries as many as 4 pairs of acrocentrics. This has evolutionary implications.

Key Words : Coleoptera, Coccinellidae, karyotype, chiasma frequency, sex chromosome system

INTRODUCTION

The family Coccinellidae is a medium-sized economically very important group of lady-bird beetles comprising about 5000 species (Richards & Davies 1979). The members of this family are also known to exhibit chromosome polymorphism. So far about 200 species are known cytologically (Smith & Virkki 1978, Mittal et al. 1989, Yadav & Gahlawat 1992, 1993a,b,c,d, Yadav et al. 1991). The present communication deals with the karyotypic details and the behaviour of chromosomes during cell division in 5 species viz., *Pharoscymnus flexibilis* Mulsant, *P. horni* Weise, *Illeis bistigmosa* Mulsant, *I. cincta* Fabricius and *Coccinella transversalis* Fabricus, of lady-bird beetles.

MATERIALS AND METHODS

Adult individuals of *Pharoscymnus flexibilis* were obtained from Biological Control Research Station, Solan (H.P) in April 1991; *P. horni*, *Illeis bistigmosa* and *I. cincta* were collected from Bangalore in November 1992 and *Coccinella transversalis* from Kurukshetra in February 1993. Adult males were utilized for making kayological preparations from the testes following the technique of Yadav & Lyapunova (1983). Table 1 shows collection data and main cytological features of the beetles under present study.

TABLE 1: A classified list showing collection data and other cytological details.

Species	Locality	Period of collection	No. of individuals sacrificed	2n	Male meio-formula	X̄	T.C.
Order : Coleoptera							
Sub-order : Polyphaga							
Family : Coccinellidae							
Tribe : Scymnini							
Pharoscymnus flexibilis	* BCRS	April 1991	12	20	$9 + Xy_p$	12.32	0.83
P. horni	Bangalore	Nov. 1992	2	22	$10 + Xy_p$	15.32	0.81
Tribe:Psyllobora							
Illeis bistigmosa	Bangalore	Nov. 1992	8	20	$9 + Xy_p$	12.00	0.74
L.cincta	Bangalore	Nov. 1992	5	-	$9 + Xy_p$	13.25	0.85
Tribe: Coccinellini							
Coccinella transversalis	Kurukshetra	Feb. 1993	4	20	$9 + Xy_p$	12.32	0.74

* BCRS = Biological Control Research Station, Solan.
X̄ = Mean chiasma frequency per nucleus.
T.C. = Terminalization coefficient.

OBSERVATIONS

***Pharoscymnus flexibilis* 2n = 20 ($9 + Xy_p$)**

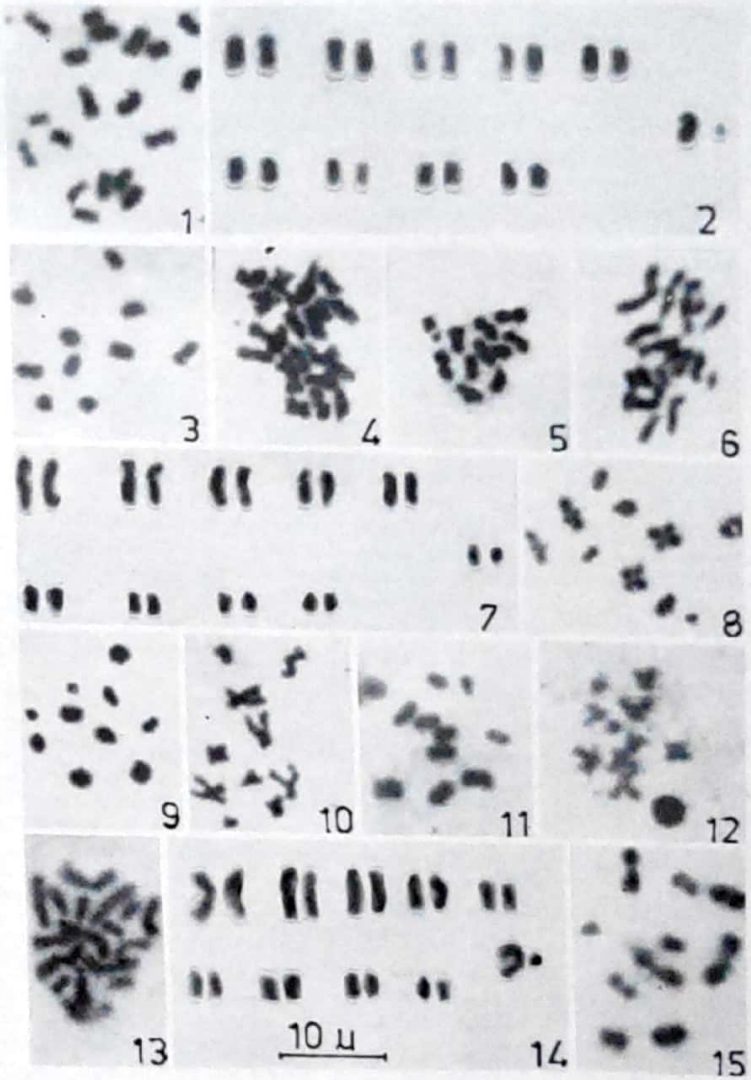
Spermatogonial metaphase exhibited a diploid set of 20 chromosomes (Fig. 1). The karyotype comprised 8 pairs of metacentric (pairs 1-8) and one pair of submetacentric (pair 9) autosomes and a metacentric X chromosome (Fig.2). While the autosomes show gradual decrease in size, the X chromosome overlaps with the autosome pair 4 (Table 2). Nine rod and ring shaped autosomal bivalents and the sex bivalent Xy_p are observed at metaphase I (Fig. 3). Two types of metaphase II, one with X and the other with y, in addition to nine autosomes, occur.

***P. horni* 2=22 ($10 + Xy_p$)**

Diploid complement of 20 chromosomes was revealed at spermatogonial metaphase (Fig. 4). Owing to overlapping of elements, karyotype could not be prepared. However, biarmed nature of most of the elements is clear. The dot-shaped y chromosome is the smallest element of the complement. Metaphase I plate showed 10 dumb-bell shaped autosomal bivalents and the sex parachute (Fig. 5). Two types of metaphase II plates were encountered, one with y and the other with X in addition to 10 autosomes.

***Illeis bistigmosa* 2n = 20 ($9 + Xy_p$)**

The diploid complement of 20 chromosomes was observed at spermatogonial metaphase (Fig. 6). The karyotype is constituted by one pair of metacentric (pair 6), 4 pairs of submetacentric



Figs. 1-15: 1-3 *Pharoscymnus flexibilis*. 1. Spermatogonial metaphase, 2. Karyotype, 3. Metaphase I. 4,5. *P. horni*. 4. Spermatogonial metaphase, 5. Metaphase I. 6-10 *Illeis bistigmosa*. 6, Spermatogonial metaphase, 7. Karyotype, 8. Diakinesis, 9. Metaphase I, 10. Metaphase II with X. 11,12 *L cincta*. 11. Metaphase I, 12. Metaphase II with X. 13-15 *Coccinella transversalis*. 13. Spermatogonial metaphase. 14. Karyotype, 15. Metaphase I.

(pairs 1-4) and 4 pairs of acrocentric (pairs 5,7-9) autosomes and an acrocentric X chromosome (Fig.7). The X chromosome is seventh in order of size (Table 2). Diakinesis revealed four crosses, three rods and two rings in addition to the Xy_p (Fig. 8). Metaphase I depicted rod - and - ring shaped autosomal bivalents and the sex parachute (Fig. 9). Two types of metaphase II plates, one with y and the other with X (Fig. 10), were observed. Owing to separation of chromatids morphology of the chromosomes was clear at this stage. The description matches the observation at spermatagonial metaphase.

TABLE 2: Percentage relative length of chromosomes and TCL of karyotypes.

Species	1	2	3	4	5	6	7	8	9	X	Y	TCL
Pharoscymnus flexibilis	11.23	11.05	10.28	9.59	9.23	8.92	8.74	8.42	8.24	9.59	4.71	43.83
Illeis bistigmosa	15.56	13.25	11.94	10.69	10.06	7.59	6.96	6.55	5.75	7.27	4.58	31.37
Coccinella transversalis	13.94	13.07	11.75	9.72	8.07	7.41	6.99	6.62	6.21	12.83	3.39	28.40

***I. cincta* 2n = 20 (9 + Xy_p)**

Karyotypic details in this species were earlier described by Yadav et al. (1991) from Kurukshetra. The present material from Bangalore depicted only meiotic stages. Metaphase I depicted 9 dumbbell and-rod-shaped autosomal bivalents and the Xy_p (Fig. 11). The first meiotic division resulted in 2 types of metaphase II plates, one with X (Fig. 12) and the other with y chromosome. At metaphase II, morphology of chromosomes was clear due to separation of chromatids revealing 2 metacentric, 5 submetacentric and 2 acrocentric autosomes and an acrocentric X. The Bangalore population, thus, differs in morphological details from the Kurukshetra population which carried 2 metacentric, 4 submetacentric and 3 acrocentric autosomes and a metacentric X.

***Coccinella transversalis* 2n = 20 (9 + Xy_p)**

Spermatogonial metaphase exhibited the diploid complement of 20 chromosomes (Fig. 13). The karyotype comprised 6 pairs of metacentric (pairs 3,5-9) and 3 pairs of submetacentric (pairs 1,2,4) autosomes and a submetacentric X chromosome (Fig. 14). The X is fifth in order of size (Table 2). Metaphase I was constituted by 9 dumb-bell-and rod-shaped autosomal bivalents and the Xy_p (Fig. 15) Two types of metaphase II plates were observed, one with y and the other with X.

DISCUSSION

A perusal of the existing literature on family Coccinellidae elucidates the cytological data of 191 species belonging to 13 tribes of subfamilies Coccinellinae and Epilachninae (Dasgupta 1977, Mittal et al. 1989, Takenouchi 1976, Yadav et al. 1991, Yadav & Gahlawat 1992, 1993a, b,c. present report) Karyotypic variation ranges from 12 in *Mulsantina hudsonica* (Smith 1963) to 28 in *Ortalia pactoralis* (Yadav & Gahlawat 1993d). This family is characterized by the possession of typical primitive polyphagan modal karyotype 2n=20 (9 + Xy_p) possessed by 85 species. Four of the species under present investigation also depicted this karyotype. *P. horni* with 2n=22, however, deviates from the modal number.

The simple male sex chromosome mechanism Xy_p is the most common and widespread system depicted by 94 species. All the species under report uniformly possess this mechanism.

Yadav & Pillai (1974) reported the diploid number of chromosomes and sex chromosome mechanism in *I. cincta*, whereas Yadav et al. (1991) described the karyotypic details in this species from Kurukshetra population. The morphological details of the karyotype in specimens from Bangalore differed (*vide supra*). This has evolutionary implications, the change in morphology of one pair of autosomes and the X chromosome suggesting the involvement of pericentric inversions. This also calls for a detailed investigation of different populations.

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EFFECT OF FUNGICIDES (BAVISTIN AND DIATHANE M45) ON SOMATIC CELLS OF *PENNISETUM AMERICANUM*

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SUMMARY

An attempt has been made to study the effects of fungicides (Bavistin and Diathane M45) on the somatic cells of pearl millet (*Pennisetum americanum* Schum). It was noted that abnormalities like fragmentation, clumping and an increase in number of chromosomes were observed at mitotic metaphase. Clumping of chromosomes, disturbed metaphase, anaphase and telophase, occurrence of bi-, tri- and tetranucleate cells and micronuclei comprised the most dominant types. Cell vacuolation, polyploid cells and anaphase bridges were found less frequently. The cytological effects decreased with the decrease of concentration of fungicides. Bavistin caused greater frequency of abnormalities than Diathane M45.

Key Words: Fungicides, mutant, mitosis,

INTRODUCTION

The introduction of high yielding varieties has necessitated the use of plant protecting chemicals and now they have become indispensable. Among chemicals used in agriculture, the use of fungicides stands next to pesticides. Increased utilization of fungicides for crop protection in modern agriculture has raised the question whether these chemicals induce any detectable cytological damage to the cells. These studies will be beneficial not only from the viewpoint of understanding the mechanism of cytological damage and recovery but also its role in environmental pollution.

In the last 2 decades, it has been established that some fungicides induce chromosomal abnormalities in higher plants (Ramel 1969, Fiskesjo 1969, Ahmad & Grant 1972, Mohan 1975, Soliman & Al-Najjar 1980).

The present study deals with the studies on cytological effects of 2 fungicides, Bavistin and Diathane M45 on pearl millet.

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TABLE 1: Mitotic index and types and frequency of cytological aberrations in cells of pearl millet treated with fungicides.

Type of treatment	Conc. (%)	Total no. of cells examined	Mitotic index	Percentage of cells with							
				Laggards	Bridges	Binucleate cells	Nuclear disntigration	Cellular vacuolation/ marginal nuclei	Fragmentation	Clumping	Micronuclei
Bavistin	Control	1888	20.0	-	-	-	-	-	-	-	-
	0.05	2054	17.5	-	-	-	-	-	0.4	0.6	-
	0.10	2256	14.6	0.36	-	0.2	-	-	1.2	4.0	0.7
	0.25	2581	12.5	2.6	1.4	0.67	1.1	-	2.6	5.8	1.5
	0.50	2549	10.3	4.1	2.6	0.92	1.3	-	5.2	10.6	5.5
	1.0	2372	8.2	6.3	3.8	2.8	2.5	1.7	6.8	11.1	9.2
Diathane M45	0.05	2213	18.2	-	-	-	-	-	0.8	0.9	-
	0.10	2377	15.0	-	-	-	-	-	1.7	3.5	-
	0.25	2448	13.4	0.72	-	-	0.4	-	3.3	5.0	2.6
	0.50	2451	11.4	3.6	1.7	0.85	1.2	-	5.6	6.1	6.4
	1.0	2537	10.4	5.9	3.2	2.2	2.1	0.7	6.1	8.4	7.5

MATERIAL AND METHODS

Dry, dormant, pure line seeds of the variety giant bajra of *Pennisetum americanum* Schum. were soaked in distilled water for 4 h. The seeds were then immersed in Bavistin and Diathane M45 solutions of 0.05%, 0.10% , 0.25% 0.50% and 1.00% concentrations. The duration of seed treatment in all the cases was 24 h with intermittent shaking. At the end of the treatment, the seeds were washed thoroughly in running tap water. Treated seeds were sown in the nursery beds along with the control. Seeds were left to germinate in the laboratory conditions to have roots with a minimum length of 1 mm for cytological studies. After pretreatment in p- dichlorobenzene the root tips were fixed in acetic-alcohal (1:3) and stained in 2% acetocarmine.

RESULTS AND DISCUSSION

The most significant radiomimetic expression of these fungicides perceived during cytological investigation was chromosome breakage. Two types of fragments observed (micro and macro) might have originated either due to single breaks occurring in the terminal region or they were the outcome of incomplete exchange. A comparative number of these fragments were centric in nature and many of them might have been lost during the subsequent cell division but the presence of micronuclei indicated that at least a few of them might have participated in their formation at various concentrations of chemicals. The micronuclei might also have arisen as a result of misdivision of the nucleus. From the results, it is quite evident that the number of micronuclei was always less than the number of fragments. This indicated that more than one fragment was involved in the formation of single micronuclei. Bavistin produced more fragments and micronuclei

than Diathane M45, Wuu & Grant (1967b) stated that pesticides and chemicals may destroy, inhibit and enhance the synthesis of enzymes and so upset the cell system. The production of broken and damaged chromosomes are the possible effects.

A change in mitotic index was also noticed due to the treatment of these fungicides. These chemicals inhibited cell division in the treated roots as shown in Table 1. The effects of higher concentration is obvious from a decline in the mitotic index value with the increase in concentration and exhibiting a marked decrease at higher concentration. The effect is more pronounced with Bavistin than Diathane M45.

The clumped configurations which were very commonly present might have resulted due to stickiness of chromosomes arising out of disturbances created by fungicides at cytochemical level. In such cases, the shape and outline of chromosomes were not firm. The effect was more due to Bavistin than Diathane M45.

Bavistin treatment has resulted in greater frequency of abnormal anaphase cells at anaphase and telophase stages as compared to that of Diathane M45. A good number of cells were with lagging chromosomes with both the fungicides in almost all the concentrations of treatment. The percentage of abnormalities increased with the increase of concentrations. It may be noted that treatment with the present chemicals induced a considerable percentage of lagging chromosomes and chromosome fragmentations which might have induced micronuclei. The phenomenon of lagging chromosomes may be attributed to hindrance of the chromosome movement accompanied by adhesion of the centromeres to the nuclear membrane or the surrounding surface of the plasma. Adhesion of contromeres of one or more chromosomes to the outer layer of the plasma and movement of the others towards the equatorial plate lead to the appearance of such lagging chromosomes (Barthelmess 1957).

Bridges at anaphase was less common than other abnormalities. The formation of bridges might have been caused due to various inversions and translocations occurring as a result of chromosome and chromatic interchanges. In some cases, stickiness appeared to give rise to bridges. A very few of the bridges persisted till late anaphase and early telophase. Mohan (1975) also reported chromosome bridges with the treatment of fungicides Plantvax and Vitavax on *Allium cepa*.

Bavistin treatment has resulted in a greater frequency of abnormalities during anaphase and telophase stages as compared to that of Diathane M45. Besides the pycnotic cells, bi-, tri and tetranucleate cells were observed. Bavistin treatment has resulted in the induction of tri- and tetraploid cells and cell vacuolation, of course, in less number of cells.

In general, the cytological effects of these fungicides resemble the aberrations caused by radiomimetic chemicals, radiations and some other pesticides. These abnormalities have been reported with many fungicides, chemicals and pesticides by Wuu & Grant 1967a, 1967b. Amer & Farah 1969, Amer & Ali 1969, Mohan 1975, Singh et al. 1977, Sharma 1982.

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KARYOLOGICAL STUDIES IN *DIPCADI ERYTHRAEUM*

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SUMMARY

Dipcadi erythraeum Webb. et Berth., endemic to deserts of Rajasthan, is studied cytologically. The somatic chromosome number is found to be 2n=22 and the karyotype is of asymmetrical nature. Meiosis is found to be normal with a low pollen fertility.

Key Words : *Dipacadi erythraeum*, karyotype, meiosis.

INTRODUCTION

Dipcadi (Liliaceae) comprises about 55 species, distributed in Mediterranean region, Africa, Madagascar, Pakistan, Nepal and it is represented in India by about 9 species of which 3 are endemic (Deb & Dasgupta 1981). *Dipcadi erythraeum* Webb. et Berth. is one of the endemic species found in deserts of Rajasthan. Cytology of 4 Indian species, *D. montanum* (2n=20,12,10), *D. saxorum* (2n=12,14), *D. ursulae* (2n=20) and *D. concanense* (2n=12) has been worked out so far (Mahabale & Chennaveeraiah 1954, 1962 Naik 1954, Kanmani 1975, Dixit et al. 1992).

In the present investigation, an attempt has been made to study the karyotype and meiotic behaviour of *D. erythraeum*.

MATERIALS AND METHODS

The bulbs of *Dipcadi erythraeum* were collected from deserts of Rajasthan (Jasalmer) and planted in gardens of Botany Department of Shivaji University. The methods for making cytological preparations and karyotype analysis are the same as presented by Dixit et al. (1989).

The gradient index (GI) and symmetry index (SI) were calculated by using the following formulae (Pritchard 1967):

$$GI = \frac{\text{Length of shortest chromosome}}{\text{Length of longest chromosome}} \times 100$$

$$SI = \frac{\text{Total length of short arms}}{\text{Total length of long arms}} \times 100$$

For analysis and comparison of the karyotype, the chromosomes of *Dipcadi* were categorised into following types on the basis of their length and centromeric position.

A : Very long chromosome (6.5-8μm) with constriction at subterminal (st) region. A1 : As above but constriction at the submedian (sm) region. A2 : As above however, constriction at median (m) region. B : Long chromosomes (5-6.5 μm) with constriction at subterminal (st) region. B1 : As above but constriction at submedian (sm) region. C : Short chromosomes (3.5-5 μm) with constriction at subterminal (st) region. C1 : As above with constriction at submedian (sm) region. D : Very short chromosomes (3.5 μm) and constriction at subterminal (st) region. D1 : As above with constriction at submedian (sm) region. D2 : As above with constriction at median (m) region.

OBSERVATIONS

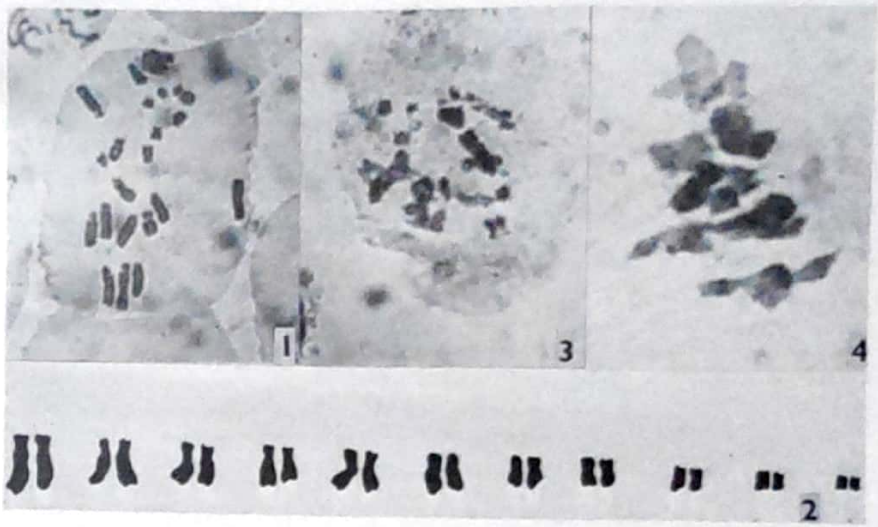
Somatic chromosome number of *D. erythraeum* is 22 (Fig.1). Its idiogram is presented in Fig. 4. By considering the above categorisation it is possible to classify *D. erythraeum* chromosomes as follows :

TYPE B : One pair of long chromosomes (5.24 ± 0.97 μm) with constriction at subterminal (st) region. TYPE C : Two pairs of short chromosomes (.17±0.84-4.51 ± 0.72) with constriction at subterminal (st) region.

TABLE 1: Karyotypic details of *Dipcadi erythraeum*.

Chrom. pair	Long arm (l) (μm)	Short arm (s) (μm)	Total length (μm)	'd 'value l-s (μm)	'r'value l/s (μm)	'i' value 100 s/c	Centromere position
I	4.23 (-0.90)	1.00 (-0.60)	5.24 (0.97)	3.23	4.21	19.16	st
II	3.53 (-0.87)	1.26 (-0.82)	4.79 (-0.69)	2.27	2.79	26.33	sm
III	3.39 (-0.75)	1.23 (-0.51)	4.63 (-0.42)	2.15	2.74	26.69	sm
IV	3.50 (-0.64)	1.01 (-0.86)	4.51 (-0.72)	2.49	3.45	22.43	st
V	3.33 (-0.80)	0.83 (-0.32)	4.17 (-0.84)	2.25	4.00	19.95	st
VI	2.21 (-0.43)	1.04 (-0.31)	3.75 (-0.78)	1.66	2.58	27.87	sm
VII	2.28 (-0.83)	0.93 (-0.33)	3.21 (-0.62)	1.35	2.45	28.91	sm
VIII	1.78 (-0.46)	0.86 (-0.28)	2.64 (-0.77)	0.92	2.07	32.47	sm
IX	1.63 (-0.63)	0.73 (-0.31)	2.37 (-0.55)	0.89	2.21	31.05	sm
X	1.24 (-0.36)	0.66 (-0.39)	1.91 (-0.38)	0.58	1.87	39.77	sm
XI	1.19 (-0.27)	0.52 (-0.07)	1.70 (-0.26)	0.62	2.32	30.04	sm

(GI = 32.49, SI = 34.49,S% = 32.49, TF% = 25.92, TCL% = 38.97)



Figs. 1-4: *Dipcadi erythraeum*. W 1. Somatic chromosomes (2n=22). 2. Idiogram. 3. Diakinensis showing 11 bivalents with nucleolus. 4. Metaphase I. (1-3x1450; 4x2500).

TYPE C1 : Three pairs as above (3.7-0.78-4.79 ± 0.42) with constriction at submedian (sm) region.

TYPE D1 : Five pairs of very short chromosomes (1.70 ± 0.07-3.21 ± 0.62) with constriction at submedian (sm) region.

Thus the karyotype formula of *D. erythraeum* is represented as : K: $2n=22 : 2B^{st} + 6C1^{sm} + 4C^{st} + 10\ D1^{sm}$

Karyotypic details of *D.erythraeum* are presented in Table 1. In meiosis, PMC showed 11 bivalents at diakinensis at metaphase I (Figs. 2.3). In general, pollen fertility was low. Precocious separation of few bivalents has been noticed in some pollen mother cells.

DISCUSSION

The majority species of the genus *Dipcadi* so far investigated from India revealed the somatic complement as 2n=12 and 20 (Machabale & Chennaveeraiah 1954, 1962, Naik 1974, Naik & Nirgude 1983, Dixit et al. 1992) and are mostly diploids. The somatic chromosome number, 2n=22, reported here for the first time in *D.erythraeum* is thus the highest number known so far in the Indian species of *Dipcadi*.

Apart from numerical variations of chromosomes, differences are also observed with respect to the number of submetacentric and subtelocentric chrosomes and haploid chromatic length, TF% GI and SI values in *D.erythraeum* (Table 1). The chromosome size varies from 5.24 to 11.70 µm in *D.erythraeum*. The length of the haploid complement is influenced by chromosome number as well as chromosome size. Analysis of data of length of chromosome is of immense value in understanding the evolutionary status of the taxa. Analytical studies of karyotype symmetry provide a valuable criterion to indicate the nature of evolutionary processes and trends in taxon in which evolution has taken place (Stebbins 1971). Low values of GI and SI in *D.erythraeum* indicate asymmetrical karyotype of '3B' category as observed in other species of *Dipcadi*. This indicates a relatively specialised status of the taxon under study.

D.erythraeum with 2n=22 appears to be secondarily evolved through aneuploidy at tetraploid level, which is further supported by its meiotic behaviour with low pollen fertility. In *Dipcadi*, meiosis is in general associated with some unusual events. This nature is not clearly understood (Levan 1944, Mahabale & Chennaveeraiah 1959, Naik & Nirgude 1982). However, Ruiz et al. 1981) attributed these irregularities to the phenomenon of gene duplication. Further it is supported by electrophoretic analysis of isozymes variability in natural population of *D. serotenum*, where 2 ADH loci and 2 esterase were found duplicated (Pascual et al. 1980, 1981).

The basic number for the genus *Dipcadi* is considered to be n=4 (Naik 1974). However, this is not tenable sicne 2n=6 is reported in *D.marlothii* from Natal (Ratter & Milne 1973) and basic karyotype proposed as 2VL + 1M pair for genus *Dipcadi*.

Considering the above view of basic karyotype, chromosome number and data from previous and present investigations it seems that genus *Dipcadi* is affected by polyploidy and structural alterations, at the same time possibility of hybridisation can not be ruled out.

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CYTOLOGICAL EFFECTS OF WATER EXTRACT OF *MORINGA OLEIFERA* ON *VICIA FABA* ROOT TIP CELLS

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SUMMARY

Aqueous extract of fruit of *Moringa oleifera* was evaluated for cytotoxicity using *Vicia* root meristem protocol. Five different concentrations of the extract were tested by treating the root tips for 2,4,6, 8 and 12 h. Decrease in mitotic indices and increase in per cent mitotic depression was found to be dependent on both concentration and the period of treatment. No significant change in prophase index, increase in metaphase index and decrease in ana- and telophase indices as compared to control suggests that the fruit extract interferes with the spindle mechanism and thus contain some stathmokinetic compound (s).

INTRODUCTION

Reports on cytotoxicity and clastogenicity of natural products in plant extract are on record (Tarkovska 1971, Srivastava et al. 1973, Hussein & Hakeem 1961, Shehab 1979, Adam & Rashad 1985, Medeiras & Takahashi 1987). *Moringa oleifera* which belongs to the family Moringaceae is grown for their fruits consumed in large quantities as green vegetable by Indian population. The gum obtained from the bark is of medicinal value and used in earache, headache and also in syphilis and rheumatism. Oil obtained from the seeds is used in acute rheumatism and gout. In this communication, we report the cytotoxicity of aqueous extract of *Moringa* fruit in *Vicia faba* root tip.

MATERIAL AND METHODS

The root tips of *Vicia faba* L. were used as experimental material. Fruit extract of *Moringa oleifera* was prepared by crushing 40 g of fruit chips in 100 ml of distilled water for 30 min in a grinder and left for complete extraction. After 12 h the extract was filtered through a fine cloth and the solution thus obtained was taken as 100% concentrated solution. Solutions of 4 different strength i.e. 10, 25, 50 and 75% was prepared by diluting 100% solution in distilled water. Actively growing young roots were treated with each concentration for 2, 4, 6, 8 and 12 h at room temperature. Parallel control was maintained in all the cases in distilled water. The control as well as treated roots were excised, thoroughly washed, fixed in methanol-acetic acid (3:1) for 24 h and stored in 70% alcohol. For cytological preparation, roots were hydrolysed, stained in 1.5% aceto-orcein and squashed on a clean slide. In each case 4500 cells were observed in 10 microscopic field from 5 slides. Data on the frequency of mitotically dividing cells and cells in different phases of mitotic cycle were recorded. Mitotic index, prophase, metaphase, anaphase and telophase indices were calculated for each treatment. Student's t-test and ANOVA were carried out for statistical analysis.

TABLE 1: Mitotic indices of *Vicia faba* root tip cells treated with different concentration of *M. oleifera fruit extract and the respective control at various time of treatment.*

Period of treatment (h)	Number of cells scored	Concentration (%)					
		0	10	25	50	75	100
0	4500	19.35	18.75	19.20	19.15	18.60	19.30
2	4500	18.65	18.35	14.10*	15.45	12.35**	10.15**
4	4500	19.10	18.20	16.30	14.20*	12.68**	9.30**
6	4500	19.15	17.15	12.20**	10.10*	9.80	7.35**
8	4500	18.60	11.30*	9.75**	7.70*	6.18**	4.19**
12	4500	19.10	13.10*	7.10**	6.15**	4.30**	2.20**

Analysis of Variance

	df	Mean square	F
Between treatments	5	84.70	15.01**
Between periods	5	87.98	15.60**
C. D. at 5%		3.56	

*,** significant at 0.05 and 0.01 levels respectively.

RESULTS AND DISCUSSION

The results showed that the treatment with the fruit extract produced significant effect on the cell division and the distribution of cells in different phases of the mitotic cycle. It can be seen in Table 1 that the treatment caused significant reduction in the mitotic index. The inhibition in the mitotic index is correlated with both the duration of the treatment and concentration of the solution. The effect of treatment was most pronounced in the 12 h duration with all the concentrations. After this period of treatment the minimum percentage of mitotic depression was 30.13 at 10% and the maximum 88.60 at 100% concentration (Fig. 1). Slow growth of roots, nuclear vacuolation, pycnosis and chromatin granulation were of frequent occurrence. No significant effect on mitotic index has been seen at 10% concentration up to 6 h but after that the mitotic depression increased considerably (Fig.1). The value of C.D. at 5% level showed that in most of the cases the mitotic index of the control and the treated group differ significantly from each other.

Table 2 shows the effect of 12 h treatment on prophase index (PI), metaphase index (MI), anaphase index (AI) and telophase index (TI). All the treatment resulted in considerable alteration in the distribution of cells is different phases of mitotic cycle. In all the treated groups M I was found to be higher than the control. Prophase indices of the treated group did not differ significantly from the control as well as from each other, nevertheless, all treated groups showed considerably low values of AI and TI.

The aqueous extract of *Moringa fruit* exerted a marked mitodepressive effect and caused imbalance in the frequency of mitotic phases in the root-tip cells of *Vicia faba*. The degree of

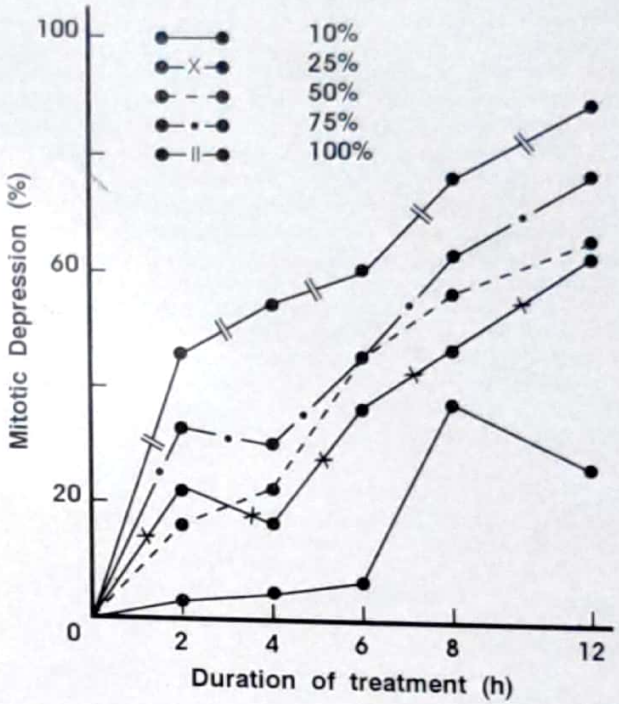

Fig. 1: Per cent mitotic depression in *Vicia* root tip cells treated with the following concentrations of *Moringa oleifera* fruit extract.

TABLE 2: Prophase, metaphase, anaphase and telophase indices after 12 hours treatment with different concentration of *M. oleifera* fruit extract.

Concentration (%)	Total number of dividing cells	Per cent of mitotic phases			
		Prophase index	Metaphase index	Anaphase index	Telophase index
0	850	57.29	22.35	15.41	4.70
10	589	56.87	26.31	12.05	4.75
25	320	58.50	30.56	7.81	3.12
50	277	58.95	38.74	10.24	2.88
75	195	55.35	40.10	4.55	-
100	99	52.45	45.25	2.20	-

mitotic depression is clearly dependent on the concentration of the extract and the duration of the treatment. The inhibition of mitosis by natural compounds present in plant extract has also been reported earlier (Tarkovaka 1971, Shehab 1979, Adam & Rashad 1984, Medeiras & Takahashi 1987). Mitotic depression has been attributed to various metabolic disturbances in the internal milieu of the cells due to effect of treatment. These disturbances generally lead to inhibition of cell division and in turn are believed to be the effect of treatment on the synthesis of DNA, RNA, protein and energy. These results in arrest of cells in G_0 stage or a retardation in the pace of events during S and G_2 phases. No significant changes in P I of the treated groups as observed in the present study indicates that the extract does not interfere with the normal metabolic processes taking place in the interphase stage. Nevertheless, considerably higher value of M and lower value of A I and T I indicates that the treatment caused blockage at metaphase stage and sufficiently large number of cells which could have entered the second division cycle are stopped from doing so, thus reducing the mitotic indices. It seems that the extract upon entering the cell, interferes with the mechanism of formation of the mitotic spindle and/or causes hindrance in the chromosome splitting from kinetochore and thus retarding their movement towards the pole. The results obtained in the present study clearly indicate the presence of some cytotoxic or mitodepressive compounds in the aqueous extracts of *Moringa* fruit. Since the effect of the compound is on spindle mechanism so it may be considered as a stathmokinetic.

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DIFFERENTIATION OF X CHROMOSOMES IN SOME INDIAN ANOPHELINES

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SUMMARY

The karyotype studies of mitotic and meiotic chromosomes of 13 Indian anopheline species have revealed significant differences in the sex chromosomes and in particular X chromosomes. These differences are largely due to the position of the centromere, and the variable position and amount of the X chromosome heterochromatin and euchromatin. These findings have revealed the X chromosome differentiation among the species under study.

Key Words: X chromosome differentiation, chromosome bandings, speciation.

INTRODUCTION

The application of chromosome banding studies on anopheline species has shown that the size and shape of heterochromatic sex-chromosome varies both at inter and intraspecific levels. However, autosomes do not show such a variation. Therefore, sex chromosome morphology has been found to be more useful than autosomal morphology for species identification (Bonaccorsi et al. 1980, Marchi et al. 1980, Baimai et al. 1984, Wibowo et al. 1984, Green et al. 1985a, Baimai & Traipakvasin 1987, Marchi & Mezzanotte 1988, 1990).

The present investigation was undertaken to analyse the metaphase chromosome karyotypes of some commonly available species of the genus *Anopheles* from South India, based on G-, Q- and R-banded patterns. It was also aimed to analyse the probable mode of X chromosomal differentiation that might have occurred during the course of the divergence.

MATERIALS AND METHODS

The various anopheline species used for the analyses and the localities of collection made are presented in Table 1. The gonadal and neuroblast cells of larvae and pupae were utilised for the preparation of chromosomes. Gravid females collected from the field were identified and transferred to the insectary, and were allowed to deposit eggs into individual vials. The larvae and pupae of individual females were reared in separate pans.

The modified air-drying technique of Hungerford (1971) was employed for the preparation of mitotic and meiotic chromosomes (Chowdaiah & Venkatachalaiah 1987). Chromosome preparations were subjected to G-, Q- and R-banding following the procedures of Sumner et al. (1971), Caspersson et al. (1971) and Lubs et al (1976) respectively. Karyotypes were prepared by following the procedure of Avirachan et al. (1969). Zeiss photomicroscope III was used for both light and fluorescence microscopy.

TABLE 1: Classified list of species belonging to the genus *Anopheles* used in the present study and their sources of collection made in South India.

Subgenus-Group/Series	Species	Collection site
Anopheles-		
Anopheles	*A. aitkenii*	The Nilgiris, Tamil Nadu
Anopheles	*A. gigas*	-do-
Myzorhyncus	*A. nigerrimus*	Bangalore, Karnataka
Cellia-Myzomyia	*A. aconitus*	Mandya, Karnataka
Cellia-Myzomyia	*A. culicifacies*	-do-
Cellia-Myzomyia	*A. fluviatilis*	-do-
Cellia-Myzomyia	*A. jeyporiensis*	Mercara, Karnataka
Neomyzomyia	*A. leucosphyrus elegans*	The Nilgiris, Tamil Nadu
Pseudomyzomyia/(Pyretophorus)	*A. subpictus*	Bangalore
Pseudomyzomyia/(Pyretophorus)	*A. vagus*	-do-
Neocellia	*A. annularis*	-do-
Neocellia	*A. stephensi*	-do-

OBSERVATIONS

The karyotype of each species investigated is characterised by the presence of 3 pairs of individual chromosomes, designated as 1, 2 and 3 based on the total length and the position of the centromere. The smallest pair is designated as sex chromosome pair (XX-female and Xy-male) and the remaining 2 pairs of mediocentric chromosomes as autosomes 2 and 3. The latter 2 pairs differ in their length. The sex chromosomes in *Anopheles stephensi*, *A. annularis*, *A. vagus*, *A. fluviatilis*, *A. culicifacies*, *A. jeyporiensis*, *A. aconitus* and *A. nigerrimus* appear to be submetacentric with arms of unequal length. In the case of *A. subpictus*, *A. aitkenii*, *A. gigas*, *A. leucosphyrus elegans* and *A. tessellatus*, the sex chromosomes appear to be acrocentric with identifiable short arms (Figs. 1-3).

The differentiatial staining of chromosome profiles (G- and Q-bands) are helpful in distinguishing the 2 autosomal pairs within the complement of each species. The banding patterns obtained by the use of both G-and Q-banding always offered corresponding results, whereas the R-banding resulted with a reverse banding pattern. In support of the observations of these results, the photograph of each metaphase complement and the corresponding karyotype of *A. stephensi* is presented (Fig. 1). For other species, only the representative G- banded complements of each species are presented (Fig. 2). In addition, a diagrammatic representation of the X chromosome of each species is also included for its corresponding banding pattern (Figs. 3-5).

The X chromosome identified by G-, Q- and R-banding patterns can be used for species identification. The Y chromosome morphology appears more condensed and higly heteropycnotic. Moreover, it does not provide clear banding patterns for species identification. In the present investigation, a detailed description of G- banded patterns of X chromosome alone is described for chromosomal characterization (Table 2).

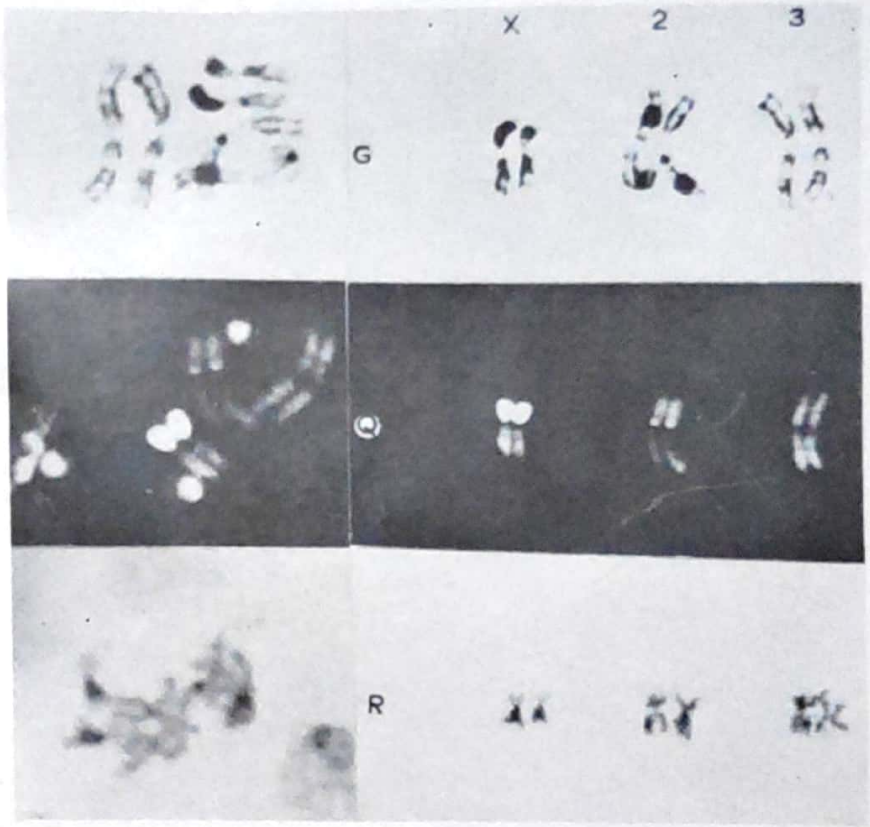

Fig. 1: Meiotic chromosomes of *Anopheles stephensi*. G-ASG-banded metaphase chromsomes and karyotype of male cells (testis) from fourth instat larvae. Q-banded metaphase chromosomes and karyotype of female cells (ovary) from fourth instar larvae. R-banded metaphase chromosomes and karyotype of female cells (ovary) from fourth instar larvae.

DISCUSSION

During the earlier cytogenetic studies of anopheline mosquitoes much emphasis was given for the role of polytene chromosome banding sequences in the understanding of speciation, systematics and evolution (Kitzmiller 1976, White 1980). It is interesting to note that the cladistic approaches employed to elucidate polytene chromosome arrangements of these species of anophelines beloinging to Afro-tropical and Oriental regions have demonstrated interspecific

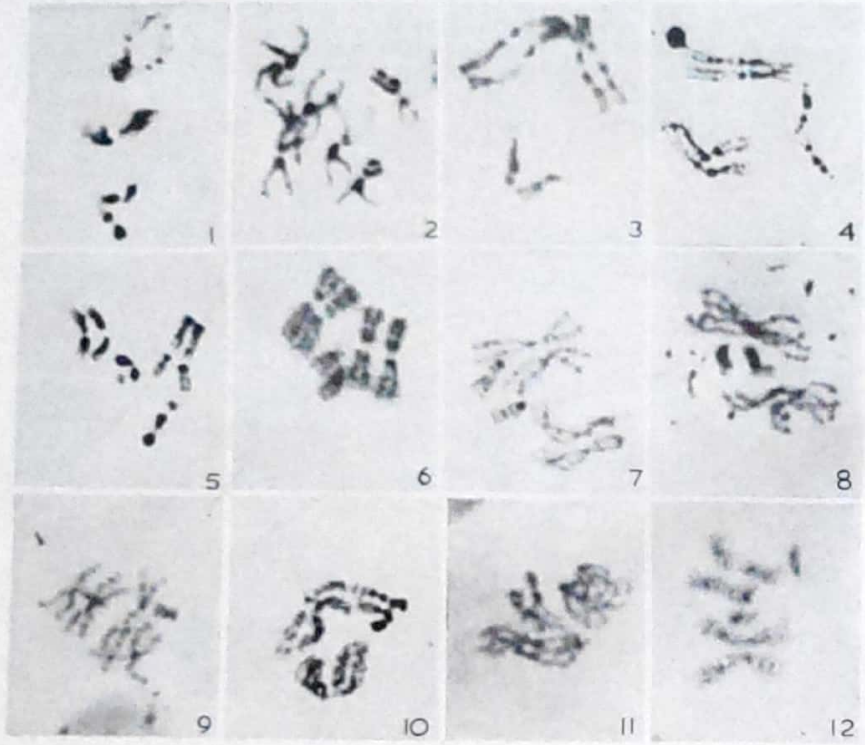

Fig. 2: Representative G-banded metaphase chromosome complement of 1. *Anopheles annularis*, 2. *A. vagus*, 3. *A. subpictus*, 4. *A. fluviatilis*, 5. *A. culicifacies*, 6. *A. jeyporensis*, 7. *A. aconitus*, 8. *A. leucosphyrus elegans*, 9. *A. tessellatus*, 10. *A. nigerrimus*, 11. *A. aitkenii*, 12. *A. gigas*.

relationships within the subgenus *Cellia*. These studies have implied the role of paracentric inversions in understanding the polytene chromosomal basis of karyotype evolution and species differentiation (Green et al. 1985a, b).

However, these polytene chromosome studies have permitted only a part of cytological analysis, since a considerable part of the genome is found in a state of under-replication during the course of polytenization. It is not obvious that the under-replicated parts of the genome represents the constituents characterised biochemically as satellite DNA of the total genome and known

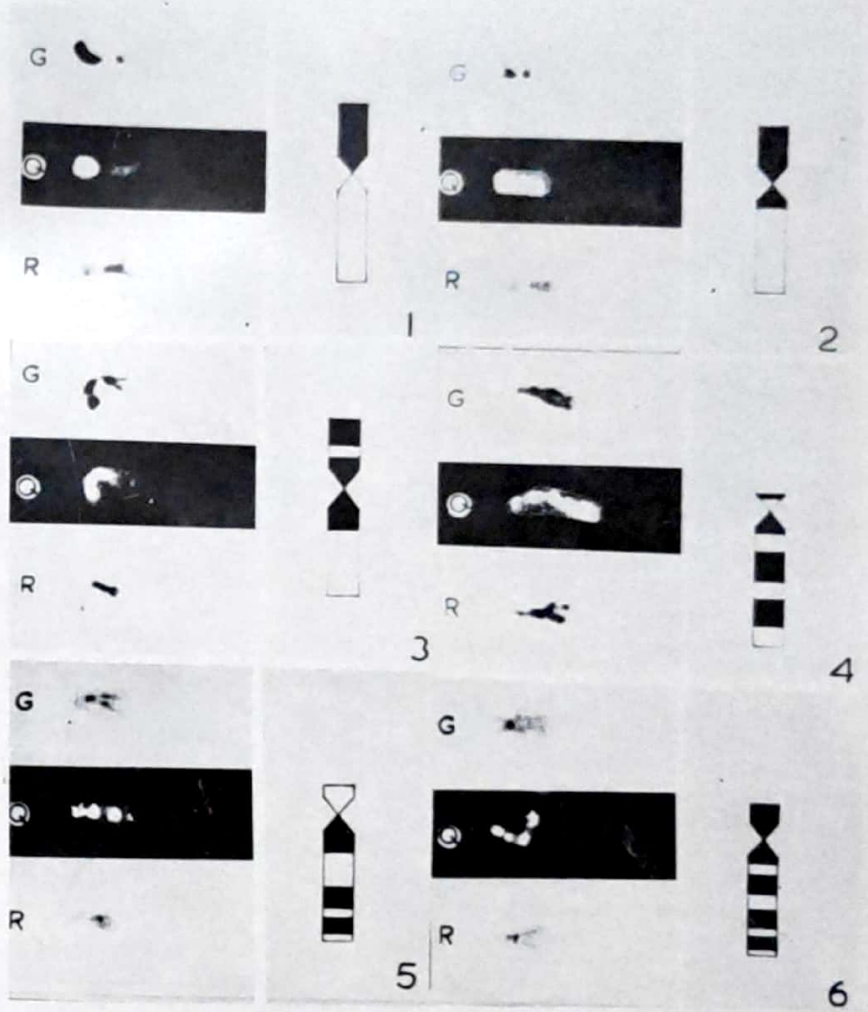

Fig. 3: Cut out X chromosome of G-, Q- and R-banding patterns with a composite diagrammatic representation of 1. *Anopheles stephensi*. 2. *A. annularis*. 3. *A. vagus*. 4. *A. subpictus*. 5. *A. leucosphyrus elegans* 6. *A. tessellatus*.

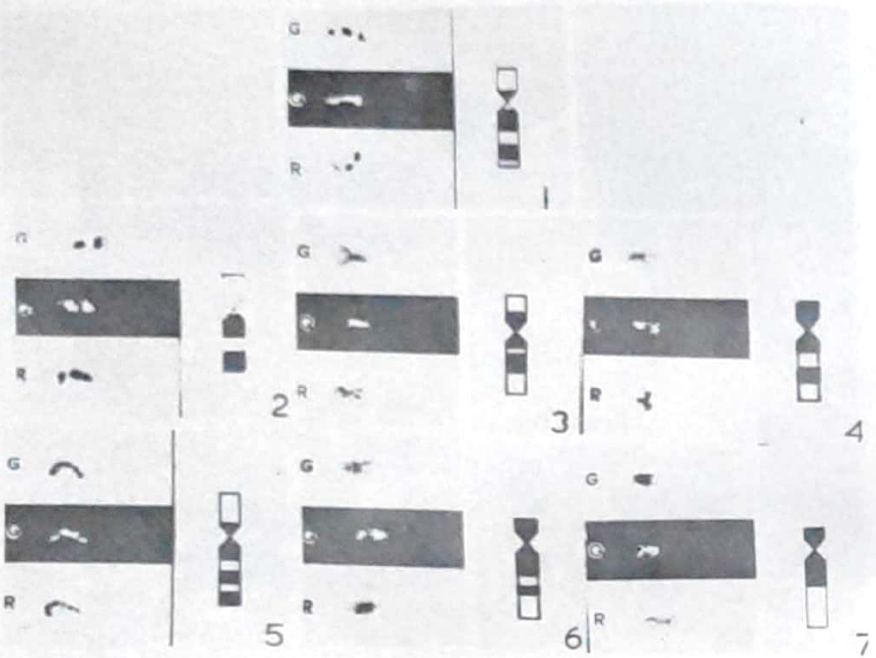

Fig. 4: Cut out X chromosome of G-, Q- and R-banding patterns with a composite diagrammatic representation of 1. *Anopheles fluviatilis*. 2. *A. culicifacies*. 3. *A. jeyporensis*. 4. *A. aconitus*. 5. *A. nigerrimus*. 6. *A. aitkenii*. 7. *A. gigas*.

cytologically as constitutive heterochromatin. Hence, these portions are not included during polytenization and as such excluded from the total genomic analysis (Redfern 1981).

In recent years, the application of Giemsa and fluorescent dye staining of mitotic and meiotic chromosomes of certain dipterans including mosquitoes have offered useful information pertaining to the variations in the amount and location of constitutive heterochromatin content for differentiating karyotypes of closely related species (Leumeniuer et al. 1978, Bonaccorsi et al. 1980, Motara 1982, Mezzanottee & Ferrucci 1983, Marchi & Rai 1986, Mays 1987).

The karyotype and heterochromatic X chromosome compositional differences observed in the present study are of primary importance for understanding the chromosomal differentiation in the sympatrically distributed South Indian anophelines. In the present study, the structural integrity of the autosomes and the plasticity of the sex chromosomes with particular reference to X chromosome has been observed.

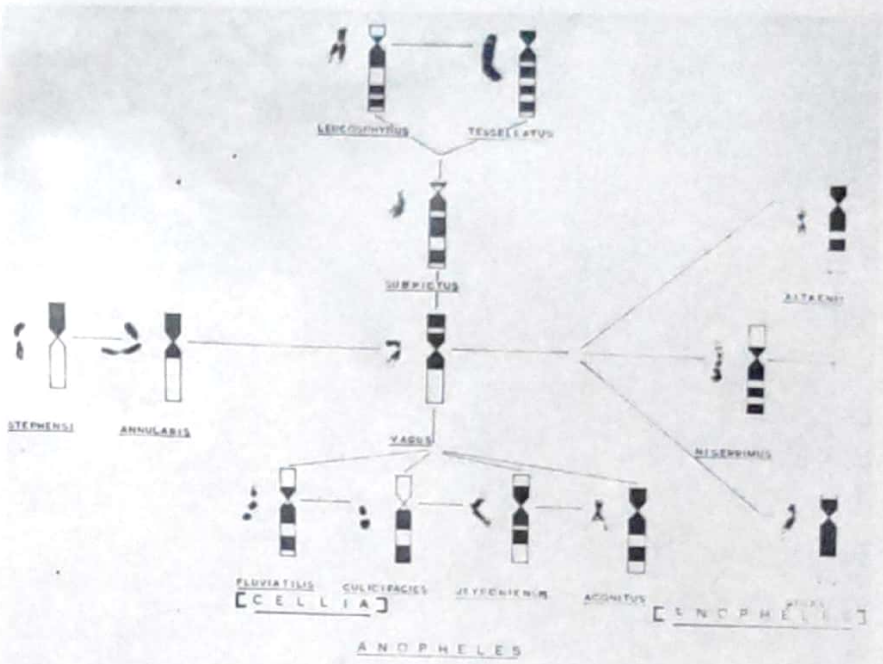

Fig. 5: Diagrammatic representation to show the probable phylogenetic relationship of the 13 anopheline species studied based on their X chromosome differentiation.

In the present case, the use of G-, Q- and R-banding patterns on metaphase chromosomes of 13 species have helped to draw a tentative scheme for X chromosome divergence (Fig. 5). The G-banding sequences of *A. stephensi* is chosen as an arbitrary standard since it possesses simple pattern through which other species could have possibly be derived. Such an assumption is based on the fact that the state of X chromosome of different species can be constituted by involving at least one pericentric inversion for a change in the centromeric position to characterise a species. This species-specific character is subsequently stabilized by the occurrence of one or more paracentric inversions.

Based on present observations, the differentiation of anopheline X chromosome could be made by deriving the *annularis* type from the *stephensi* type by involving 1 pericentric inversion during the course of X chromosome evolution. *A. annularis* state can then be used to derive the *vagus* type by involving both 1 pericentric and 1 paracentric inversion. As such *vagus* appears to be a common point for the origin of other species of the genus by the involvement of inversions at different times during the course of evolution. Thus, the X chromosome of *subpictus* can be derived

from the *vagus* by involving 1 pericentric and 2 or more paracentric inversions. At the same time, the *subpictus* type can be used for the X chromosome derivation of *leucosphyrus elegans* and *tessellatus* through one pericentric inversion to begin with and subsequently by 2 paracentric inversions. However, other variable modes of arrangements of paracentric inversions leading to 2 different patterns characterising each status is also possible. Thus, the *vagus* type of X chromosome might be the possible basis for deriving *fluviatilis, culicifacies, jeyporiensis* and *aconitus* status involving 1 peri- and 2 or more paracentric inversions.

The *vagus* X chromosome can also be used as a common point of origin for deriving the species belonging to subgenus *Anopheles* including *nigerrimus, aitkenii* and *gigas*. As such the *nigerrimus* type can be derived from the *vagus* type through 1 pericentric and 3 or 4 paracentric

TABLE 2: The X chromosome G-banding patterns were designated as dark, medium and light band based on their staining profiles of intense, moderate and lightly-stained portions respectively. The banding description starts from the distal end of the short arm (P) to the distal end of the long arm (Q). X chromosome type is shown is parenthesis sm = submetacentric, a = acrocentric.

Species	Short arm (P)	Long arm (Q)
A. stephensi (sm)	Consists of dark band	Includes a medium band
A. *annularis* (sm)	Includes a dark band	Proximal end includes dark band and the following portion consists of medium band
A. *vagus* (sm)	Prominent dark bands with a middle light band	Proximal portion includes dark band and the remaining portion of medium band
A. *subpictus* (a)	A distal instense band	The proximal, middle and distal portions contain a dark band, each separated by a light band
A.*leucosphyrus-elegans* (a)	Includes a light band	Includes proximal dark, then a light, followed by two dark bands
A. tessellatus (a)	Is represented by a dark band	Four prominent dark bands, each separated by a light band on the distal end
A. *fluviatilis* (sm)	Distal end includes a medium and proximal dark band	Includes a proximal and a middle dark band, each followed by a distal light band
A. culicifacies (sm)	Includes a light band	Is characterised by the presence of proximal dark, middle light and distal dark bands
A. *jeyporiensis* (sm)	A dark, light and a proximal dark band	Proximal to middle region consists of two prominent dark bands followed by a light and a distal medium band
A. aconitus (sm)	Includes a light band	Includes proximal dark, middle a light and two dark bands followed by a distal light band
A. nigerrimus (sm)	Distal to middle by a medium & proximal by dark band	Characterised by three distinct dark bands, each separated by a light band
A. aitkenii (a)	Consists of a dark band	Has a proximal prominent dark band, followed by a light band and the distal region includes a dark and a light band
A. gigas (a)	Includes a dark band	Proximal to middle by a prominent dark band followed by a light band

inversions and in the case of *aitkenii* and *gigas*, through 1 pericentric and 2 or 3 paracentric inversions in appropriate combinations.

Thus, it can be stated that the repositioning of X chromosome heterochromatin in relation to euchromatin in the total chromosome morphology will reflect the derived state of species-specificity. King (1980) while endorsing White's (1978) hypothesis of 'Karyotypic Orthoselection' has generalized the chromosome lineages assuming that a particular type of chromosome rearrangement could have dominated over the other types during the process of speciation. In the light of the above theory and also other related works carried out on the interspecific differences in the polytene chromosomal vaiations for anopheline mosquitoes, it is suggested that the paracentric variations may form the major force for chromosome rearrangement during the process of speciation. Based on the present data, it can also be suggested that the pericentric and paracentric inversions may be the causative factors in the X chromosome heterochromatin differentiation.

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EVALUATION OF F_1 HYBRIDS USING NEWLY EVOLVED SEX-LIMITED BIVOLTINE SILKWORM RACES OF *BOMBYX MORI*

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SUMMARY

In the present investigation, through hybridization 15 quantitative characters of the F_1 hybrids were analysed for the first time involving newly evolved Chinese 8605, 8615 sex-limited and 8608 sex-limited and 8618 normal marking Japanese varieties of *Bombyx mori* with known genotype ZW+^{P} PP, ZW+ ^{P}+^{P} females and ZZ PP, ZZ +^{P} +^{P} males. The quantitative characters of the parents and their hybrids were compared with the check variety hybrid currently used in summer and autumn breeding programmes. The use of sex-limited varieties in the breeding programme has been discussed.

Key Words: *Bombyx, mori*, sex-limited hybridization, quantitative characters.

INTRODUCTION

China is the first country in the world to have known the rearing of silkworm, *Bombyx mori* with mulberry leaves, to reel cocoons and to weave silk fabrics. In China, change of traditional silkworm breeding method to scientific techniques evolved during the recent years has enabled synthesis of high productive supersilk silkworm varieties suitable to local conditions and agronomical practices in China with desirable genotypes of known genetic constitution with an objective to increase not only quantity of silk but also quality of superior silk to cater the international market. Hence, after the reform, Chinese silk industry has made a remarkable progress in both silk production and exploration. In the present investigation, through hybridization, an attempt has been made to analyse 15 quantitative characters such as fecundity, hatching percentage, duration of all instars, duration of the fifth instar, cocoon crops per 10000 fourth instar silkworm, larvae cocoon shell weight per 10000 fourth instar silkworm, cocooning ratio to the fourth instar silkworm larvae, rate of dead worms and cocoons, larva-pupa rate, cocoon weight, cocoon shell weight, cocoon shell rate, filament weight, filament length and size of the filament of sex-limited varieties of Chinese 8605, 8615 and sex-limited Japanese 8608, normal marking 8618 silkworm *B. mori* and their hybrids. Further, there is no documentary evidence at present with reference to use of these sex limited varieties of *B. mori* in breeding programmes for commercial exploitation. Hence, in the present paper a comparative sequential investigation on above 15 quantitative

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characters of parents and their hybrids has been made and compared with the performance of the present check varieties Xinhang x Keming hybrids. This investigation may provide important evidence to use these sex-limited varieties in the breeding programme of summer and autumn rearing in the coming year to produce superior high grade silk.

MATERIALS AND METHODS

The 4 silkworm varieties of *Bombyx mori* viz., 8605, 8615, 8608 sex-limited and 8618 normal marking with known genotypes and phenotypes were used as the parent materials for the present investigation. Among them, 8605 and 8615 are Chinese bivoltine varieties spinning white oval cocoons. The remaining 8608 and 8618 are Japanese bivoltine varieties spin white dumber cocoons.

The positive and negative crosses were made by utilizing the above 4 varieties. The first positive cross involves Chinese normal marking sex-limited XW+p pp 8605 female and Japanese normal marking ZZ+p+p 8618 male. The negative cross involves Japanese normal marking ZW+p+p 8618 female and Chinese plain ZZ pp 8605 male.

The second positive cross involves Chinese normal marking sex- limited ZW+p pp 8615 female and Japanese plain ZZ pp 8608 male. The negative cross involves Japanese normal marking ZW+p pp 8608 female and Chinese plain ZZ pp 8615 male.

During the preparation of hybrid layings synchronisation of moth emergence was achieved by brushing and commencing the rearing of the above parent varieties at the same time. The sexes were separated during pupal stage and the moths were isolated immediately after emergence for the crossing experiments. After copulation the moths were despaired and kept in the dark for egg laying at 25°C and relative humidity 70-80%. The eggs were collected and subjected to acid treatment within 20 h of oviposition.

The disease free laying of the F_1 hybrids and respective parents varieties were incubated at a temperature 25±1°C with 75-85% (R.H.) until hatching. The cellular rearing of replicates of 5 each was conducted by feeding Hu Sang 197 mulberry variety leaves harvested from the garden maintained in the Zhejiang Agricultural University campus following the general rearing methods. While rearing disinfectant No. 1 powder was sprinkled on the body of the ant worms immediately after brushing. This procedure is applicable to all the age silkworm larvae immediately after respective moults and also to the matured worms before placing on to the montages. This powder was used to prevent muscardine disease during the larval development.

The rearing data pertain to the 15 quantitative characters namely - fecundity, hatching percentage, duration of all instars, duration of fifth instar, cocoon crops per 10000 fourth instar silkworm, cocoon shell weight per 10000 fourth instar silkworm, cocooning ratio to the fourth instar silkworm larvae, rate of dead worms and cocoons, larva-pupa rate, cocoon weight, cocoon shell weight, cocoon shell rate, filament weight, filament length and size of the filament were compiled to assess the performance of the parents and their F_1 hybrids.

The data assembled were analysed by employing the following statistical methods: The data pertaining to the expression of heterosis and overdominance in F_1 hybrids with regard to quantitative characters under study in the respective crosses were estimated by employing the formula:

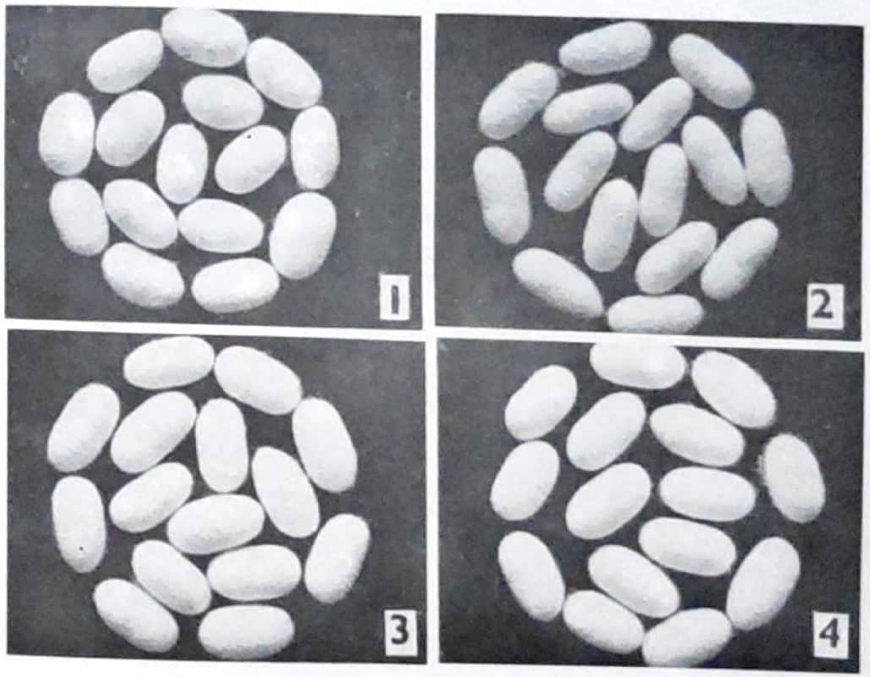
$$H(\%) = \frac{F_1 - MP}{MP} \times 100 \qquad OD(\%) = \frac{F_1 - BP}{BP} \times 100$$

Where H (%) = percentage of heterosis; OD (%) = percentage of overdominance; F_1 = mean value of the hybrid; MP = mean value of the mid parent; BP = mean value of the better parent.

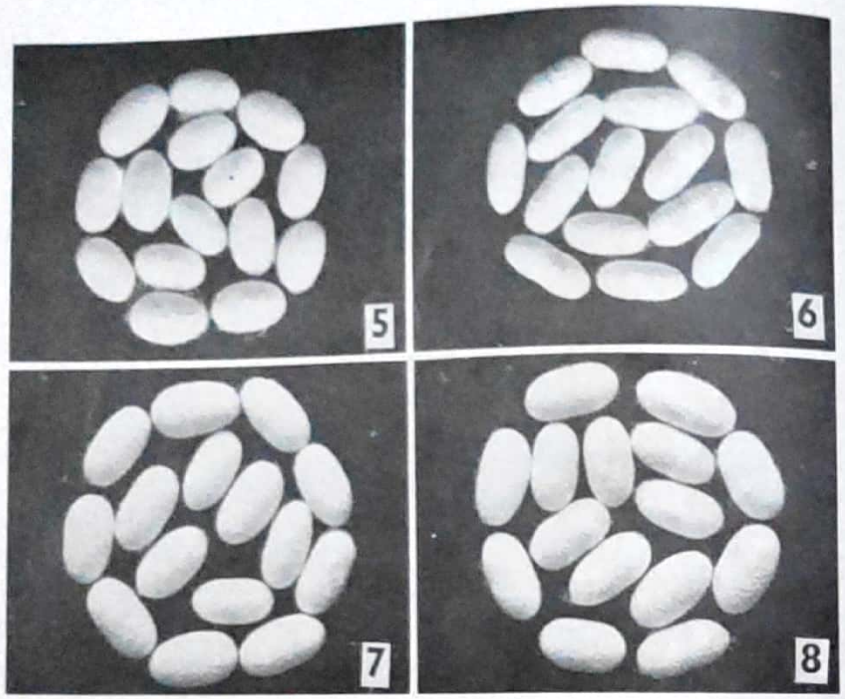
OBSERVATIONS

The mean values of 15 quantitative characters of parental varieties and 2 single cross F_1 hybrids along with heterosis and overdominance are presented in Tables 1 and 2. In addition, the photographs of cocoons spun by the parents 8605, 8615, 8608, 8618 and their hybrids are presented in Figs. 1-8. The genotypic and phenotypic expressions of the larval characters were represented in Figs. 9 and 10.

The performance of the parent varieties used in the breeding experiments and their hybrids obtained during summer and autumn rearing season in China with respect to increase or decrease of 15 quantitative characters were presented below:



Figs. 1-4: Cocoons of *Bombyx mori*. 1. P1 8605 Chinese variety 2. P2 8618 Japanese variety 3. F_1 hybrids of the positive cross 8605 x 8618 4. F_2 hybrids of the negative cross 8618 x 8605.



Figs. 5-8: Cocoons of *Bombyx mori*. 1. P1 8615 Chinese variety 2. P2 8608 Japanese variety 3. F_1 hybrids of the positive cross 8615 x 8608 4. F_2 hybrids of the negative cross 8608 x 8615.

Fecundity

The F_1 hybrids of the 2 single crosses 8605 x 8618 and 8615 x 8608 revealed a mean fecundity rate of 663.67 eggs, heterosis 21.32%, overdominance 5.17% and 659.33 eggs and heterosis 31.16% with overdominance 30.99% respectively (Tables 1 & 2). On the other hand, comparison of fecundity rate among the parent varieties, the Japanese 8608 and 8618 revealed highest mean fecundity rate.

Hatching percentage

The F_1 hybrids of the single cross 8695 x 8618 showed a mean hatching percentage of 98.52%, heterosis 1.58% with overdominance 0.73% (Table 1). The F_1 hybrids of the other cross 8615 x 8608 revealed a mean hatching percentage of 97.25% with heterosis 1.12% and overdominance -0.092% (Table 2). Further, hatching percentage recorded in Chinese parents was very high.

POSITIVE CROSS

CHINESE		JAPANESE
8605 FEMALE		8618 MALE
SEX-LIMITED	X	NORMAL
NORMAL MARKING		MARKING
$ZW+^{P}$ PP		$ZZ+^{P}+^{P}$

F_1 HYBRIDS

$ZW+^{P}+^{P}P$ FEMALE	$ZZ+^{P}P$ MALE

ALL NORMAL MARKING

DARK	LIGHT

NEGATIVE CROSS

JAPANESE		CHINESE
8618 FEMALE		8605 MALE
NORMAL MARKING	X	PLAIN
$ZW+^{P}+^{P}$		ZZ PP

F_1 HYBRIDS

$ZW+^{P}P$ FEMALE	$ZZ+^{P}P$ MALE

ALL NORMAL MARKING

Fig. 9: Genotypic and phenotypic expressions of the parents (8605, 8618) and their F_1 hybrids.

Duration of all instars

The F_1 hybrids of the two single crosses 8605 x 8618 and 8615 x 8608 showed 560 h, heterosis -0.70%, overdominance 0.70% and 559 h, heterosis -0.99% and overdominance 0.88% respectively (Tables 1, 2). There was no significant variation observed among the parental varieties but marginal variation was noticed in hybrids.

POSITIVE CROSS

CHINESE		JAPANESE
8615 FEMALE		8608 MALE
SEX-LIMITED	X	PLAIN
ZW+^{P} PP		ZZ PP

F_1 HYBRIDS

ZW+^{P} PP FEMALE	ZZ PP MALE
NORMAL MARKING	PLAIN

NEGATIVE CROSS

JAPANESE		CHINESE
8608 FEMALE		8615 MALE
SEX-LIMITED	X	SEX-LIMITED
NORMAL MARKING		PLAIN
ZW +^{P} PP		ZZ PP

F_1 HYBRIDS

ZW +^{P} PP FEMALE	ZZ PP MALE
NORMAL MARKING	PLAIN
SEX-LIMITED	SEX-LIMITED

Fig. 10: Genotypic and phenotypic expressions of the parents (8608, 8615) and their F_1 hybrids.

Duration of fifth instar

The mean fifth instar larval duration of the F_1 hybrids of the cross 8605 x 8618 revealed that 192 h with heterosis 1.31%, overdominance -2,040% (Table 1). The other cross 8615 x 8608 showed 19 h following heterosis -2.55% with overdominance -2.55% (Table 2). There was no significant variation observed among the parental varieties.

Cocoon crops per 10000 fourth instar silkworm

The mean cocoon crops per 10000 fourth instar silkworm of the F_1 hybrids of the cross 8605 x 8618 showed 19.671 kg with heterosis 58.41% and overdominance 52.09% (Table 1). The F_1 hybrids of the other cross 8615 x 8608 revealed mean value of 17.92 kg following 38.48% heterosis and overdominance 29.58% (Table 2). There was an increase in the trait observed in Chinese varieties.

Cocoon shell weight per 10000 fourth instar silkworm

The F_1 hybrids of the cross 8605 x 8618 registered a mean cocoon shell weight of 4.46 kg with 69.73% heterosis and overdominance 59.94% (Table 1). The hybrid of the single cross 8615 x 8608 revealed a mean cocoon shell weight 3.94 kg following heterosis 38.47%, overdominance 26.70% (Table 2). Further, the Chinese varieties revealed highest cocoon shell weight.

TABLE 1: Mean values of the quantitative characters of the parent races and their hybrids in F_1 generation

Metric traits	Parent (8605) (P1)	Parent (8618) (P2)	Mid parent	Better parent	Hybrids (F_1)	Hetersis (%)	Over - dominance (%)
Fecundity (No.)	463.00	631.00	547.00	P1	663.67	21.32	5.17
Hatching (%)	97.80	96.17	96.98	P1	98.52	1.58	0.73
Duration f all instars (h)	564.00	564.00	564.00	P1	560.00	-0.70	-0.70
Duration fo fifth instar (h)	183.00	196.00	189.50	P2	192.00	1.31	-2.04
Cocoon crops per 10000 fourth instar silkworm (kg)	12.93	11.90	12.41	P1	19.67	58.41	52.09
Cocoon shell weight per 10000 fourth instar silkworm (Kg)	2.79	2.46	2.63	P1	4.46	69.73	59.94
Cocooning ratio to the fourth instar larvae (%)	73.67	73.12	73.39	P1	96.28	31.18	30.69
Rate of dead worm and cocoons (%)	4.00	9.00	6.50	P1	0.00	-100	-100
Larva-pupa rate (%)	70.72	66.54	68.673	P1	95.82	39.61	35.49
Cocoon weight (mg)	1636.00	1615.00	1650.50	P1	2046.00	23.96	21.35
Cocoon shell weight (mg)	362.00	331.00	346.50	P1	459.00	32.46	26.79
Cocoon shell rate (%)	21.58	20.74	21.16	P1	22.70	7.27	5.19
Filament weight (g)	0.26	0.26	0.26	P2	0.40	51.51	49.81
Filament length (m)	1003.87	981.95	992.91	P1	1261.25	27.02	25.63
Size of the filament (D)	2.35	2.44	2.40	P2	2.87	19.74	17.39

Cocooning ratio to the fourth instar silkworm

The F_1 hybrids of the cross 8605 x 8618 and 8615 x 8608 revealed 96.28%, 31.18%, 30.69% and 98.27% and 4.05% mean cocooning ratio, heterosis and overdominance respectively (Tables 1, 2). The highest cocooning ratio was observed in Chinese varieties.

TABLE 2: Mean values of the quantitative characters of two parent races and their hybrids in F_1 generation.

Metric traits	(Parent (8615) P1	Parent (8608) P2	Mid parent	Better parent	F_1 hybrid	Heterosis (%)	Over-dominance (%)
Fecundity (No.)	502.00	503.33	502.67	P2	659.33	31.16	30.99
Hatching percentage	97.34	95.00	96.17	P1	97.25	1.12	-0.09
Duration of all instars (h)	564.00	564.00	564.00	P1	559.00	-0.88	-0.88
Duration of the fifth instar	196.00	196.00	196.00	P1	191.00	-2.55	-2.55
Cocoon crops per 10000 fourth instar silkworm (kg)	13.83	12.05	12.94	P1	17.92	38.48	29.58
Cocoon shell weight per 1000 fourth instar silkworm(kg)	3.58	2.58	2.85	P1	3.94	38.47	26.70
Cocooning ratio to the fourth instar larvae (%)	94.44	84.36	89.40	P1	98.27	9.92	4.05
Rate of dead worm and cocoons (%)	6.00	7.00	6.50	P2	1.25	-80.76	-82.14
Larva-pupa rate (%)	88.78	78.46	83.62	P1	97.05	16.06	9.31
Cocoon weight (mg)	14442.00	1360.00	1401.00	P1	1814.00	29.47	25.79
Cocoon shell weight (mg)	321.00	290.00	305.50	P1	395.00	29.29	23.05
Cocoon shell rate (%)	22.53	21.45	21.99	P1	22.03	0.18	-2.21
Filament weight (g)	0.26	0.25	0.25	Pl	0.33	28.04	25.85
Filament length (m)	1179.00	952.50	1065.75	P1	1131.56	6.17	-4.02
Size of the filament (D)	2.04	2.42	2.23	P2	2.63	18.05	8.82

Rate of dead worms and cocoons

The mean rate of dead worms and cocoons of the above cross showed 0% -100%, -1000% and 1.25%, -80.75^ and -82.14% with regard to heterosis and overdominance respectively (Tables 1, 2). This is very high in Japanese parent varieties.

Larva-pupa rate

Perusal of Table 1 and 2 reveals that F_1 hybrids of the cross between 8605 x 8618 recorded 97.82% pupation rate following 39.61% heterosis, 35.49% overdominance (Table 1). The other F_1 hybrid of the cross 8615 x 8608 showed pupation rate 97.05% with 16.06% heterosis and overdominance 9.31% (Table 2).

Cocoon weight

The F_1 hybrids of the cross 8605 x 8618 revealed a mean cocoon weight of 2046 mg with 23.96% heterosis and 21.35% overdominance (Table 1). The other cross F1 hybrids registered that 181 mg following 29.47% heterosis and 25.79% overdominance (Table 2).

Cocoon shell weight

The F_1 hybrids of the cross 8605 x 8618 was observed to have registered cocoon shell weight of 459 mg with 32.46% heterosis followed by 26.79 overdominance. The F_1 hybrids of the other cross 8615 x 8608 revealed a mean cocoon shell weight of 395 mg with 29.29% heterosis and 23.05% overdominance. These hybrids of the above crosses showed a significant difference with regard to cocoon shell weight. The Chinese varieties showed highest cocoon shell weight when compared to Japanese varieties (Tables 1, 2).

Cocoon shell rate

The F_1 hybrids of the cross 8605 x 8618 showed mean cocoon shell rate of 22.70% with 7.27% heterosis and 5.19% overdominance. The F_1 hybrids of another cross revealed cocoon shell rate of 22.03% with heterosis 0.18% and overdominance -2.21%. The Chinese parent varieties showed highest cocoon shell rate than that of the Japanese parents (Tables 1, 2).

Filament weight

The F_1 hybrids of the cross 8615 x 8618 showed mean filament weight of 0.4 g with 51.51% heterosis and 49.81% overdominance. The F_1 hybrids of the cross 8615 x 8608 revealed that cocoon filament mean weight 0.33 followed by 28.04% heterosis and 25.85% overdominance. The Chinese parents revealed highest filament mean weight than Japanese parents (Tables 1, 2).

Filament length

The mean cocoon filament length of the F_1 hybrids of the cross 8605 x 8618 shows 1267.25 meters, heterosis 27.02% with overdominance 25.63%. The F_1 hybrids of the cross 8615 x 8608 registered the mean filament length 1131.56 meters, heterosis 6.17% and overdominance -4.02%. This was the highest filament length observed in Chinese parents (Tables 1, 2.

Size of the filament

The F_1 hybrids of the cross 8605 x 8618 and 8615 x 8608 revealed the mean size of the filament 2.87D, 19.74% heterosis overdominance 17.39% and 2.63D with 18.05% heterosis and 8.82% overdominance respectively. The Japanese parents showed highest size of the filament than that of the Chinese parents (Tables 1,2).

DISCUSSION

In the present conventional cross breeding programme the known genotype used as a choice of mating system is of vital importance to obtain high productive hybrids for commercial utilization. Generally, in cross breeding programme two or more varieties are used, which are good in some characters and poor in others to obtain F_1 hybrids with a hormonious blend of desired quantitative characters (Rajanna 1989).

In view of the above, 4 silkworm varieties of Chinese and Japanese newly evolved bivoltine varieties, which are known for their distinct phenotypic expression with respect to commercial characters have been utilized in positive and negative crosses in order to extract the most promising

F_1 genotype. Further, the phenotypic expression of the variety is determined by the interaction between genetic and environmental factors (Mather & Harrison 1949, Robertson 1955, Sengupta 1969, Barlow 1981, Levins 1968, Rajanna 1989). Similarly, the expression of the quantitative characters in silkworm *Bombyx mori* is largely dependent on the complex interaction between polygenes governing them and environmental factors to which they are exposed (Rajanna 1989). Therefore, suitability of the variety is dependent on its genotypic response to produce consistently high phenotypic expression in F_1 hybrids under different environmental conditions. However, the performance of the varieties itself will be the best indicator of the suitability of the genotype to known environmental conditions (Rajanna 1989).

In view of the above factors, both Chinese and Japanese bivoltine sex-limited varieties of known genetic background have been utilized in the present investigation in order to study the performance of the parents and their hybrids during summer and autumn rearing seasons for the first time.

Analyses of the 15 quantitative characters of the F_1 hybrids revealed the expression of varied degrees of heterosis and overdominance. For example, the highest heterosis of all the F_1 hybrids of the two single crosses with regard to productivity characters such as cocoon crops per 10000 fourth instar silkworm (58.41%, 38.48%), cocooning ratio to the fourth instar silkworm (31.18%, 9.92%), cocoon weight (23.96%, 29.47%). Further, comparatively lesser heterosis for larva-pupa rate (39.61%, 16.04%) was observed (Tables 1, 2). They reported higher heterosis for productivity character compared to viability character and suggested that heritability plays an important role in the expression of these characters. A close analysis of the mean values of the quantitative characters in the F_1 hybrids of both the crosses reveal that the heterosis depends on the mid parent value irrespective of the parents involved. This agrees with the findings of Osawa & Harad (1944). They showed that greater the mid parents value, lesser will be the effect of heterosis. The expression of heterosis is influenced by two genes one acting directly on the characters and another influencing the expression of the characters indirectly as suggested by Barlow (1981).

The analysis of the higher cocoon weight, cocoon shell weight and cocoon shell rate in the F_1 hybrids can be attributed to the inheritance of sex linked dominant maturity gene (Im). The marginal increase in the hatching percentage in F_1 hybrids may be due to physiological states of male and female moths and also temperature, light and humidity during incubation (Tazima 1978). The short larval duration in the F_1 hybrids may be due to higher rate of metabolism and speed of development of the heterozygous larvae.

From the rearing results (Table 1) of 8605 x 8618 during summer and autumn seasons showed that the cocoon yield for 10000 larvae reached 19.67 kg, cocoon qualities such as cocoon weight 2046 mg, cocoon shell weight 459 mg, cocoon shell rate 22.70% and length of the silk filament 1261.25 meters in the F_1 hybrids when compared to F_1 hybrids of the cross 8615 x 8608. This shows that F_1 hybrids obtained by 8605 x 8618 is superior than that of the F_1 hybrids of the cross 8615 x 8608. This further indicates 8605 and 8618 parents have reached high level. Because of this achievement of the parents they can be used as supersilkgeneous basic breeding materials for summer and autumn rearing to produce superior grade silk. The performance of the check variety

hybrid during summer and autumn was compared with our present rearing results and noticed that parents involved in this breeding programme are superior than the present check varieties.

This indicates that the sex-limited variety 8605 for normal marking can be utilized with 8618 all normal marking variety to produce a high quality silk. Further, sex-limited varieties can be utilized in the breeding programme because the identification of sex at the larval stage is very easy. This helps the egg producing industry. The silk size of the sex-limited male cocoon is small, silk quality is very good, neatness is very high. Therefore, it is suggested that the future, the sex-limited races can be disseminated for commercial production of silk in China and also in other silk producing countries.

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ON POLLEN DEGENERATION IN CYTOPLASMIC MALE STERILE RADISH

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SUMMARY

The present report deals with the analysis of cytoplasmic male sterility (CMS) in the anthers of radish. Sterility is postmeiotic. The first sign of variation appears in the tapetum immediately after the microspores are released from the tetrad condition. Radial walls of some tapetal cells rupture and the contents form an intratapetal syncytium. The syncytium and microspores degenerate simultaneously. Occasionally, the tapetum at tetrad stage becomes hypertrophied and consequently the microspore tetrads are crushed in the locule.

Key Words: *Raphanus*, pollen sterility.

Male sterility can be described as the failure or inability of the plant to produce functional gametes. This is different from that of self-incompatibility where viable gametes are formed but unable to fertilize. There are 3 types of male sterility in plants viz., genic, cytpolasmic and cytoplasmic-genic. Genic male sterility results by the action of nuclear genes and its inheritance is strictly according to Mendelian principles. Cytoplasmic male sterility (CMS) is due to cytoplasmic factors. Cytoplasmic- genic male sterility is due to genic as well as cytoplasmic factors and follows non-Mendelian inheritance. Cytoplasmic-geneic male sterility (CGMS) is highly useful in plant breeding programmes to generate hybrids, because it eliminates the expense of hand emasculation procedures. It has been reported in 342 species (Kaul 1988). The cause for pollen sterility has long been attributed variously to the malfunctioning of tapetum, untimely dissolution of callose, poor vasculature, biochemical disturbance, mutation in mitochondrial genome and the presence of viruses (Graybosch et al. 1984, Shivanna & Johri 1989, Grant et al. 1986, Sawhney & Bhadula 1988, Kakihara et al. 1988, Kaul 1988, Thesis & Robbelen 1990).

While working on histochemical and biochemical aspects of CMS in crop plants, we came across some striking abnormalities during microsporogenesis hitherto not observed in radish. Radish *(Raphanus sativus)* is an important member of the economically useful family, Cruciferae. CMS was first reported by Ogura (1968) in this plant.

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Seeds of CMS and male fertile lines of radish were obtained from Cruciferae Genetics co-operative, Wisconsin, U.S.A. The plants were raised and maintained in the pots. Different stages of anthers were fixed in Carnoy's fixative, processed by using routine microtechnique methods. The microtome sections of 6 μm were stained with mercuric bromophenol blue (Vijayaraghavan & Shukla 1990).

The microsporogenesis in CMS and fertile lines follow the same course of development up to the formation and release of microspores from the tetrad. In male fertile line, the anther at pollen mother cells stage consists of an epidermis, endothecium, a middle layer and the tapetum. The tapetum in fertile another remains at parietal region even after the formation of pollen grains. In CMS line, the first sign of abnormality in the anther appears in tapetum. Following the release of microspores from the tetrad, the radial walls of tapetal cells begin to rupture, allowing the tapetal contents to mix together. The tapetal cell contents remain enclosed within a continuous inner tapetal wall and form and intratapetal syncytium. Later, intratapetal syncytium and microspores start degenerating simultaneously. Meiosis in the pollen mother cells proceeds normally until tetrad formation, after which the microspores degenerate either during tetrad formation or immediately after their separation. The tapetum becomes plasmodial. The plasmodium enlarges and invade the anther locule and consequently the developing microspores are crushed to death. The constituent tissues of the anther locule are completely collapsed.

Number of histological studies have demonstrated that the aberrations leading to male sterility may occur at any stage of anther development (Kaul 1988). It is difficult to define exactly the stage at which the abortion is initiated because of wide range of histological disturbances and their varying expression. The present study on *Raphanus* revealed that the sterility is post- meiotic. Although CMS has been reported in large number of species, the tapetal development, the course of microsporogenesis and its breakdown stages are known only in 12% of CMS plants studied of them in 16% the tapetum is normal, in 35% it is abnormal and in 49% it is persistent, however the breakdown of microspoogenesis is observed in all the cases (Kaul 1988). The tapetal tissue may remain intact for a longer duration or breaks down earlier and becomes hypertrophied or even develops intratapetal syncytium. Intratapetal syncytium and hypertrophic nature of tapetum has been reported in CMS lines of *Sorghum* and sugarbeet (Overman & Warmke 1972, Chauhan & Kinoshita 1980), *Raphanus* (Ogura 1968, Theis & Robbelen 1990), *Allium* (Konvicka et al. 1978) and *Brassica* (Banga et al. 1984). In the present study, hypertrophy associated with the formation of intratapetal syncytium is observed. This deviation in the tapetum is observed before the formation of aberrant microspores. This leads to the assumption that whether the male sterile genes come into effect only due to the disturbance of the tapetum or the tapetum first suffers due to the expression of male sterile genes. It is possible that both tapetum and microspores suffer from the deficiency of the same gene product (Graybosch et al. 1984).

Occasionally a complete hypertrophy of tapetum is noticed in the radish at tetrad stage. Consequently, the microspore tetrads are crushed in the locule. In the CMS line of *Raphanus* (Theis & Robbelen 1990) occasional release of microspores from the tetrad is also inhibited and tetrads degenerate. Similar type of observations were also made in pepper (Horner & Rogers 1974) and

sunflower (Horner 1977). There is an unequivocal agreement in the literature that a co-ordinated development of tapetum and microspores is a prerequisite for the development of fertile pollen grains (Shivanna & Johri 1985). The importance of the tapetum results from the fact that the nutrients for the developing microspores have to pass through this cell layer. Furthermore, the synthesis of various substances is shown to take place in the tapetum. Therefore, the male sterility is often associated with the aberrant behaviour of the tapetum. Further insights into the initial effects of CMS may lead to a more comprehensive understanding of the regulation and expression of male sterility controlling gene (s) and additionally it may also provide strategies for the introduction or induction of male sterility in commercially important plants.

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COVER : Cocoons of *Bombyx mori*. Clockwise (*from left*): P_1 8605 Chinese variety; P_2 8618 Japanese variety; F_2 hybrid of negative cross 8618 x 8605; F_1 hybrid of Positive cross 8605 x 8618.