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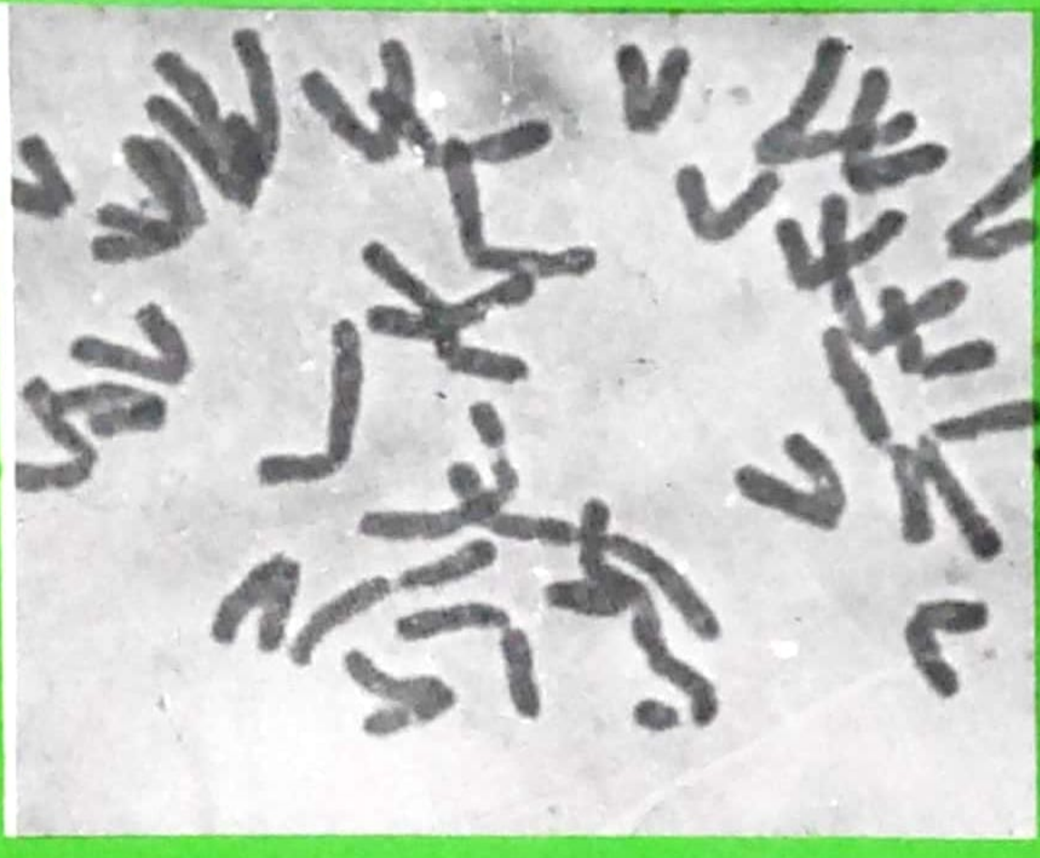

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FLORAL BIOLOGY AND BREEDING BEHAVIOUR IN *BAMBUSA ARUNDINACEA**

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SUMMARY

Bambusa arundinacea (Retz.) Willd. was found in full bloom in Pune in 1989-91. Anthesis occurred between 5.30 a.m. and 2.00 p.m. depending on temperature and relative humidity. Androecium and gynoecium matured simultaneously. Stigmas remained at a higher level than stamens, when the reproductive structures were fully exerted. Pollen fertility was about 93%. Pollination was effected by wind. Geitonogamy and adelphogamy were possible. Insects visited the florets and collected large quantities of pollen. However, they did not act as pollinating agents. There was profuse seed production in clumps under open pollination. Bagging the florets without emasculation and artificial selfing resulted in seedset indicating only sexual reproduction. Bagging the florets without emasculation and artifical selfing resulted in seedset pointing towards possible absence of self-incompatibility. Seedling populations showed a wide variation.

Key Words : *Bambusa arundinacea*, breeding behaviour, floral biology, flowering.

INTRODUCTION

Bamboos are amongst the most economically important multipurpose plant species (Mc Clure 1966). They can be divided into 3 categories on the basis of their flowering behaviour: species which flower (i) annually, (ii) gregariously and periodically and (iii) irregularly. Most bamboo species flower at the end of their vegetative growth phase, ranging betwsen 3 and 120 years (Janzen 1976). Nearly 1250 species of bamboos occurring worldwide present rich genetic diversity. Their peculiar flowering makes the transfer of desirable traits between varieties, species and genera by conventional methods difficult. Studies on their reproductive biology are meager. In vitro induction of flowering (Nadgauda et al. 1990) can help in obtaining predictable and synchronous flowering in 2 or more species. It is necessary to compare the in vitro flowering with in vivo flowering, so that any changes brought about by in vitro conditions can be detected and rectified. With this view also in mind we studied the reproductive biology in *Bambusa arundinacea* (Retz.) Willd.

B. arundinacea is one of the 2 most important bamboos found throughout India (Kondas 1973). Its different cohorts have intermast periods ranging between 30-54 years (Janzen 1976). Though exact age of the clumps studied is not known, they are known to be more than 30 years old.

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The culms of this species are very useful in construction work (Yudodibroto 1987, Vivekanandan 1987, Widjaja & Risyad 1987) and is one of the major sources of raw material for the paper industry in India.

MATERIAL AND METHODS

B.arundinacea growing in the Empress Botanical Garden and the Poona University Campus, Pune mass flowered in 1989-91. During this period, observations on the processes involved in seed production were made.

The time of anthesis, anther dehiscence and pollen release as well as stigma receptivity were studied by making regular and periodic visits to the field. The time when the 'lemma' and 'palea' were separated fully and the reproductive structures exerted completely was considered as the time of anthesis. When the stigma was fully exerted, its 3 lobes were well expanded and the papillae were turgid. Such stigmas were considered as receptive. The temperature and relative humidity, and the time of anthesis during different seasons were recorded. Multiple regression coefficient analysis was carried out (Snedecor & Cochran 1967). Observations on insect visitors, their identity, seasons and frequency of their visits were recorded. The florets at anthesis were tagged with thread and the younger and older florets were removed from the spikelet. Some of these florets were left open to be pollinated by the natural agents and the remaining florets were bagged a day before anthesis. Some of the bagged florets were emasculated 2 or 3 h before anthesis and the remaining ones were left unemasculated. The following day the latter were either allowed to open inside the bags or pollinated with pollen grains from anthers of the same floret. Pollen was collected by tapping the anthers on to a piece of butter paper. The emasculated florets were either left unpollinated or pollinated with pollen from the same or a different plant.

The pollen grains were stained with Alexander's stain (Alexander 1969) to check pollen fertility. The methods followed for in vitro pollen germination were similar to those described earlier (Nadgauda et al. 1993). Observations on the seed predators were made when the seed shedding took place in the months of April and May. Observations on the growth habit of the seedlings were made from a large number of seedlings established after the first few rains of the ensuing monsoon.

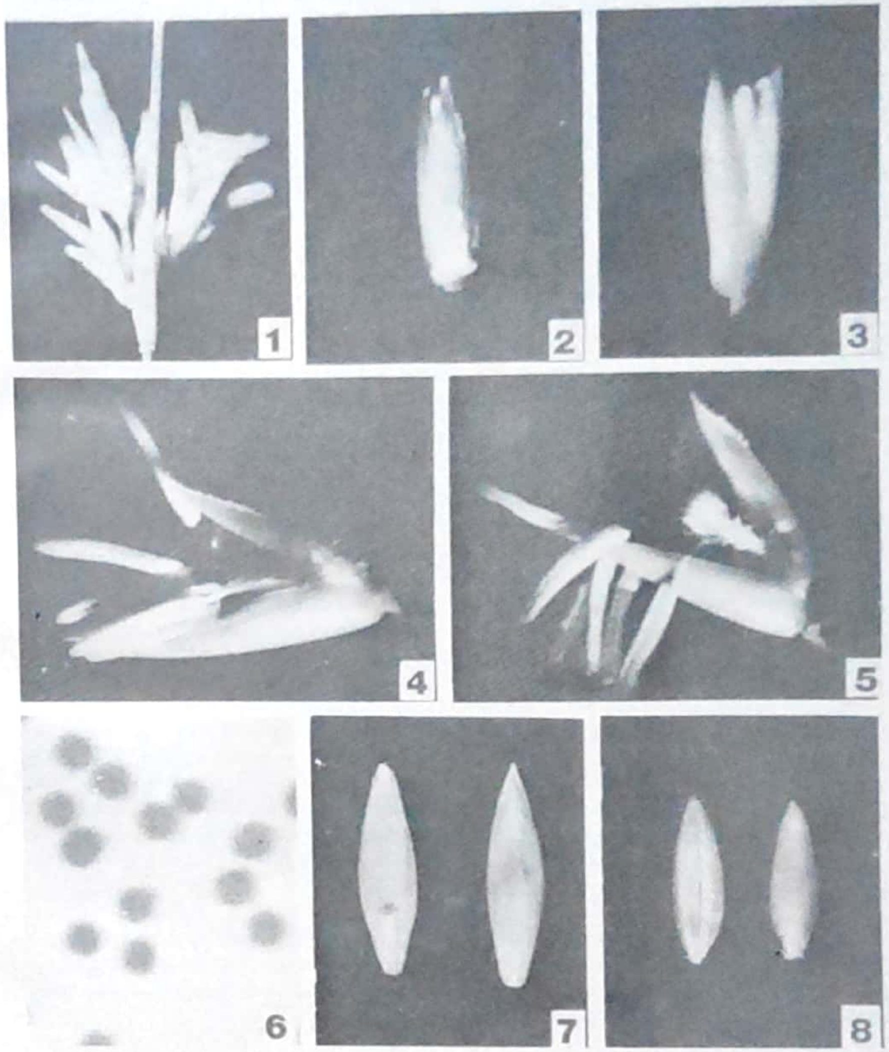
OBSERVATIONS

In the Empress Botanical Garden, there were many clumps whereas in Poona University campus there were only 3 clumps. Each clump consisted of approximately 15-20 culms. The flowering took place in many flushes with short resting periods in between. Flowering commenced in October-November, 1989 when very young spikelets were seen on the culms. The florets started opening by the end of November and continued till May-June 1991.

Time of anthesis, anther dehiscence and stigma receptivity

The florets in *B.arundinacea* are chasmogamous. The 'lemma' and 'palea' opened and exposed androecium and gynoecium which matured at the same time. Young florets grew rapidly during the 5-6 h preceding anthesis. Thereafter, the 'lemma' and 'palea' gradually separated from each other and the stigma became exerted. At anthesis, the anthers were found situated at a lower level as compared to that of the stigma (Figs. 1-5). The length of florets ranged between 9 and 10 mm. Individual anthers (with filaments) were around 5 mm and gynoecium around 9 mm long.

Pollen from a culm pollinated stigma of florets, at a lower position on the same culm (geitonogamy), florets on a different culm from the same clump (adelphogamy) or florets on a different clump (cross pollination). The mature stamens dangled at the tips of long filaments, anthers dehisced by apical pores and the pollen released from many florets at the same time formed a 'pollen cloud' which moved in the direction of the wind. Each floret required about 2 h for the completion of anthesis. Both on natural and artificial pollination the receptive stigma attached large number of pollen grains.



Figs. 1-8: 1. Spikelets. 2. A young floret 3. One floret at the start of anthesis. 4. A floret at an advanced stage of anthesis. 5. A floret at anthesis; note the differential position of anthers and stigma. 6. Pollen grains. 7. Seeds with husk. 8. Seeds without husk.

Influence of climatic conditions

Anthesis in all florets opening in a given day took place in a span of 3-4 h when majority of the florets opened. The time of anthesis was dependent on atmospheric temperature and relative humidity (Table 1). Floret opening in different months of the year was as follows: in summer (April-May) between 5.30 a.m. and 9.00 a.m., during monsoon (June-September) between 8.30 a.m. and 12.30 p.m., in September-October and early November between 8.00 a.m. and 12.00 noon and in winter (late November to the beginning of February) between 11.00 a.m. and 2.30 p.m.

Insect visitors

Two species of insects, *Apis mellifera* and *Allodapha marginata* visited the florets at anthesis, only in seasons when the climate was not extremely hot, cold or raining. They fed on and collected large quantities of pollen. The insect visitors damaged the young florets by eating pollen from even immature anthers. However, they did not act as pollinators.

TABLE 1: Influence of climatic factors (Maximum , minimum and mean air temperature and humidity) on the time of anthesis in *Bambusa arundinacea* on the basis of regression analysis.

Time of anthesis regressed on to the climatic condition	R^2	F	Regression equation
Maximum air temperature	73.3%	19.15*	Time of anthesis = 21.2-0.340 Maximum air temperature
Minimum air temperature	53.2%	7.94*	Time of anthesis = 13.8-0.224 Minimum air temperature
Mean air temperature	6.4%	0.48	Time of anthesis = 11.5-0.046 Mean air temperature
Maximum humidity	77.1%	23.61*	Time of anthesis = 3.68+0.167 Maximum humidity
Minimum humidity	1.7%	0.12	Time of anthesis = 9.81+0.0108 Minimum humidity
Mean humidity	17.2%	1.45	Time of anthesis = 6.84+0.05523 Mean humidity
Mean temperature and Mean humidity	26.1	1.06	Time of anthesis = 8.06-0.0554 Mean temperature + 0.0594 Mean humidity

R^2 = Multiple correlation coefficient.
F = Test statistics for regression coefficient.
* = Significant.

Breeding system

Table 2 summarizes the details of breeding experiments. Under open pollination seed production was high. On bagging without emasculation (to exclude wind) seedset was low and bagging after emasculation resulted in the absence of seedset. On cross-pollination seed production was high and on artificial self-pollination the seedset was lower.

Pollen fertility

The pollen fertility was 92.88 ± 0.4% (Fig.6). Among the sugars tried, sucrose (1%) optimally supported pollen germination, a lower or a higher percentage of which greatly reduced the percentage of it. Fructose, glucose and maltose were ineffective. Fresh coconut milk also promoted pollen germination. However, the pollen tubes were highly coiled in their appearance and the results were inconsistent depending on the batch of coconut milk used. For sustained growth of the pollen tubes, other ingredients of the Brewbaker & Kwack (1963) medium were also necessary. A modified medium standardized for in vitro pollen germination in this species contained 100 ppm H_3BO_3, 200 ppm Ca $(NO_3)_2$, 200 ppm Mg SO_4 and 100 ppm KNO_3. In this medium, pollen germination was 86±0.99% and pollen tubes grew approximately 1 mm in length.

TABLE 2: Effect of different pollination treatments on seedset in *Bambusa arundinacea**.

Treatment	Number of florets	Seedset observed	Percentage seedset
Open pollination	571	433	75.81 ±1.77
Bagging without emasculation	354	62	17.50 ±0.69
Bagging after emsculation	286	00	00.00
Artificial cross-pollination	241	159	65.85 ±1.85
Artificial self-pollination	192	68	35.49 ±1.75

* Mean of three observations ± standard error

Seed predators

The seeds (Figs. 7, 8) when ripe fell from the culms and formed a thick layer on the ground underneath the clumps.The seeds started falling by January-February. Most of the seeds matured in March-May. In Pune, the main predators of the bamboo seeds were rats, squirrels and sparrows. Sparrows and squirrels were seen all through the day, eating bamboo seeds. Rats were seen mostly in the late evenings, nights and early mornings. At times, stray rats were observed collecting bamboo seeds even during the day. Since both the sites were frequented by humans, there were no jungle animals in their vicinity.

Seedling variability

The seeds readily germinated after the first 2 or 3 showers of rains and formed a thick carpet of seedlings. The seedlings showed a high degree of variation in their growth pattern. There were 4 types: short-slender, medium-statured, tall-slender and tall-vigorous. In one of the collections 17.50±0.81% of the seedlings were albinos. The albinos did not survive beyond the four leaf stage.

DISCUSSION

In *B. arundinacea*, differential position of androecium and gynoecium prevents self-pollination. However, geitonogamy and adelphogamy, bringing in the same effect as selfing are possible. Dichogamy and protogyny are reported in some bamboos which effectively prevent self pollination (Venkatesh 1984, Nadgauda et al. 1993). As in other grasses, wind-pollination and cross-pollination were possible. The highly feathery nature of the receptive stigma could help in capturing air borne pollen. In the present species, anthesis was found dependent on temperature and humidity. In *Dendrocalamus strictus*, however, though temperature plays a decisive role, relative humidity had no apparent influence on anthesis (Nadgauda et al. 1993). In insect visits, *B. arundinacea* was comparable with *Ochlandra travancorica* (Venkatesh 1984) and *Dendrocalamus strictus* (Nadgauda et al. 1993). Bodekar (1930) reported insect visitations of *Bambusa polymorpha* florets in Burma. Gunckel (1948) noted that a species of *Chusquea* in Chile was pollinated by wind and also "by some small insects". In these species also, possibly the insects may not be acting as pollinators.

The lower percentage of seedset in bagged florets, as compared to those under open pollination may be due to obstruction to the wind and the absence of large quantities of cross pollen available to the latter group. Seedset in these florets indicates the possible absence of self-incompatibility. Seedset in artificially self-pollinated florets also points towards the absence of self-incompatibility. Pollination by pollen from the same floret can be done only when anther dehiscence takes place in that floret, which may be slightly later than the time of stigma receptivity. Artificial cross pollination can be done slightly earlier than the time of stigma receptivity. Pseudoself-incompatibility also reduces seedset on self-pollination. In *B.arundinacea*, reduction in seedset on artificial self-pollination may be due to one of these or both. From their observation of seed set in inflorescences which were bagged, Kondas et al. (1973) and Indira (1988) inferred this species to be self-compatible. Absence of seedset in floret bagged after emasculation can be considered as indicating only sexual reproduction in this species. Nearly 93% pollen fertility and higher percentage of pollen germination and good gr~wth of the pollen tubes point towards regular meiosis and production of viable pollen in sufficient numbers in this species. One way of overcoming the barrier placed by nature on hybridization betwen bamboos is cryopreservation of pollen. The method standardized for in vitro pollen germination and pollen tube growth is useful in checking viability of stored pollen.

Seed predators are thought to be selecting heavily against the tails of the bamboo seeding. Also against small cohorts and sporadically seeding individuals (Janzen 1976). Heavy seed predation in the forests can help in maintaining synchrony of mast crop within a cohort. However, this may not be true in protected habitats.

Observations in this study point towards cross-pollination prevalent in this species. Kondas et al. (1973) also reported a similar observation. Absence of self-incompatibility enables seed production at times of sporadic flowering, which may be lower and the variability produced may also be less. Mc Clure (1973) noted that it is common for bamboos to flower without setting seeds. An isolated wild clump that is flowering well out of phase with the main mast crop may set little or no seed (J C D 1883, Gamble 1902, 1904).

The results of our investigation are useful in planning breeding programmes in bamboos and making a comparison between in vivo and in vitro flowering in *B. arundinacea* which would help in further refining the in vitro flowering methods for perennial seed production and hybridizations.

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INFRASPECIFIC KARYOTYPE EVOLUTION IN *CYANOTIS VILLOSA* SCHULT. F. (COMMELINACEAE) BY CENTRIC FUSION

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SUMMARY

Cytology of plants of *Cyanotis villosa*, considered to be the most primitive species of the genus, collected from 9 localities in South India was investigated. Plants from Silent Valley, Wyanad, Udagamandalam and Munnar showed n = 12 and 2n=24. Plants from Kodaikanal, Courtallam, Vandiperiyar, Upper Kothayar and Ponmudi showed n = 13 and 2n = 26. Karyotype formulae in the above taxa were n = 12 = 3 m + 2 sm + 7 st and n=13 = 2 m + 2 sm + 9 st respectively. From a comparative study of the karyotypes, it is suggested that plants with n= 12 were derived from plants with n= 13 by a centric fusion and a loss of one centromere. An analysis of the distribution of the 2 cytotypes suggested that the cytotype with n = 12 has evolved in Kodaikanal and that it is more adapted to less warmer areas, away from the equator.

Key Words : *Cyanotis*, karyotype, centric fusion, dysploidy.

INTRODUCTION

The tropical genus *Cyanotis* is considered to have evolved from a *Belosynopsis* ancestry with n=13 and *C. villosa* (*C. lanceolata* Wt.) with n=13 is reported to be the most primitive member of the genus (Faden & Suda 1980). *C. villosa* is a perennial spreading plant with blue flowers. In South India this species occurs at high altitudes. Cytotypes with n=12 (Shetty & Subramanyam1962, Rao 1970, Renugadevi & Sampath Kumar 1986), n=12 + 1 (Rao 1970), n=13 (Raghavan & Rao 1965, Rao 1970, Jones & Kukkonen 1971) and 2 n = 26 + 1B (Owens 1981) have been reported in this species. Reports of the existence of different cytological races of *C. villosa* and the pivotal position occupied by the species in the evolution of the genus have prompted a detailed cytological analysis of the species from South India.

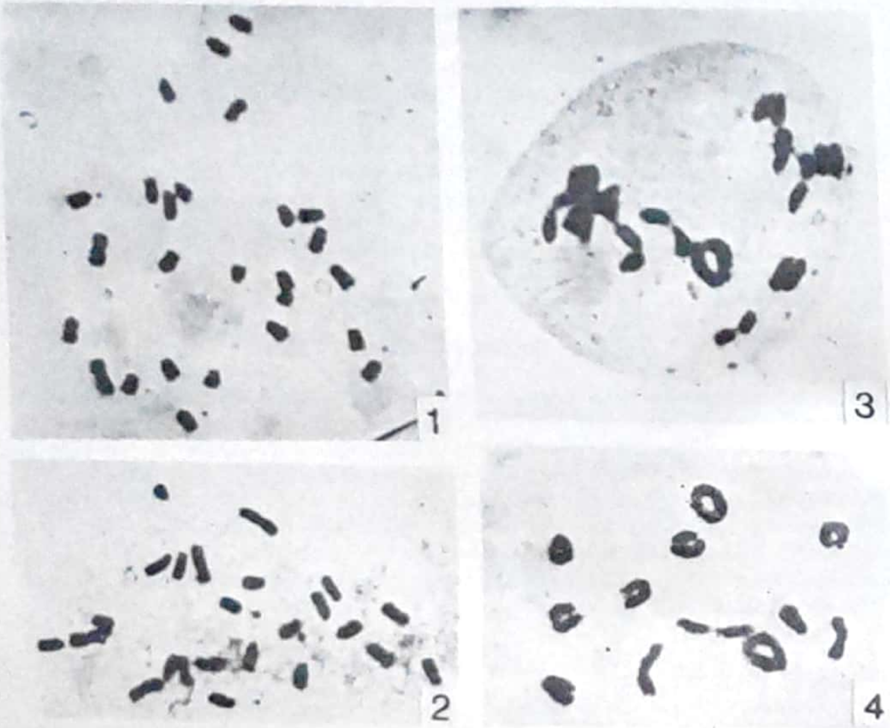
MATERIALS AND METHODS

During this study, plants of *C. villosa* were collected from Courtallam, Ponmudi, Upper Kothayar, Vandiperiyar, Munnar, Wyanad, Silent Valley and Udagamandalam. These were grown in the Botanical Garden, University of Kerala. Flower buds and root tips for cytological studies were fixed in ethanol-acetic acid (3 : 1) with a trace of ferric acetate. The root tips were pretreated in 0.002 M aqueous solution of 8-hydroxyquinoline at about 5°C for 2 h. The root tips and anthers were squashed in 1% acetocarmine.

Measurements of somatic chromosomes for karyotype study were made from photographs magnified to 2000 times. The relative chromosome length (RCL) was calculated by multiplying the percentage length of each chromosome by ten. The chromosomes were arranged in 3 groups, M, sm and st based on the position of the centromere. (Levan et al. 1964).

OBSERVATIONS

Chromosome numbers in the different collections were determined from root tip cells and / or PMCs. Root tip cells of the materials from Kodaikanal, Courtallam, Vandiperiyar, Upper Kothayar and Ponmudi showed 2n = 26 chromosomes (Fig.1). The haploid set consisted of 2 m + 2 sm + 9 st chromosomes with a n.f. value of 30. One of the large st pairs is satellited on the short arm. The chromosomes range between 1.5 µm and 3.7 µm in length in the Courtallam accession with a total chromosome length (TCL) of 65 µm for the diploid complement.



Figs.1-4 : Somatic and meitoc chromosomes of *Cyanotes villosa* (All Figs. x 1400) 1. *C. villosa* (Courtallum). a root tip cell showing 2n = 26. 2. *C. villosa* (Udagamandalam). a root tip cell showing 2n = 24. 3. *C. villosa* (Ponmudi). a PMC showing 13 bivalents. 4. *C. villosa* (Munnar). a PMC showing 12 bivalents.

The accessions from Silent Valley, Wyanad, Udagamandalam (Fig.2) and Munnar revealed 2n = 24. The haploid set of the accessions from Udagamandalam and Silent Valley consists of 3 m + 2 sm + 7 st chromosomes with a n.f. value of 30. A long pair of st chromosomes revealed

satellites attached to the short arms. The chromosomes range between 1.5 µm and 4.5 µm in length in the accession from Udagamandalam with a TCL of 65.4 µm. The st pairs in both accessions show a gradual decrease in length.

Meiotic studies revealed n = 13 bivalents in the accessions from Ponmudi (Fig. 3), Upper Kothayar, Courtallum, Vandiperiyar and Kodaikanal and n = 12 bivalents in the accessions from Munnar (Fig. 4), Udagamandalam, Silent Valley and Wyanad.

DISCUSSION

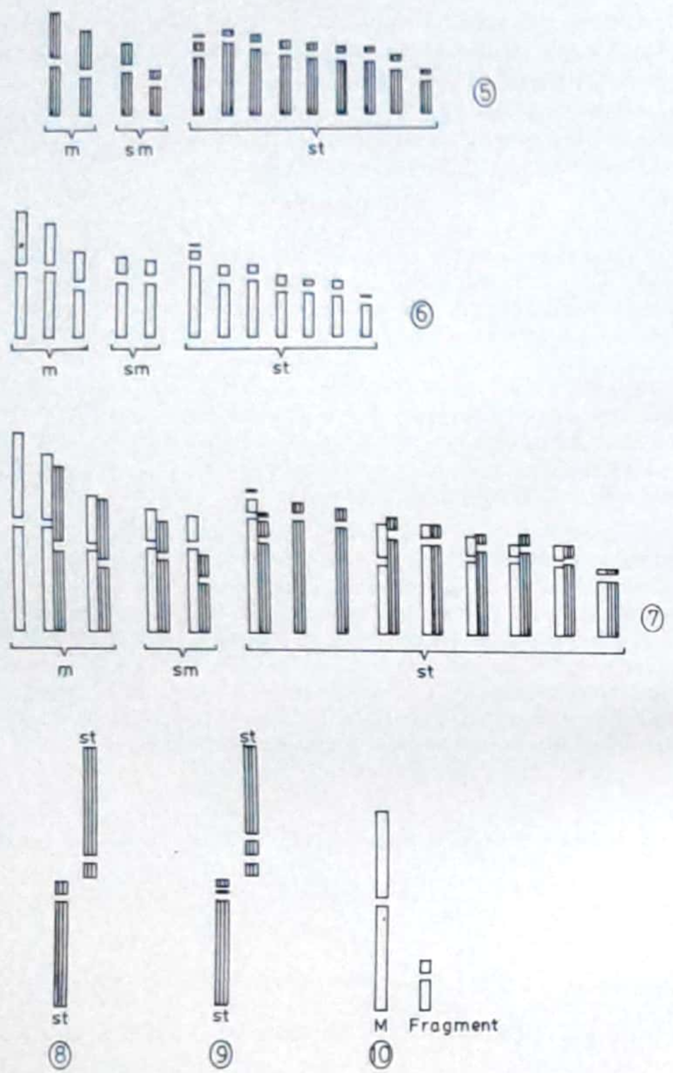
Rao (1970) observed plants of *C. villosa* with n = 13 and n = 12 from Kodaikanal and suggested that the n = 13 taxon is derived from the race with n = 12 as a result of tetrasomy followed by differentiation of the tetrasomic by structural changes. However, comparative studies by the authors (unpublished) on karyotypes in *Cynotis* have shown that n = 13 is a more primitive number than n = 12 in the genus and that the general tendency of karyotype evolution in the genus is towards reduction in chromosome number as evidenced by the reports of n = 15, 14, 13, 12, 11, 10 and 8 in various species (Murthy 1934, Islam & Baten 1952, Sharma 1955, Kammathy & Rao 1961, Raghavan & Rao 1961, 1965, Shetty & Subramanyam 1962, Lewis 1964, Jones & Kukkonen 1971, Renugadevi & Sampathkumar 1986, Bai et al. 1984). In this background n = 13 has to be considered as the primitive number and n = 12 as derived in *C. villosa*.

A comparison of the karyotype of the 2 races (Figs. 5, 6) showed that both have the same n.f. value of 30 and almost equal TCL. But n = 12 race has one m chromosome more and 2 st chromosomes lesser than the n = 13 race. From the composite idiogram (Fig. 7) it is evident that the 2 m chromosomes in the n = 13 plants are similar in size to the 2 short m chromosomes in the n = 12 plants. It is also seen that the latter taxon has no st chromosomes of comparable morphology to the sixth and seventh st chromosomes of the n = 13 taxon. Further, it is seen that the arms of the additional m chromosome in the n = 12 taxon is almost equal in length to the long arm of the seventh st chromosome of the n = 13 taxon (Table 1). The short arm of the above additional m chromosome is slightly shorter than the long arm of the sixth st chromosome of the n = 13 cytotype.

TABLE 1: RCL of the long and short arms of the chromosomes 6 and 7 of the n = 13 - race and of the additional m chromosome of n = 12 - race of *C. villosa*.

Chromosome	Long arm (µm)	RCL	Short arm (µm)	RCL
n = 13 race 6th st	2.6	4.0	0.4	0.6
7th st	2.4	3.7	0.4	0.6
n = 12 race additional m	2.4	3.7	2.1	3.2

These findings clearly indicate that the large m chromosome in the n = 12 race is perhaps formed as a result of the centric fusion between 6th and 7th st chromosomes of the n = 13 race as shown in Figs. 8, 9, 10.



Figs. 5-10: Idiograms and scheme of evolution of the additional chromosome in *C. villosa*. 5. Idiogram of *C. villosa* 2n = 26 (Courtallum). 6. Idiogram of *C. villosa* 2n = 24 (Udagamandalam). 7. Composit idiogram of the two cytotypes (2n = 26 and 2n = 24). 8. Two original st chromosomes. 9. Simultaneous breaks in the st chromosomes. 10. Products of the centric fusion.

Besides a reduction in st chromosomes and addition of m chromosomes, such a centric fusion will result in a small centric fragment chromosome. It is interesting to note that a small chromosome which divides regularly during mitosis and which could not be traced beyond first metaphase in PMCs is reported in plants of *C. villosa* having n = 12 (Rao 1970). It is likely that Rao has struck a clone of *C. villosa,* which still has the small centric fragment and this would lend support to the suggested role of centric fusion in the origin of the n = 12 taxon. However, the small chromosome has not been reported in any other collection of the n = 12 cytotype of *C. villosa*. This may be because, such small chromosomes may be eliminated during meiosis and a population with n = 12 may be established in accordance with the 'dislocation hypothesis' (Stebbins 1971).

The report of 2n = 26 + 1B in a specimen of this species (Owens 1981) is of considerable taxonomic interest in the context of the present findings. Cytological analysis of a larger sample from the whole range of distribution of the species may perhaps show that the B chromosome in Owen's specimen is a small centric fragment formed by an earlier centric fusion in an ancestral stock with n = 14. In such an event, the *Belocynapsis* ancestry of *Cyanotis* (Faden & Suda 1980) will have only very feeble cytological support, because it is based on the assumption that x = 13 is the original basic number of *Cyanotis.*

Rao (1970) could raise both the chromosomal races having n = 12 and n = 13 from a small bunch of vegetative shoots collected from Kodaikanal and has identified Kodaikanal, where the two races are sympatric, as the area of racial differentiation in *C. villosa.* This inference is supported by the occurence of a centric fragment in some plants with n = 12.

The identification of the possible location of origin of the n = 12 race of *C. villosa* offers a chance to study the relative distribution of the two races and the probable factors influencing it. The cytotype with n = 13 is reported from places in South India such as, Kodiakanal, Courtallum, Vandiperiyar, Upper Kothayar, Ponmudi (present study) Thenmalai (Raghavan & Rao 1965) and Ceylon region (Jones & Kukkonen 1971). The n = 12 race is reported from Silent Valley, Wyanad, Munnar, Udagamandalam (present study), Yercaud and Marudamalai (Shetty & Subramanyam 1962). It is seen that the 2 races have sympatric distribution in South India, with overlapping at the Kodaikanal area. A critical examination of the pattern of distribution shows that the original n = 13 race is seen in areas nearer to the equator while the derived race with n = 12 is seen in Kodaikanal and regions north to Kodaikanal, which are away from the equator. It has been shown that *C. villosa* has floral characters which promote cross-pollination (Owens 1981). It is also known that in cross-fertilized species, restriction of genetic recombination by dysploid reduction in chromosome number will work as an 'infective principle' in colonization of new habitats (Stebbins 1971). It might be that the dysploid change has conferred on the n = 12 races of *C. villosa,* a preference to less warmer habitats and consequently it has succesfully spread to places away from the equator.

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KARYOMORPHOLOGICAL ANALYSIS IN *HEVEA BRASILIENSIS*

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SUMMARY

Karyomorphological analysis of 4 clones of *Hevea brasiliensis* Muell. Arg. viz., RRIM 600, GT 1, PB 235 and PB 314 was carried out. The karyotype formula for RRIM 600, GT 1, PB 235 and PB 314 was 9 m + 8 sm + 1 st, 10 m + 7 sm + 1 st, 12 m + 5 sm + 1 st and 8 m + 8 sm + 2 st respectively. All the 4 clones are diploid with 2n = 36 and fall under 2A karyotype category. Even though there is gross similarity in the karyotype, in critical analysis there is a significant difference in chromosome morphology with reference to centromeric position and total chromosome length. More isobrachial chromosomes are seen in PB 235, while the highest number of heterobrachial chromosomes are found in PB 314. The karyotype of PB 314 is found to be more specialized than the other 3 clones studied here.

Key Words : *Hevea brasiliensis*, karyomorphology, clones.

INTRODUCTION

Hevea brasiliensis Muell. Arg., the para rubber tree, belonging to the family Euphorbiaceae is the most important source of natural rubber. A large number of clones are extensively grown for natural rubber. The somatic chromosome number of 2n = 36 has been reported previously by a host of authors for this species (Bangham 1931, Ramaer 1935, Paddock 1943, Baldwin 1947, Ross 1959, Majumdar 1964, Ong 1981, Saraswathyamma et al. 1984). The chromosomes are very small, therefore, only very few attempts have been made so far to study their morphology. Ong (1975, 1981) made a preliminary study on the pachytene analysis and karyomorphology of this species from Malaysia. However, karyomorphological studies at the clonal level have not so far been reported in this taxon. The present study deals with detailed karyotype analysis in four clones of *H. brasiliensis* viz., RRIM 600, GT 1, PB 235 and PB 314 to find out the interclonal relationships.

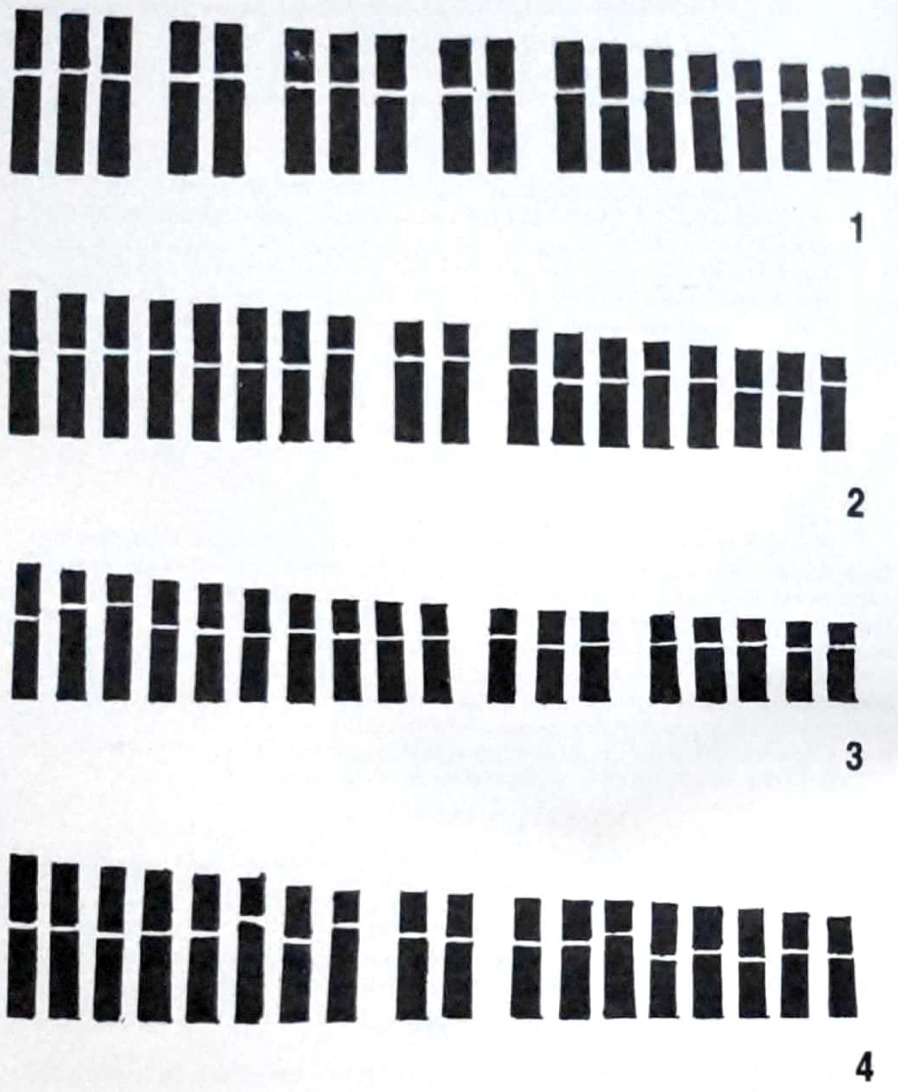
MATERIALS AND METHODS

Fresh shoot tips of the clones RRIM 600, GT 1, PB 235 and PB 314 were collected from RRII experimental field. The shoot tips were pretreated with saturated solution of paradichlorobenzene a 10°C for 3 h and fixed in 3 : 1 : 1 ethyl alcohol-acetic-chloroform mixture. After hydrolysing in 1 N HCL for 5 min at 60°C, it was kept overnight in 2% acetocarmine solution. Preparations were made by squashing the shoot tips in 45% acetic acid.

The system proposed by Stebbins (1958), Huziwara (1962) and Levan et al. (1964) were followed for karyotype analysis. The ANOVA for total chromosome length and arm ratio was carried out from 5 replicates of each clone.

OBSERVATIONS

All the 4 clones, RRIM 600, GT 1, PB 235 and PB 314 showed 2n = 36 in their somatic complement. Details of chromosomes are shown in the idiograms (Figs. 1-4).



Figs. 1-4 : Idiograms of somatic chromosomes of *H. brasiliensis*. 1. RRIM 600. 2. GT 1. 3. PB 235. 4. PB 314.

In RRIM 600, the length of the chromosomes ranged from 1.8 µm to 3.5 µm. The total chromosome length (TCL) of the haploid complement was 49.08 µm. The F% ranged from 22.2 to 46.2. The TF% was 36.9. The karyotype formula (KF) was 9 m + 8 sm + 1 st in the haploid complement. It belonged to 2A karyotype category.

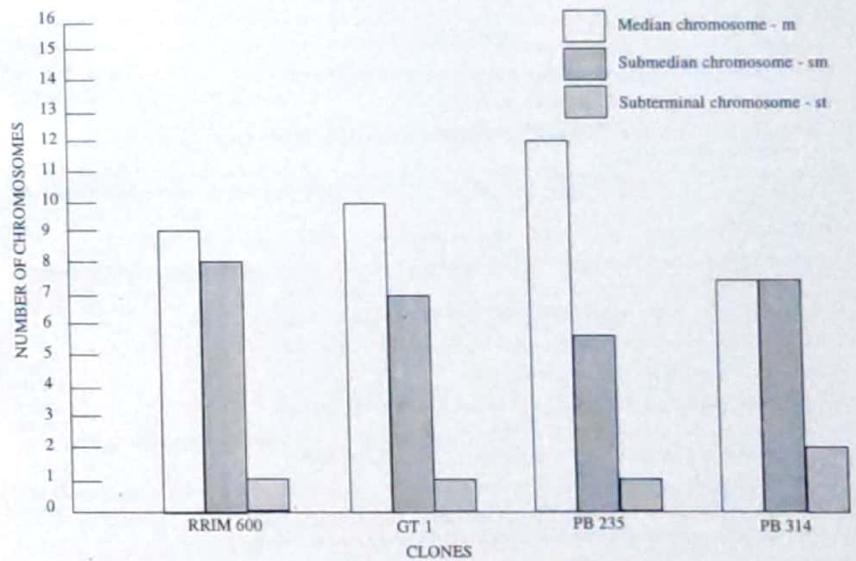
In GT 1, the chromosome length varied from 1.7 µm to 3.3 µm. The TCL was 45.04 µm. The F% ranged from 23.5 to 44.8 and TF% was 36. The KF was 10 m + 7 sm + 1 st and it falls under 2A karyotype category.

In PB 235, the chromosome length ranged from 1.6 µm to 3.1 µm. The TCL was 40.5 µm. The F% ranged from 21 to 47 and TF% was 38.1. The karyotype belonged to 2A category and the KF of the haploid complement was 12 m + 5 sm + 1 st.

In PB 314, the range of chromosome length was between 1.8 µm and 3.5 µm. The TCL was 47.66 µm. The F% varied from 21.7 to 45.5 and TF% was found to be 36.8. The karyotype belonged to 2A category and the KF was 8 m + 8 sm + 2 st.

DISCUSSION

Chromosomes of the 4 clones studied here are smaller and their length ranged from 1.6 µm to 3.5 µm. The clone PB 235 is characterized by both smaller size and lower TCL content. The highest TCL is noted in RRIM 600 followed by PB 314, GT 1 and PB 235. All of them belong to 2A



Figs. 5: Frequency of m, sm and st types of chromosomes in clones of *Hevea brasiliensis*.

karyotype category. Though there is some gross similarity in karyotype category, in critical analysis they differ in chromosome morphology with reference to the centromeric position. Total short arm length (TSL) is highest in RRIM 600 (18.1 μm) followed by PB 314 (17.5 μm), GT 1 (16.2 μm) and PB 235 (15.4 μm). The average chromosome length values are 2.7 μm, 2.5 μm, 2.2 μm and 2.6 μm in RRIM 600, GT 1, PB 235 and PB 314 respectively. The frequency of m, sm and st types of chromosomes also vary among these clones (Fig. 5). In PB 235, 12 out of 18 chromosomes in the haploid complement are found to be isobrachial whereas the highest number of heterobrachial chromosomes are found in PB 314. Besides these differences, variations can be noticed in F% and TF%. These differences within clones were found to be statistically significant as revealed by the ANOVA test.

According to Stebbins (1971), the 2 basic features which bring about karyotype asymmetry are (i) shifting of the centromere from median to sub-median and sub-terminal positions and (ii) increasing intrakaryotypic size difference of chromosomes. Of the 4 clones studied here, 2 sub-terminal chromosomes are noticed in PB 314 while the rest have only one sub-terminal chromosome in their haploid complement. PB 314 also possessed the least number of metacentric chromosomes (8). The karyomorphological evidence thus reveals that PB 314 is more specialized than the other 3 clones studied. It is suggested that the structural changes might have brought about the change in the chromosome morphology of the different clones studied in the present investigation.

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HETEROCHROMATIN VS MEIOTIC STABILITY AND KERNEL CHARACTERS IN TRITICALE

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SUMMARY

A comparative study of various hexaploid triticales, which differ widely in their heterochromatin contents were made with reference to the role of heterochromatin content on meiotic stability and agronomic characters. Triticales with lesser amounts of heterochromatin exhibited relatively more stable meiosis and more desirable agronomical characters as compared to the triticales with higher heterochromatin contents. Implication of heterochromatin in meiotic stability and kernel fertility was discussed.

Key Words: Triticale, heterochromatin, meiotic stability, kernel characters.

INTRODUCTION

Triticale (X *Triticosecale* Wittmack), the first man-made cereal, combines the qualities of its parents wheat and rye. Despite enormous progress made in breeding of triticale as a commercial cultivar, it suffers from a variety of disorders which often reflected in reduction of its yield and commercial acceptability. Cytological instability and kernel shrivelling were among the 2 prominent undesirable traits still associated with triticale. These traits to a large extent are correlated with heterochromatin content in triticales. The present paper reports the effect of variation in heterochromatin content on meiotic instability and kernel characters in 2 groups of triticales which largely differ in heterochromatin contents.

MATERIALS AND METHODS

The following 16 hexaploid triticale cultivars, in 2 groups which were previously analysed for their heterochromatin contents (Renu Edwin 1994) were selected to evaluate the role of heterochromatin on meiotic stability and various agronomic characters (Table 1).

TABLE 1: Heterochromatin content in different hexaploid triticales.

Triticale varieties	Heterochromatin content (%)
GROUP-1	
Almos	12.62
Arabian	11.89
Badiel 'C'	13.93
Borba	12.56
Beagle	13.04
Carman	13.84
Aseret	10.11
Mizar	10.28

TABLE 1: (concluded)

Triticale varieties	Heterochromatin content (%)
GROUP-II	
Bacum	6.49
T 1116	3.76
TL 2624	3.90
6TA 118	4.51
DTS 30-32	4.69
Cinnamon	4.18
Maxi Tol	6.58
DTS 139	5.02

Cytological studies were made according to standard procedures. Data on chromosome associations and other cytological parameters were recorded at appropriate meiotic stages. Data on various agronomical characters were recorded taking 10 plants from each of the cultivar. Duncan's new multiple range test ($P < 0.05$) was applied to compare the means of various parameters.

RESULTS AND DISCUSSION

Among the 2 groups of hexaploid triticales which differ widely in heterochromatin content, there was a clearcut difference in the meiotic stability (Table 2). Meiotic stability in triticale reflects through the occurrence of quadrivalents, rod bivalents, univalents, and consequently reduction in the chiasma frequency. Meiotic abnormalities like laggards, bridges, fragments, micronuclei and sterile pollen are other cytological parameters indicating the cytogenetic instability of a genotype. In the present study, triticales which contain relatively higher amounts of heterochromatin showed significantly a higher meiotic instability as compared to the triticales which had a lower per cent of heterochromatin content. Heterochromatin, particularly telomeric heterochromatin of rye chromosomes in triticale has been implicated in meiotic instability in view of its late replicating nature (Lima-de-Faria & Jaworska 1972). Thomas & Kaltskies (1974) observed that heterochromatic telomeres did not participate in chromosome pairing until they had finished replication. Gupta & Fedek (1985) also found that the relative amounts of heterochromatin in different *Secale* species determines the degree of chromosome pairing in intergeneric hybrids. Frequent occurrence of rye chromosomes (which had major heterochromatic bands) as univalents in triticale meiotic metaphase (Thomas & Kaltskies 1974) also supports the role of heterochromatin in meiotic instability. However, in addition to heterochromatin other genic factors in controlling chromosome pairing in triticale also could not be ruled out.

The consequential effect of meiotic instability in triticale is aneuploidy which directly affects the plant fertility. Various agronomic characters recorded on both the groups of triticales (Table 3) indicate that triticales which had lower amounts of heterochromatin are agronomically superior in having significantly higher number of spikelets, florets and increased grain yield, grain weight, and reduction in kernel shrivelling.

Plant fertility in triticale was significantly improved, when selections were made for reducing the heterochromatin content (Kaltsikes et al. 1980). The reduction of heterochromatin seems to reduce the univalent formation and consequently the aneuploidy. Hulgenhof & Schlegel (1985) noticed that when heterochromatin reduced from specific rye chromosomes in triticale, there was a

TABLE 2: Data on various cytological character in different hexaploid triticales (First line is mean and second line is range).

Variety	IV	II		I	Chiasmata	Laggards	Bridges/ fragments	Micro- nuclei	Sterile pollen
		Rod	Ring						
Group I									
Almos	0.28^{ab} (0-1)	2.82^{ab} (0-8)	16.40^{f} (15-19)	1.50^{ab} (0-6)	36.74^{cd} (34-38)	1.36^{a} (0-4)	0.68^{ab} (0-2)	0.56^{a} (0-4)	5.36
Arabian	0.32^{a} (0-1)	2.86^{a} (0-8)	16.26^{fg} (14-19)	1.56^{a} (0-6)	36.66^{cd} (34-40)	1.32^{a} (0-4)	0.70^{a} (0-2)	0.68^{a} (0-4)	5.42
Badiel 'C'	0.24^{abc} (0-1)	2.68^{abcd} (0-7)	16.72^{e} (15-19)	1.36^{abcd} (0-8)	37.08^{c} (34-40)	1.40^{a} (0-5)	0.72^{a} (0-2)	0.64^{a} (0-3)	5.20
Borba	0.32^{a} (0-1)	2.82^{ab} (0-8)	16.46^{ef} (15-19)	1.40^{abc} (0-6)	37.02^{c} (34-41)	1.28^{ab} (0-5)	0.68^{ab} (0-2)	0.72^{a} (0-3)	5.28
Beagle	0.28^{ab} (0-1)	2.80^{ab} (0-8)	16.36^{f} (15-18)	1.56^{abc} (0-6)	36.64^{cd} (34-40)	1.44^{ab} (0-6)	0.72^{a} (0-2)	0.72^{a} (0-4)	5.62
Carman	0.24^{abc} (0-1)	2.68^{abcd} (0-8)	16.48^{ef} (15-18)	1.60^{a} (0-6)	36.60^{cd} (33-40)	1.52^{a} (0-5)	0.68^{ab} (0-2)	0.68^{a} (0-3)	5.68
Aseret	0.28^{ab} (0-1)	2.88^{ab} (0-8)	16.40^{g} (14-18)	1.80^{a} (0-6)	36.08^{d} (34-38)	1.28^{ab} (0-5)	0.76^{a} (0-2)	0.68^{a} (0-4)	5.82
Mizar	0.20^{abc} (0-1)	2.84^{ab} (0-7)	16.40^{f} (15-19)	1.56^{a} (0-8)	36.44^{cd} (34-42)	1.28^{ab} (0-6)	0.68^{ab} (0-2)	0.60^{a} (0-4)	5.72
Group II									
Bacum	0.04^{c} (0-1)	1.92^{abcd} (0-3)	18.12^{cd} (17-20)	0.92^{cde} (0-4)	38.28^{b} (36-42)	0.40^{d} (0-3)	0.20^{c} (0-2)	0.36^{ab} (0-4)	3.02
T1116	0.12^{abc} (0-1)	2.00^{abcd} (0-3)	17.92^{d} (17-20)	0.96^{bcde} (0-4)	38.32^{b} (35-42)	0.44^{d} (0-4)	0.24^{bc} (0-1)	0.16^{b} (0-2)	2.82
T2624	0.04^{c} (0-1)	1.76^{abcd} (0-3)	18.24^{bc} (17-20)	0.96^{bcde} (0-4)	38.40^{ab} (36-42)	0.48^{c} (0-2)	0.16^{c} (0-1)	0.12^{b} (0-2)	2.74
6TA 118	0.04^{c} (0-1)	1.60^{cd} (0-3)	18.52^{a} (16-20)	0.84^{cde} (0-4)	38.78^{ab} (37-41)	0.52^{bcd} (0-4)	0.40^{abc} (0-1)	0.32^{ab} (0-2)	2.68
DTS 30-32	0.04^{c} (0-1)	2.04^{abcd} (0-3)	18.16^{cd} (17-20)	1.76^{a} (0-4)	38.52^{ab} (37-42)	0.52^{bcd} (0-4)	0.36^{abc} (0-1)	0.32^{ab} (0-3)	2.72
Cinnamon	0.04^{c} (0-1)	1.68^{bcd} (0-3)	18.48^{ab} (18.20)	0.80^{de} (0-4)	38.80^{ab} (37-42)	0.56^{bcd} (0-4)	0.44^{abc} (0-1)	0.28^{ab} (0-2)	2.58
Maxitol	0.08^{bc} (0-1)	1.52^{d} (0-3)	18.60^{a} (18-21)	0.80^{a} (0-4)	39.04^{ab} (37-42)	0.52^{bcd} (0-4)	0.40^{abc} (0-1)	0.28^{ab} (0-2)	2.54
DTS 139	0.04^{c} (0-1)	1.84^{abcd} (0-3)	18.60^{a} (17-21)	0.52^{a} (0-4)	39.20^{a} (37-42)	0.52^{bcd} (0-3)	0.32^{abc} (0-1)	0.36^{ab} (0-2)	2.40

Mean in each column followed by the same letters are not significantly different accroding to Duncan's New Multiple Range Test ($P < 0.05$).

TABLE 3: Data on various kernel characters in different hexaploid triticales (First line is mean and second line is range).

Variety	No. of spikelets/ spike	No. of florets/ spike	Florets fertility/ plant (%)	Seeds/ spike	Grain yield/ plant (g)	1000-grain weight (g)	Kernel shrive-lling
Group I							
Almos	14.20^{bc} (13-15)	42.60^{b} (39-45)	46.70^{e} (45-51)	30.50^{d} (29-34)	10.10^{c} (8-13)	36.70^{bc} (34-39)	Medium
Arabian	14.25^{b} (13-15)	42.75^{b} (39-45)	47.40^{e} (45-51)	30.60^{d} (29-33)	10.30^{c} (8-12)	35.30^{bc} (33-38)	High
Badiel 'C'	14.55^{b} (14-15)	43.65^{b} (42-45)	48.30^{de} (46-52)	31.60^{cd} (30-34)	10.60^{c} (9-13)	36.70^{bc} (35-39)	Medium
Borba	14.45^{b} (14-15)	43.35^{b} (42-45)	49.50^{d} (47-53)	32.60^{c} (30-36)	10.20^{c} (8-13)	35.80^{bc} (34-39)	High
Beagle	13.90^{bc} (13-15)	41.70^{bc} (39-45)	46.40^{e} (45-49)	31.10^{cd} (30-35)	9.80^{c} (8-12)	36.90^{b} (35-40)	Medium
Carman	14.20^{b} (13-15)	42.60^{b} (39-45)	48.80^{d} (47-51)	39.90^{b} (38-43)	10.30^{c} (9-13)	36.70^{bc} (34-39)	Medium
Aseret	14.10^{bc} (13-15)	42.30^{b} (39-45)	49.80^{d} (47-53)	30.80^{cd} (29-34)	10.64^{c} (9-13)	36.15^{bc} (34-39)	High
Mizar	13.45^{c} (13-15)	40.35^{c} (39-42)	46.60^{e} (45-50)	31.10^{cd} (30-35)	10.00^{c} (8-12)	36.80^{bc} (34-39)	Medium
Group II							
Bacum	21.90^{a} (21-23)	65.70^{a} (63-69)	5[illegible].90^{bc} (58-65)	62.90^{a} (62-69)	15.50^{b} (14-17)	46.20^{a} (45-48)	Low
TIll6	22.00^{a} (21-23)	65.50^{a} (63-69)	59.40^{c} (58-66)	61.40^{a} (60-68)	15.20^{b} (14-17)	46.00^{a} (45-49)	Low
TL 2624	22.05^{a} (21-23)	64.70^{a} (63-69)	59.40^{c} (58-65)	61.40^{a} (62-68)	15.35^{b} (14-18)	46.20^{a} (45-49)	Low
6TA 118	22.00^{a} (21-23)	65.50^{a} (63-69)	60.60^{abc} (59-66)	62.25^{a} (61-69)	15.65^{ab} (14-18)	46.45^{a} (46-49)	Low
DTS 30-32	21.90^{a} (21-23)	65.70^{a} (63-69)	61.65^{ab} (60-67)	62.65^{a} (61-69)	15.70^{ab} (14-18)	46.80^{a} (46-49)	Low
Cinnamon	22.00^{a} (21-23)	66.00^{a} (63-69)	62.55^{a} (61-69)	63.25^{a} (62-70)	16.15^{ab} (15-18)	47.10^{a} (45-49)	Very Low
Maxitol	22.05^{a} (21-23)	66.15^{a} (63-69)	62.55^{a} (61-69)	63.00^{a} (62-70)	16.80^{a} (15-18)	47.20^{a} (45-49)	Very Low
DTS 139	22.15^{a} (21-23)	65.45^{a} (63-69)	62.05^{a} (60-67)	63.30^{a} (62-69)	16.85^{a} (16-18)	47.50^{a} (46-48)	Very Low

Mean in each column followed by the same letters are not significantly different according to Duncan's New Multiple Range Test ($P < 0.05$).

reduction of 30% univalents than the original karyotype. On the other hand, selection of agronomically superior plants not only reduced the frequency of univalents and aneuploidy, but also improved the meiotic activity (Malik & Mittal 1988). Heterochromain which ultimately affect the grain yield, grain weight and grain shape. Anaphase bridges in coenocytic endosperm in triticale suggested to be the result of delayed replication of heterochromatin segments (Bennett 1977). Skovmand et al. (1984) established a positive correlation between improved seed type and a decrease in aberrant endosperm nuclei.

In view of the above, it is concluded that, heterochromatin content in triticales had a role in determining the meiotic stability and agronomical characters. Efforts, therefore, should be aimed to select the triticales with reduced heterochromatin content either by conventional plant breeding or by induced mutagenesis.

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MITOTIC STUDIES ON SOME MEMBERS OF BACILLARIACEAE

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SUMMARY

Karyological investigations on 15 diatoms revealed chromosome numbers ranging from 2n=6 in *Nitzschia pseudofonticola* to 2n=48 in *N. obtusa*. Chromosomes organized into an equatorial ring during metaphase. Existence of chromosomal counts as multiples of 3,4 or 5 in the taxa investigated currently indicate the role of polyploidy in speciation.

Key Words: Diatoms, cytology, polyploidy.

INTRODUCTION

The diatoms (Bacillariophyceae), comprising more than 10000 valid species are difficult to examine karyologically (Duke & Reimann 1977) and chromosome numbers are available for only 43 taxa (Kociolek & Stoermer 1989). The reasons for slow progress are the rapidity of mitosis, hard cell walls impregnated with silica, minute size of the chromosomes and lack of genetically homogeneous populations in nature (Geitler 1973). There have been a few mitotic studies in recent times on these microorganisms pertaining to cell division pattern and microtubule organization (Pickett-Heaps et al. 1978, 1984, Soranno & Pickett-Heaps 1982, Spurck et al. 1986, Wordeman et al. 1986) but none seems to focus on chromosome behaviour, organization and numbers. The present investigation is an attempt made to study chromosome numbers in 15 taxa belonging to family Bacillariaceae (Nitzschiaceae).

MATERIALS AND METHODS

Fifteen diatom taxa viz., *Hantzschia amphioxys* f.(Ehr.) Grun. f. *capitata* Muel., *H. amphioxys* (Ehr.) Grun. v. *densestriata* Front., *Bacillaria paradoxa* Gmel., *Nitzschia pseudofonticola* Must., *N. palea* (Kg) W.sm., *N. fonticola* Grun., *N. heufleriana* Grun., *N. philippinarum* Hust., *N. regula* Hust/. v. *fennica* A. cl., *N. subrostrata* Hust., *N. gandershemiensis* krasske, *N. acicularis* W.sm., *N. clausii* Hantzsch., *N. obtusa* W. sm.v. *scalpelliformis* Grun. and *N. obtusa* W. Sm were collected from freshwater ponds in Varanasi. They were identified using monographs of Hustedt (1930-66), Foged (1979) and Sarode & Kamat (1984).

Axenic cultures of experimental algae were raised in Chu-10 medium (Chu 1942) at 25 ± 1°C with an irradiance of 50 μ mol m^{-2} S^{-1} and a photoregime of 16:8 h. Inoculum from stationary phase cultures (15 day old) was subcultured to grow for 48 h under aforesaid standard culture conditions. Subsequently, the materials were fixed in 1:3 acetic acid-alcohol mixture at an interval of 10 minutes during 24 h cycle in order to excise the cell cycle and kept in fixative for 24-48 h to ensure complete pigment extraction. Before preparing the material for cytological observations, they were subjected to acid pretreatment for curing the cell wall. Since the cell wall in diatoms is highly impregnated with silica, they were given acid bath with 0.5 M hydroflouric acid for 3-5 min followed by water rinsing to remove acid traces. After the

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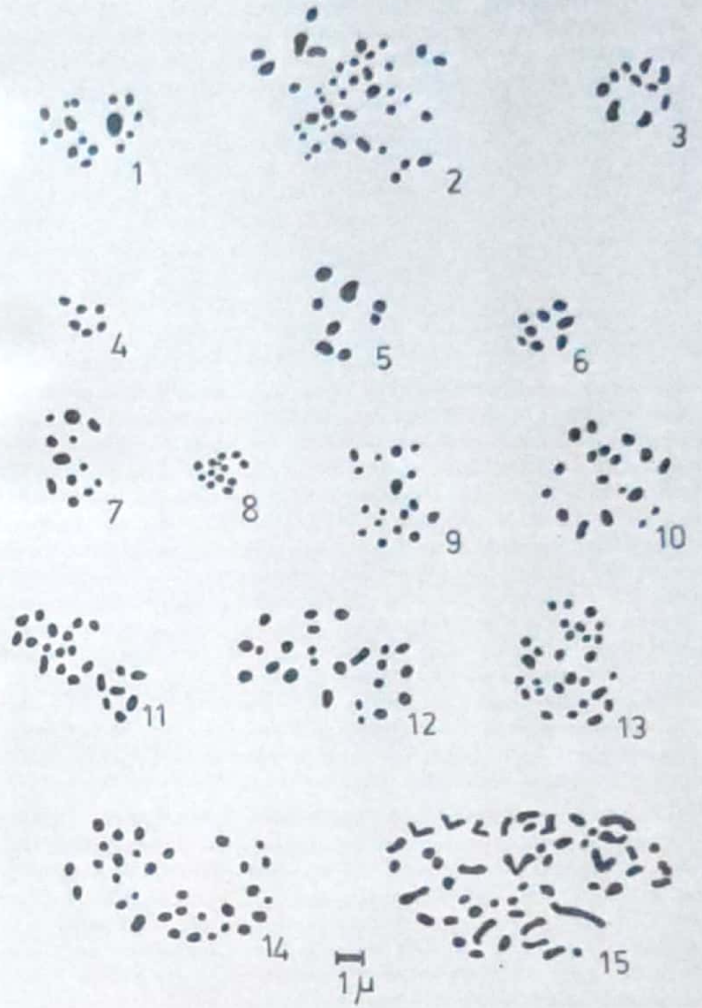
acid treatment, the cells were squashed in 2% warm acetocarmine compounded with iron alum. Karyological characteristics and chromosome numbers (2n) of a taxon were inferred from 25 mitotic squash preparations under occular divider fitted to compound microscope.

OBSERVATIONS

The interphase nucleus was spherical and lies in the central area during cell division in all the taxa except in *Bacillaria paradoxa* and *Nitzschia fonticola* where it was localized towards the polar region and parietal layers of cytoplasm respectively. Approximately 100-150% increment in the nuclear diameter was recorded during prophase over its interphase size. A distinct nucleolus (1.8-2.0 μm) was observed in *Hantzschia amphioxyx* var. *densestriata* and persisted till the end of prophase. Metaphase chromosomes were organized into a ring or plate in the equatorial region. Chromosomes appeared as dot-or rod-like structures devoid of structural organization into centromere and chromatids. Table 1 summarizes nuclear characteristics of different taxa during cell division while Figs. 1-15 depict metaphase chromosomes.

TABLE 1: Karyological characteristics of some taxa of Bacillariaceae.

Name of the taxa	Interphase nucleus		Prophase nucleus	Metaphase		Chromosome		
	Shape	Diameter (μm)	Diameter (μm)	Arrange-ment	Diameter (μm)	Shape	Size (μm)	Number (2n)
Hantzschia amphioxys f. *capitata*	Spherical	4.0-4.8	5.0-7.0	Ring	6.0-6.4	Dot	0.3-1.2	16
H. amphioxys v. *densestriata*	Spherical	3.5-4.0	3.5-8.0	Plate	4.8-5.0	Dot	0.3-0.8	40
Bacillaria paradoxa	Spherical	3.2	5.8	Ring	4.8-5.0	Dot	0.3-0.9	10
Nitzschia pseudofonticola	Spherical	2.0	3.0	Ring	2.0	Dot	0.25-0.6	6
N. palea	Spherical	2.0-3.0	4.4-5.0	Ring	3.2	Dot	0.5-0.8	8
N. fonticola	Spherical	1.8-2.0	3.8-4.2	Ring	2.5-2.8	Dot	0.5-1.0	8
N. heufleriana	Spherical	4.0-4.2	6.0-6.6	Plate	5.0	Dot	0.8-1.5	12
N. philippinarum	Spherical	3.0	5.0	Ring	3.2-4.0	Dot	0.5-0.8	12
N. regula v. *fennica*	Spherical	3.2-4.0	6.0-6.6	Ring	4.5-5.0	Dot	0.25-0.8	18
N. subrostrata	Spherical	3.0-3.5	5.0	Ring	4.0	Dot	0.5-0.8	18
N. gandershemiensis	Spherical	2.0-3.0	5.0-6.0	Ring	4.0-4.5	Dot	0.5-0.8	22
N. acicularis	Spherical	3.0-3.4	6.0-6.4	Plate	4.8	Dot	0.5-1.0	24
N. clausii	Spherical	2.0-3.0	6.0	Ring	5.0	Dot	0.8-1.5	28
N. obtusa v. *scalpelliformis*	Spherical	3.8-4.2	7.0	Ring	5.0-6.0	Dot	0.3-1.0	32
N. obtusa	Spherical	4.0-5.0	7.0-7.5	Plate	6.0-7.0	Dot-Rod	0.5-1.0	48



Figs. 1-15: Metaphase chromosome configurations in some bacillarian taxa. 1. *Hantzschia amphioxys* f. *capitata* (2n=16). 2. *H. amphioxys* v. *densestriata* (2n=40). 3. *Bacillaria paradoxa* (2n=10). 4. *Nitzschia pseudofonticola* (2n=6). 5. *N. palea* (2n=8). 6. *N. fonticola* (2n=8). 7. *N. heufleriana* (2n=12). 8. *N. philippinarum* (2n=12). 9. *N. regula* v. *fennica* (2n=18). 10. *N. subrostrata* (2n=18). 11. *N. gandershemiensis* (2n=22). 12. *N. acicularis* (2n=24). 13. *N. clausii* (2n=28). 14. *N. obtusa* v. *scalpelliformis* (2n=32). 15. *N. obtusa* (2n=48).

DISCUSSION

All the taxa of the family Bacillariaceae were characterized by spherical nucleus located in the central area prior to onset of the cell division. Even though, spherical nucleus exists in most of the diatoms, other nuclear shapes such as girdle in *Surirella ovalis* (Drum & Pankratz 1964), lenticular in *Melosira varians* (Crawford 1973) and discoid in *Pinnularia gibba* (Giri & Chowdary 1991a) are also not uncommon. Central disposition of nucleus during cell division although characteristic of diatom cells, presence of excentric nucleus as recorded in *Bacillaria paradoxa* and *Nitzschia fonticola* may be encountered rarely (Giri & Chowdary 1991a). This was also reported earlier by Manton et al. (1969) in *Lithodesmium undulatum* and *Melosira varians* (Crawford 1973). Though, nucleus is characterized by the presence of one nucleolus, occurrence of heteromorphic nucleoli was reported in *S. ovalis* (Drum & Pankratz 1964) and *M. varians* (Crawford 1973). Nucleolus usually appears conspicuously till the end of prophase as in conventional mitotic cells. However, it may persist through larger part of the cell division as in case of few naviculoid diatoms, *Gyrosigma kutzingii* and *Navicula cuspidata* var. *ambiqua* (Giri 1992).

Most of the taxa examined exhibited ring-like arrangement of chromosomes in the equatorial region at metaphase. Geirler (1932) termed such arrangement as 'equatorial ring' and considered such an arrangement in diatoms more an animal character than plants. Wordeman et al. (1986) attributed this organization to microtubules radiating from all directions of the cell. Ring-like arrangement of chromosomes is predominant among diatoms although plate like organization of chromosomes at metaphase is not rare. Arrangement of chromosomes in the equatorial region need not be a consistent factor at higher taxonomic level such as family or in lower taxonomic units such as genera (Giri & Chowdary 1991a). Both plate- as well as ring-like organization of chromosomes were seen in different taxa of *Hantzschia* and *Nitzschia* in the present study. Chromosomes in most of the taxa under investigation possess dot-like configuration. They were devoid of centromeres and chromatids, similar to the type of chromosomes reported in *Navicula halophila* (Subramanyan 1945), *Lithodesmium undulatum* (Manton et al. 1969) and *Synedra ulna* (Roy & Sarma 1977). *Nizschia obtusa* has dot-rod-like and v-bent chromosomes (Neocentric?). Similar types of chromosomes were also reported in *Pinnularia* species (Spurck et al. 1986) and in *P. gibba* (Giri 1992). Earlier, Iyengar & Subramanyan (1944) reported long rod-like metacentric, sub-metacentric and telocentric chromosomes in *Cyclotella meneghiniana*.

The lowest chromosome number recorded for this family is 2n=6 in *Nitzschia pseudofonticola* and the highest count is 2n=48 in *N. obtusa*. Morphologically distinct taxa of *Nitzschia* revealed same chromosome number of 2n=8 in *N.palea* and *N.fonticola*; 2n=12 in *N. heufleriana* and *N. philippinarum* and 2n=18 in *N. regula* var. *fennica* and *N. subrostrata*. Sarma (1982) stated that such instances of existence of same chromosome numbers in taxa belonging to different families or different taxa belonging to same genus are of prime significance in establishing evolutionary relationships. In another study, occurrence of same chromosome number in different naviculoid diatoms was encountered by Giri (1992).

Nitzschia obtusa had chromosome numbner of 2n=48 whereas, *N. obtusa* var. *scalperlliformis* had 2n=32. A similar relationship in other diatom taxa namely, *Cyclotella meneghiniana* (2n=60) and *C. meneghiniana* f. *unipunctata* (2n=18); *Navicula cryptocephala* and *N. cryptocephala* var. *exilis* (2n=10) was reported by Giri & Chowdary (1991b) and Giri (1992) respectively.Further

chromosomes in the current bacillarian taxa exist in multiples of 3, 4 or 5 except in *Nitzschia gandersheimiensis* (2n=22). This suggests that speciation in diatoms was effected by spontaneous polyploidization. Recently, Kociolek & Stoermer (1989) also supported this view of evolution of diatoms.

Due to close structural affinities between *Nitzschia* and *Bacillaria*, Boyer (1927) merged both the genera. Hustedt (1930) pointed out the differences in transapical planes of these 2 genera and treated them as distinct genera. Hendey (1964) also favoured the retention of the genus *Bacillaria* until a revision of all the species having a central keel has been worked out. Karyological features reveal close interrelationship between these 2 genera. Equational ring arrangement of chromosomes at metaphase, existence of chromosome number as a multiple of 5 and their dot-like appearance without structural organization are some of the common features shared by these 2 genera. Nucleus is not placed in the central area during cell division in *Bacillaria paradoxa* which is a predominant character in various taxa of *Nitzschia*. Anyhow, current status of karyological information as regards these 2 genera does not justify any discussion on their retention as separate genera or merger into one. More information on few more species of *Bacillaria* is awaited.

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I dedicate this article to the memory of my supervisor late, Prof. Y.B.K. Chowdary.

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KARYOMORPHOLOGICAL ANALYSIS IN *VISCUM* LINN.

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SUMMARY

Karyomorphological analysis on 3 species of *Viscum* was carried out for the first time. The cytology of *V.verruculosum* (Wt. & Arn.) is the first report. The present report of n=12 in *V. angulatum* Heyne and n=13 in *V.orientale* Willd. differed from the previous reports. The primary basic chromosome number for *Viscum* is suggested as x=7. The chromosome number n=14 might be a polyploid origin from x=7 and the numbers x=13, 12 and 10 are aneuploid derivatives from n=14.

Key Words: *Viscum*, karyomorphology, speciation.

INTRODUCTION

The genus *Viscum* comprises about 100 species of which chromosome number reports are available for only 15 species (Wiens & Barlow 1971). Cytology of the Indian representatives of the genus remains largely unworked.

The present study was undetaken as part of an attempt to characterise the Indian representatives of Viscaceae in the hope that this will expand the picture of evolutionary trend of the family. Detailed karyomorphological analysis on 3 species of *Viscum* is reported here.

MATERIALS AND METHODS

Materials for the present study were collected from Udhagamandalam and Kodaikanal in Tamil Nadu. Meiotic studies were made from pollen mother cells (PMC). Mitotic chromosomes of *V. orientale* and *V. verruculosum* were prepared from young leaf tips. Mitotic studies on *V. angulatum* (as there are no leaves in this species) were carried out using endosperm tissue of young fruits. The materials for mitotic studies were pretreated in 0.002 M 8-hydroxyquinoline at 8°C for 4 h. The materials were fixed in acetic-alcohol (1:3). Squashes were prepared in 2% acetocarmine. Karyological analysis was made from photographs taken from paraffin-sealed temporary slides. Karyomorphological analysis was done as per the systems proposed by Stebbins (1958) and Levan et al. (1964).

RESULTS AND DISCUSSION

The PMCs of *V.orientale* exhibited 13 bivalents at metaphase I (Fig. 1) and the somatic cells showed 26 chromosomes at metaphase (Fig.2). The gametic number of *V. verruculosum* was found to be n=14 (Fig.3) and the somatic number was 2n=28 (Fig. 5). One B-chromosome was observed in about 3% of the PMCs (Fig.4). B-chromosomes of different sizes and number (0-6) were frequently observed in somatic cells also (Fig.6). *V.angulatum* exhibited a meiotic number of n=12 (Fig.7) and a somatic number of 2n=24 (Fig.9). Sticky association of bivalents was of common occurrence in this species, sometimes persisting up to late anaphase I (Fig.8). The details of

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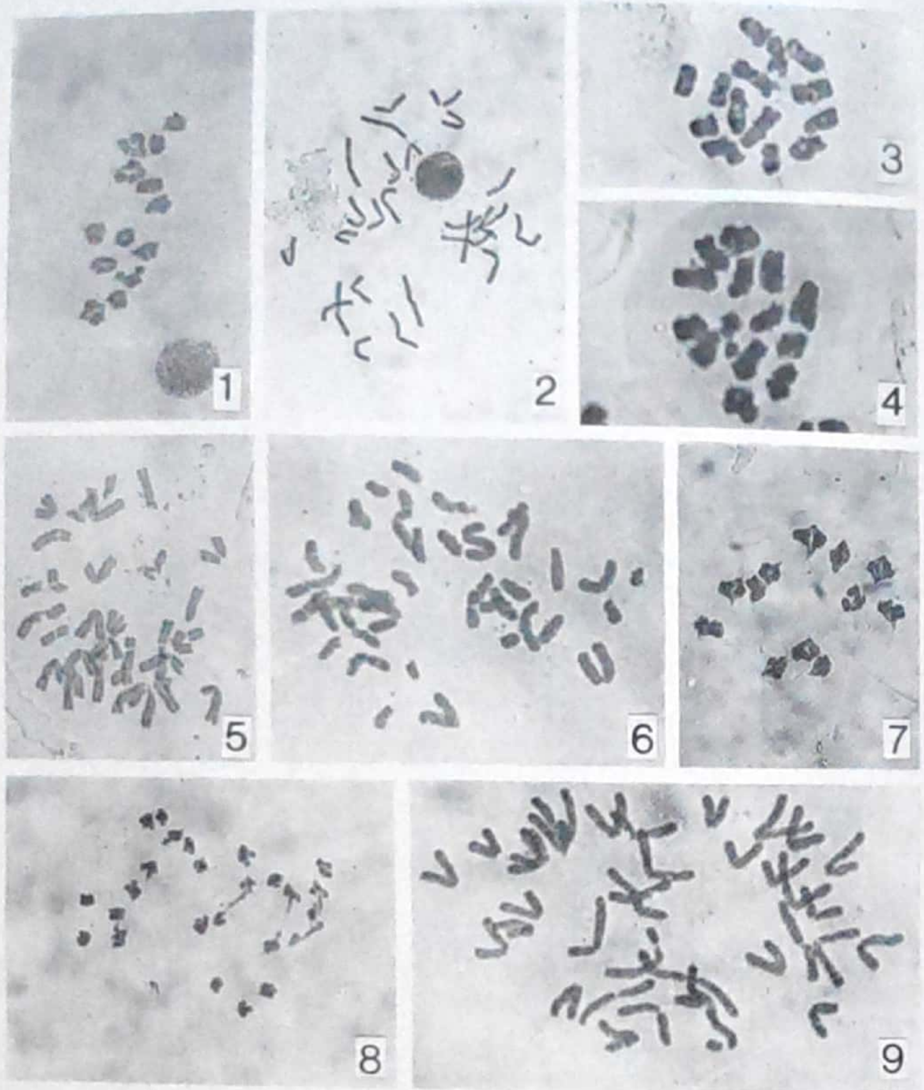

Figs. 1-9: Cytology of *Viscum*. 1. *V. orientale* (n=13). 2. *V. orientale* (2n=26). 3. *V. verruculosum* (n=14). 4. *V. verruculosum* (n=14+1B). 5. *V. verruculosum* (2n=28). 6. *V. verruculosum* (2n=28+6B). 7. *V. angulatum* (n=12). 8. *V. angulatum* anaphase I with sticky bridges. 9. *V. angulatum* (3n=36). (1,2,7 x 450; 3,4,6 x 900; 5,8 x 675).

TABLE 1: Characterisation of chromosomes in three species of *Viscum*

Species	Chromosome number 2n	Chromosome length (µm)	Frequency of chromosome types in the haploid set M	m	Sm	St	t	TCL (µm)	ACL (µm)	Karyotype category
V. orientale	26	5.55 - 11.32	1	12	0	0	0	421.90	16.22	1A
V. verruculosum	28	2.77 - 11.11	0	8	5	1	0	349.06	12.46	2B
V. angulatum	24	2.46 - 8.38	1	10	1	0	0	249.10	12.25	2A

karyomorphology of 3 species studied here are shown in Table 1.

The karyomorphological analysis of a viscaceous taxon (*Viscum capitellatum*) was first carried out by Soman & Bhavanandan (1993). The present investigation revealed 3 different gametic numbers of n=12, 13 and 14 in the 3 species examined. The chromosome number n=14 recorded in *V. verruculosum* is the first report for the genus. The present report of n=12 for *V. angulatum* differs from Feuer (1965) who has reported a gametic number n=11 (in Wiens & Barlow 1971). Likewise n=13 recorded in *V. orientale* differs from the previous count of n=10 reported by Schaeppi & Steindl (1945).

Difference of opinion exists among the cytologists regarding the basic chromosome number in *Viscum*. Wiens & Barlow (1971) stated that the direction of aneuploid changes is not clear in *Viscum* and the basic number of the genus can be any of the numbers in between 10 and 13.

As far as the authors are aware, chromosome number reports are available in 6 of the 7 genera of Viscaceae out of which 4 (*Arceuthobium, Korthalsella, Phoradendron* and *Dendrophthora*) possess a basic number of 14. Barlow & Martin (1984) porposed a basic number of 14 for *Viscum* also. However, Raven & Kyhos (1965), Rudenberg (1967), Ehrendorfer et al. (1965) and Walker (1972) have suggested that angiosperms had monophyletic origin from progenitors with x=7. Goldblatt (1980) has also suggested that almost all angiosperms with gametic numbers above 9 probably had polyploidy in the evolutionary history. So, it is suggested that n=14 (as observed in *V. verruculosum*) might have originated from an ancestral number of n=7 through polyploidy followed by stabilisation. The gametic numbers of 13, 12 and 10 might be a step-wise aneuploid derivation from n=14. The chromosomal evidence also suggests that aneuploidy has played a significant role in speciation in *Viscum*.

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A LINE X TESTER ANALYSIS FOR COMBINING ABILITY AND GENETIC COMPONENTS IN TASAR SILKWORM

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SUMMARY

A line x tester analysis involving diverse 8 females and 3 males of *Antheraea mylitta* D. was conducted for estimating combining ability effects and genetic components which may be useful in planning future breeding programmes for yield and yield components. Genetic parameters were estimated for 8 characters. The progenies differed significantly for all the characters. The variation due to general combining ability were significant for only 4 characters viz., absolute silk yield, fecundity, hatching percentage and effective rate of rearing. The specific combining ability variation were also significant for 4 characters. Overall estimates of nonadditive genetic variances were higher than additive variances. The best general combiners and specific cross combinations for absolute silk yield, fecundity, hatching percentage and effective rate of rearing were S_4, GF_3, S_{17}, and N_1 and S_4 x R.S., N_2 x R.S., GE_2 x N_5 and N-2 x Raily respectively.

Key Words: *Antheraea mylitta*, combining ability, genetic components.

INTRODUCTION

Silk yield potential in the varieties of *Antheraea mylitta* D. is poor, therefore, it needs to be imporoved for commerical exploitation. Effective improvement in complex trait like yield may be brought by understanding their genetic make up for improving yield. In *A. mylitta*, several hybridization programmes had been taken up earlier like diallel, single, doube and 3 way crossess (Bardaiyar et al. 1976, Jolly et al. 1969, 1972, Siddiqui et al. 1988a, b). Of these, Line x Tester analysis of combining ability is fairly effective and popular. No such effort has so far been made in tropical tasar producing species. Therefore, the present investigation was undertaken to know the genetic architecture of the populations which may be helpful in choosing the promising parents to be used in hybridization programmes to improve the yield potential.

MATERIALS AND METHODS

The materials consisted of 8 diverse females viz. GE_1, GE_2, GF_3, N-1, N-2, L_9, S_4 and S_{17}, 3 males N_5, Raily, R.S. and their 24 F_1 hybrids of tasar silkworm *A. mylitta*. The females were selected from genetic stocks maintained on the basis of their *per se* performance. Thirty-five progenies (11 parents and 24 hybrids) were reared in randomised block design with 4 replications at C.T.R. & T.I., Nagri, Farm, Ranchi during commercial (Oct-Nov) crop, 1988. Data were collected on 8 metric traits namely, absolute silk yield (g), fecundity (No), hatching (%), larval weight (g), larval duration (days), effective rate of rearing (E.R.R.%), cocoon weight (g) and shell weight (g). The estimates of combining ability effects, genetic components and variances were estimated according to the method developed by Kempthorne (1957).

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OBSERVATIONS

Pooled analysis of variance for 8 characters has been presented in Table 1. All the progenies differed significantly for the characters studied.

The estimates of variances of general combining ability effects due to males (lines) and females (tester) and specific combining ability effects of lines and tester were significant for fecundity, hatching percentage, effective rate of rearing and absolute silk yield while it was found nonsignificant for larval weight, larval duration, cocoon and shell weights. (Table 2).

TABLE 1: Analysis of variance for 8 characters in *A. mylitta.*

Source of variation	D.F	Mean sum of square: Absolute silk yield (g)	Fecundity (No)	Hatching (%)	Larval weight (g)	Larval duration (days)	E.R.R. (%)	Cocoon weight (g)	Shell weight (g)
Replication	3	3065.92	2661.80	78.88	8.18	2.26	297.33	0.70	0.02
Treatment	34	11836.96**	6325.60**	371.53**	10.11**	10.12**	513.84**	1.83*	2.08*
Parent	10	6872.76	11060.27**	296.63	5.11	20.40**	244.25	2.12*	0.25**
Lines	7	4841.78	12809.96**	117.34	6.89	28.63**	303.75	3.12*	0.21**
Testers	2	11487.19	9637.75	4 1.97	4.29	6.75	127.94	4.41**	0.14
LxT	1	11860.74	1657.51	1260.96	0.22	4.13	60.36	0.53	0.13
Crossess	23	13446.76**	4541.99	415.26**	12.71**	6.06	624.22**	0.70	0.14**
Parent x crossess	1	24453.78	12.02	114.81	0.48	0.64	671.06	0.82	0.11
Error	102	6501.12	3804.04	210.20	6.08	6.21	254.61	0.77	0.05

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

TABLE 2: Estimate of variances due to combining ability effects.

Source	•D.F.	Fecundity (No)	Hatching (%)	Larval weight (g)	Larval duration (days)	ERR (%)	Cocoon weight (g)	Shell weight (g)	Absolu-silk yield (g)
GCA (Line)	8	317.00**	17.52**	0.51	0.52	21.22**	0.064	0.004	541.76**
GCA (Tester)	3	118.88**	6.57**	0.19	0.19	7.96**	0.024	0.002	203.16**
SCA	24	951.00**	52.55**	1.52	1.55	63.66**	0.192	0.013	1625.28**

** Significant at 1 % level.

General combining ability affects

The estimates of general combining ability effects for all the eight characters are presented in Table 3.

TABLE 3: Estimates of general combining ability effects for 8 characters.

Progenies	Fecundity (NO)	Hatching (%)	Larval weight (g)	Larval duration (g)	E.R.R. (%)	Cocoon weight (g)	Shell weight (g)	Absolute silk yield (g)
Lines								
GE_1	-12.20	-1.26	+0.08	-0.35	+3.05	-0.22	-0.10	-28.60
GE_2	-6.03	+4.82	+1.59**	+0.23	+4.12	-0.09	-0.05	+32.71**
GF_3	+55.14**	-4.03	-1.46	+1.31**	-12.68	-0.07	+0.04	-31.91
N-1	-13.62	-0.13	-0.30	-1.27	+11.64**	-0.11	+0.01	+34.58**
N-2	-7.28	-0.64	-0.03	+0.73	-0.50	+0.06	+0.07**	+9.94
L-8	-17.62	+0.42	_1.28	-0.69	-3.91	+0.15	-0.02	-28.66
S_4	-0.12	-4.90	+0.44	-0.35	-7.55	+0.16	+0.04	-16.35
S17	+1.72	+5.72**	+1.11**	+0.40	+5.81**	+0.12	+0.004	+29.21**
Testers								
N_5	+6.91*	-0.60	+0.21	-0.01	+0.06	-0.11	-0.05	-3.75
Raily	-6.81	+0.05	-0.20	-0.04	-5.08	+0.07	+0.02	-22.74
R.S.	-6.09	+0.55	-0.01	+0.05	+5.02	+0.04	+0.03	+26.48

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

Absolute silk yield

Three female parents N_1, GE_2 and S_{17} exhibited highly significant positive general combining ability effects for absolute silk yield. The female parent N_1 followed by parent GE_2 were the best general combiners for absolute silk yield. The parents GF_3, L_8 and GE_1 showed significant negative general combining ability for absolute silk yield.

Fecundity

One female parent GF_3 and male parent N_5 exhibited positive and significant general combining ability effects. Remaining parents showed negative general combining ability effects excepts S_{17}.

Hatching percentage

Only one parent showed significant and positive general combining ability effects. Remaining male and female parents possessed either positive or negative but nonsignificant general combining ability effects.

Larval weight

Two female parents showed significant general combining ability effects for larval weight. None of the male parents showed significant general combining ability effects. The best general combiner for larval weight was GE_2.

Larval duration

One female parent GF_3 showed positive and significant general combining ability effect. Rest of the parents exhibited positive or negative but nonsignificant general combining ability effects.

Effective rate of rearing

Two female parents exhibited significant general combining ability effects for this character. Remaining parents (female and male) were either positive or negative but nonsignificant general combining ability effects.

Cocoon and shell weight

None of the female and male parents showed positive and significant general combining ability effects for cocoon weight and shell weight except N-2 which showed positive and significant general combining ability effects.

Specific combining ability effects

The specific combining ability for all the 8 characters are presented in Table 4.

Absolute silk yield

Two hybrids namely S_4 x R.S. and S_{17} x N_5 out of 24 hybrids showed significant specific combining ability effects for higher silk yield. One hybrid viz., S_4 x N_5 exhibited significant negative specific combining ability effects.

The best cross combination was S_4 x R.S.

Fecundity

None of the hybrids exhibited positive and significant specific combining effects. Numerically the best hybrids were N_2 x R.S. and GF_3 x N_5.

Hatching percentage

Two hybrids exhibited significant and positive specific combining ability effects. Only One hybrid exhibited negative and significant specific combining effects, for hatching percentage. The good specific combiner crosses were GE_2 x N_5 and GF_3 x R.S.

Larval weight

Only 1 hybrid, GE_2 x R.S. was exhibited positive and significant specific combining ability effects. Remaining hybrids showed either positive or negative but nonsignificant specific combining ability effects.

Larval duration

No significant specific combining ability effect was observed for any cross combination for larval duration. Maximum specific combining ability effect were found in the cross L_8 x Raily.

TABLE 4: Estimates of specific combining ability effects for 8 characters.

Hybrid	Fecundity (No.)	Hatching (%)	Larval weight (g)	Larval duration (g)	E.R.R. (%)	Cocoon weight (g)	Shell weight (g)	Absolute silk yield (g)
1	2	3	4	5	6	7	8	9
GE_1 x N5	+17.01	+1.48	-1.96	+0.01	-6.99	+0.24	+0.10	-18.98
xRaily	-3.52	+11.21	-0.33	-0.46	-6.31	-0.33	-0.08	+12.58
xR.S.	-13.49	-12.69	+2.29	+0.45	+13.30	+0.09	-0.02	+6.40
GE_2 x N_5	-24.16	+19.41**	+1.86	+1.68	+3.58	+0.38	+0.02	+62.86
xRaily	+31.06	-12.43	-1.15	-1.79	-1.18	-0.16	+0.06	-20.69
xR.S.	-6.91	-6.98	+3.01**	+0.12	-2.40	-0.54	-0.06	-42.16
GF_3 x N5	+42.68	-12.06	+1.31	+0.34	+4.65	-0.25	+0.01	+5.47
x Raily	+5.90	-3.70	+0.35	-0.88	-6.26	+0.47	+0.09	-22.33
x R.S.	-48.57	+15.76**	-1.48	+0.53	+1.61	-0.22	-0.11	+16.86
N-1 x N5	+32.68	-19.95	-0.57	-0.32	+0.71	0.32	-0.10	-49.53
x Raily	-17.10	+11.19	-0.84	+0.21	+8.06	-0.25	-0.02	+52.63
x R.S.	-15.57	+8.76	+1.41	+0.12	-8.78	+0.57	+0.11	-3.10
N-2 x N5	-24.16	+5.14	+1.57	-0.07	-5.60	+0.58	+0.06	-24.30
x Raily	-28.19	-3.64	+0.92	+0.96	+17.99**	+0.19	+0.04	+76.24
x R.S.	+52.34	-1.50	-2.49	-0.89	-12.39	-0.77	-0.10	-51.94
L-8 x N5	-34.32	+0.15	+0.41	-1.91	+8.49	-0.46	-0.06	+20.96
x Raily	+10.65	+6.49	-0.62	+2.13	-12.99	+0.32	-0.04	-29.00
x R.S.	+23.68	-6.64	+0.21	-0.22	+4.50	+0.14	+0.10	+8.04
S_4 x N5	-9.32	+1.09	+1.49	+1.01	-16.71	-0.11	-0.03	-80.47
x Raily	+5.15	-5.14	+0.79	-0.21	+7.52	-0.54	-0.11	-9.40
x R.S.	+4.18	+4.05	-2.27	-0.80	+9.19	+0.65	+0.14	+89.87**
S17 x N5	-0.41	+4.75	-0.22	0.74	+11.87	-0.05	+0.02	+83.99**
x Raily	-3.94	-3.99	+0.40	+0.04	-6.83	-0.02	+0.06	-60.02
x R.S.	+4.34	-0.76	-0.68	+0.70	-5.04	+0.07	-0.07	-23.97

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

Effective rate of rearing

Only one hybrid out of 24 hybrids showed positive and significant specific combining ability effects for higher effective rate of rearing. Remaining hybrids were either positive or negative but exhibited nonsignificant specific combining ability effects.

Cocoon and shell weight

None of the cross combinations exhibited significant specific combining ability effects for both the characters. The hybrids S_4 x R.S., N_2 x N_5 and N-1 x RS for cocoon weight and hybrids S_4 x RS and N-1 x RS were found numerically superior.

Genetic components/variance and heritability

The estimates of genetic components/variances and heritability are presented in Table 5. The estimates of nonadditive genetic components/variances (D^2) were found higher than the additive genetic variance (A^2) for absolute silk yield, fecundity, hatching percentage, larval wiehgt, effective rate of rearing and shell weight, but the estimates of additive components/variance were higher than nonadditive components/variance for larval duration and cocoon weight. Moderate heritability (narrow sense) was recorded for all the characters ranging from 23.08% in shell weight to 33.73% in effective rate of rearing.

TABLE 5:Estimates of genetic variances and heritability for 8 characters.

Genetic component/ variance	Fecundity (No.)	Hatching (%)	Larval weight (g)	Larval duration (g)	E.R.R. (%)	Cocoon weight (g)	Shell weight (g)	Absolute silk yield (g)
A^2	-1.77	46.06	-0.61	0.15	21.34	-0.07	0.00	98.98
D^2	51.38	96.54	1.99	-0.09	71.65	0.06	-0.00	1880.24
Heritability	24.89	27.73	24.89	23.94	33.73	28.75	23.08	29.10

DISCUSSION

Any breeding programme aiming to evolve an outstanding widely adopted variety must be based on the estimates of nature and magnitude of genetic variances present in the population. The variances due to general combining ability effects of females and males and specific combining ability effects were significant for 4 characters viz., absolute silk yield, fecundity, hatching percentage and effective rate of rearing. This indicates that both additive and nonadditive gene effects control the different characters (Siddiqui et al. 1988a, Subba Rao 1983). The estimates of genetic variance A^2 (additive) and D^2 (nonadditive) also revealed the importance of both additive and non-additive gene effects, although the estimates of the latter were higher in case of absolute silk yield, fecundity, hatching percentage larval weight, effective rate of rearing and shell weight whereas, additive part was higher in the case of larval duration and cocoon weight. Under such a situation it is suggested that the population breeding approach would be highly remunerative because the method is ideally suited to exploit both additive and nonadditive gene actions. The selection would also maintain certain flexibility for advantage. The best parent for absolute silk yield and effective rate of rearing is S_4. The outstanding cross combination for absolute silk yield, cocoon weight and shell weight was S_4 x R.S. The best parent was always not the best combiner. It is, therefore, suggested that the parents should be chosen on the basis of their *per se* performance, combining ability and genetic diversity.

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KARYOTYPE DISTINCTION IN INDIAN TAROS

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SUMMARY

Detailed karyomorphological analysis was made in 21 diploid ($2n=28$) and 24 triploid ($2n=42$) morphotypes of taro (*Colocasia esculenta* (L.) Schott.) recognised from 239 accessions assembled from all over the Indian subcontinent. The data showed that 4 chromosomes (Chrom. 1,3,7 and 9) in both the ploidy groups exhibited marked variation in their morphology, possibly due to pericentric inversions at the diploid level. A dozen karyotypically distinct cytotypes were recognized based on the morphology of these 4 marker chromosomes, 6 among diploids and 6 among triploids. The magnitude of karyotype distinction is very striking between the first and the fifth diploid cytotypes; all the marker chromosomes being metacentric (m-type) in the former and subtelocentric (st-type) in the latter. Plants of these 2 cytotypes differed for a number of qualitative characters such as petiole colour, leaf shape, leaf margin, corm shape and cormel shape. We postulate that these 2 cytotypes may have evolved either from a common ancestor or the latter may have evolved from the former during the early evolutionary history of the species.

Key Words: Taro, *Colocasia esculenta*, morphotype, karyotype distinction, marker chromosome, cytotype.

INTRODUCTION

Taro (*Colocasia esculenta* (L.) Schott.), belonging to the family Araceae, is an important tuber crop extensively cultivated in the tropical world. Throughout the islands of the Pacific, Asian Archipelago, Central Africa, West Indies and the islands of Caribbean and Central America, taro forms an important item of diet. In India, the corm, cormels and leaves of this crop are used as vegetable. Innumerable morphologically distinct plant types occur in this vegetatively propagated species which exists at 2 ploidy levels, diploid with $2n=28$ and triploid with $2n=42$(Sharma & Das 1954, Mookerjea 1955, Yen & Wheeler 1968, Vijaya Bai et al. 1971, Ramachandran 1978) and in addition, a few aneuploid variants in both the ploidy groups are also known (Rao 1947, Delay 1951, Sharma & Sarkar 1963). Despite the usefulness of karyomorphological information in assessing the nature and magnitude of karyotype variation and its possible bearing on plant morphological diversity in species complexes, the data available on this polymorphic species are scanty and scattered, covering only a limited number of plant types from narrow distributional ranges (Sharma & Sarkar 1963, Kuruvilla & Singh 1981, Sreekumari & Mathew 1989, 1991a, 1991b) barring the study on the South Pacific taro forms (Coates et al. 1988). This paper concerns the results of detailed karyotype analysis in a very large number of accessions from all over the Indian subcontinent representing different latitudinal, longitudinal and altitudinal regions. The study has revealed the existence of a number of karyotypically distinct cytotypes among the Indian taro forms.

MATERIALS AND METHODS

The materials for the study constituted 239 accessions of taro assembled from all over India and maintained at the Central Tuber Crops Research Institute, Trivandrum. Initial screeding for chromosome number showed that 120 accessions were diploids and 119 triploids. Various quantitative and qualitative characters of the diploid and triploid accessions were analysed, and on the basis of recognizable plant morphological differences they were grouped into 45 morphotypes (21 diploid and 24 triploid). Detailed karyomorphological study was made in the total assemblage of morphotypes at the rate of 5 accessions per morphotype. Somatic chromosome preparations were made from root tips fixed in Carnoy's fluid after pretreatment with 0.002 M 8- hydroxyquinoline at 4°C for 2 h. Karyotype analysis was made based on mean chromosome measurements from 5 metaphase plates from each plant (3 plants/accession). Morphological classification of the chromosomes was made following the system proposed by Levan et al. (1964), by which chromosomes with arm ratio r=1 were designated as M type (absolute metacentric), those with arm ratio between 1.0 and 1.7 as m-type (nearly metacentric), between 1.7 and 3.0 as sm-type (submetacentric), between 3.0 and 7.0 as st-type (subtelocentric) and those exceeding 7 as t-type (nearly telocentric or acrocentric). Karyotype category was determined following Stebbins (1971) according to which there are 4 categories based on the position of centromere (1,2,3,4) and 3 categories based on the size difference between the longest and shortest chromosome of the complement (A,B,C) together constituting 12 categories (1A, 1B, 1C; 2A, 2B, 2C; 3A, 3B, 3C; 4A, 4B, 4C).

In the diagramatic representation of the various cytotypes with respect to the 4 marker chromosomes represented in Figs. 1 and 2, the 6 cytotypes of the diploid group are designated as 2-a, 2-b, 2-c, 2-d, 2-e and 2-f and those of the triploid as 3-a, 3-b, 3-c, 3-d, 3-e and 3-f where 2 and 3 stand for diploid and triploid respectively and a to f stand for 6 cytotypes in each of the ploidy groups.

OBSERVATIONS

The chromosomes of morphotypes of both the ploidy groups were medium-sized ranging in length from 1.33 - 4.55 µm. The chromosomes of the triploids, however, were from 2.20 - 2.88µm as against 2.30 - 3.05µm in the diploid counterparts. The karyotypes were of the graded type showing no sharp intrakaryotypic chromosome size difference, and they were of medium asymmetry (karyotype categories 2A and 2B in the diploids and 2A, 2B and less frequently 3A and 3B in the triploids), and in both, the 2B category outnumbered. Although the morphotypes within each of the ploidy groups were apparently similar in gross karyomorphology, they differed from one another in finer details of chromosome morphology, mainly due to diminution of size of chromosome arms in varying magnitude and changes in the relative position of the centromeres. This is seen to have resulted in recognizable difference in the morphology of different chromosomes in terms of their r-values, leading to corresponding difference in the frequency of different chromosome types between and among the morphotypes.

The nature and extent of chromosome structural changes was found to be more obvious in the relatively larger chromosomes of the complement, and hence this size class of chromosomes (1-9) were subjected to closer examination. The overall data in the entire assemblage of morphotypes showed that 4 chromosomes of this group (chrom, 1,3,7 and 9) were the most altered in morphology. Based on the differences in morphology of these marker chromosomes it was possible to recognize a dozen karyotypically distinct cytotypes, 6 in the diploid (2-a to 2-f) and 6 in the triploid (3-a to 3-f) group of morphotypes (Figs. 1, 2). In most of the morphotypes of the diploid group all the 4 marker chromosomes were metacentric (m-type). In a few others, one of the marker chromosomes was subtelocentric (st-type), chromosome 3 in one morphotype and chromosome 7 in 6 others. Plants of yet another morphotype showed 3 of the marker chromosomes (3,7 and 9) subtelocentric, and in 2 others all the 4 marker chromosomes subtelocentric. However, in one of the morphotypes

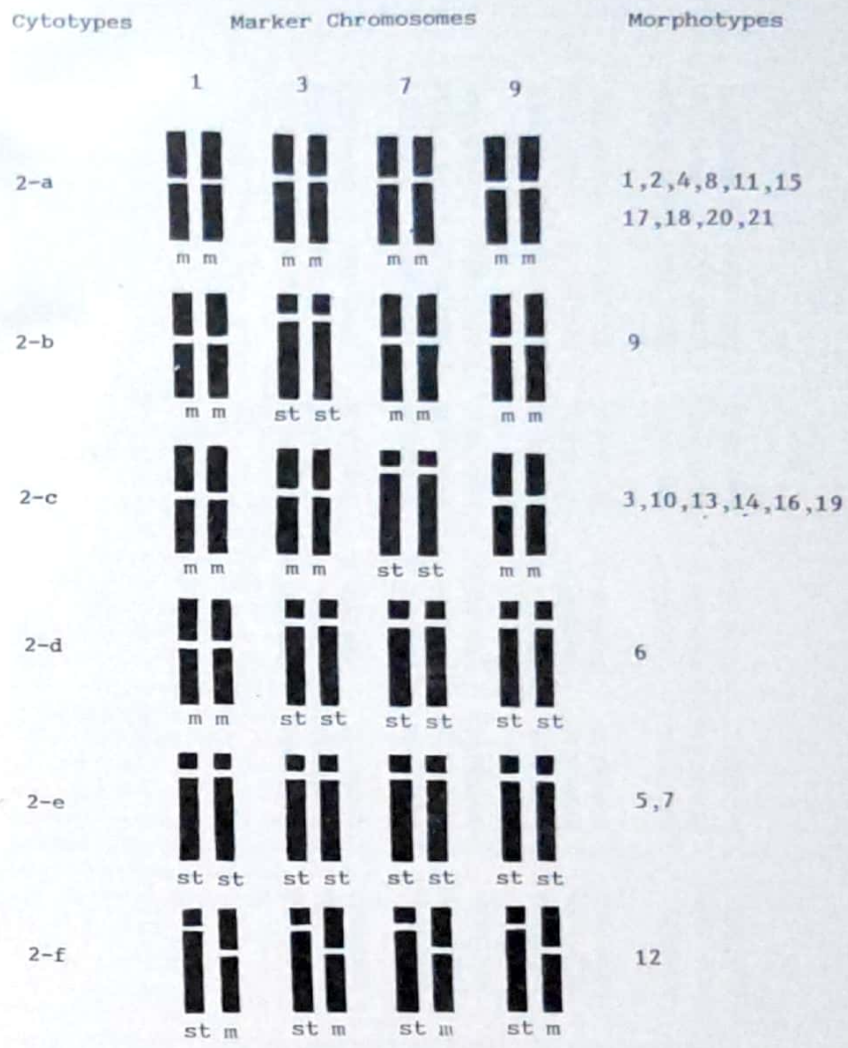

Fig. 1: Cytotypes in diploid taro (2-a to 2-f). 2.a All the marker chromosomes are of m-type. 2-b Marker chromosomes 1, 7 and 9 m-type and 3 st-type. 2-c Marker chromosomes 1, 3 and 9 m-type and 7 st-type. 2-d Marker chromosome 1 is m-type, and 3, 7 and 9 st-type. 2-e All the marker chromosomes are of st-type. 2-f All the marker chromosomes are heteromorphic (one member st-type and the other m-type).

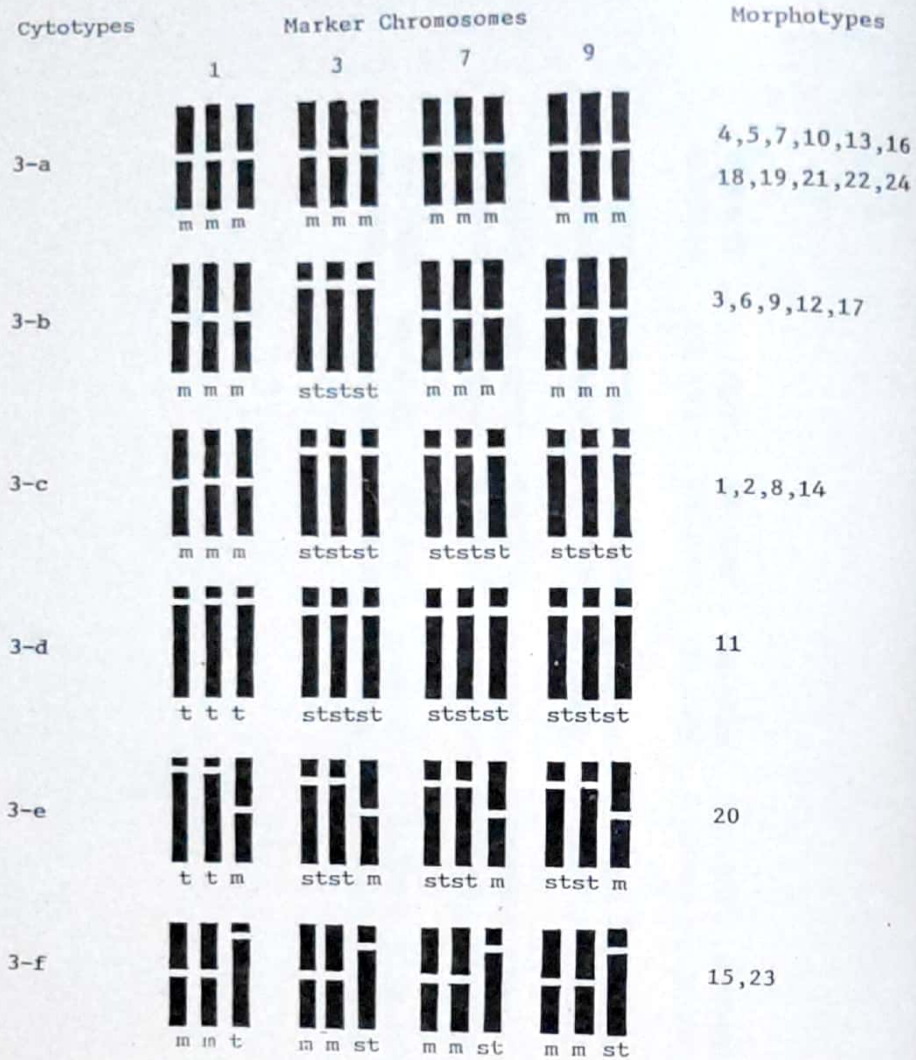

Fig. 2: Cytotypes in triploid taro (3-a to 3-f). 3-a All the marker chromosomes are of m-type. 3-b Marker chromosomes 1, 7 and 9 m-type and 3 st-type. 3-c Marker chromosome 1 m-type and others st-type. 3-d Marker chromosome 1 is t-type and others st-type. 3-e All the marker chromosomes are heteromorphic with one member m-type. In marker 1, two members are of t-type and in 3, 7 and 9 they are of st-type. 3-f All the marker chromosomes are heteromorphic with two mambers m-type, and one member t-type in marker1, and st-type in the others.

all the 4 marker chromosome pairs showed heteromorphism with respect to position of the centromere, one of their members being metacentric and the other subtelocentric.

In the triploid groups of morphotypes also similar types of karyotype differences were noticed. Of the 24 triploid morphotypes, 11 had all the 4 marker chromosome pairs metacentric, while in 5 others chromosome 3 was subtelocentric and the other marker chromosome metacentric. In a group of 4 other morphotypes (1,2,8 and 14) chromosomes 3,7 and 9 were subtelocentric and the other marker chromosome (1) was metacentric. In one morphotype (11) chromosome 1 was nearly acrocentric (r=6.30), whereas the other 3 marker chromosomes in this were subtelocentric. In 3 morphotypes (20,15 and 23) all 4 marker chromosomes were heteromorphic with respect to centromere position (Fig.2). In morphotype 20, one member each of the 4 marker chromosomes was metacentric and the other members telocentric in marker chromosome 1 and subtelocentric in marker chromosomes 3, 5 and 7. In morphotypes 15 and 23, the reverse condition was noticed in which 2 members each of the marker chromosomes were metacentric, and the other member telocentric in marker chromosome 1 and subtelocentric in marker chromosomes 3,7 and 9.

DISCUSSION

Most of the previous karyotype studies in taro were confined to a limited number of forms from restricted distributional ranges such as a few types from the North-east India (Sharma & Sarkar 1963, Kuruvilla & Singh 1981)and South and Central India (Sreekumari & Mathew 1989, 1991a, 1991b). The study reported from the South pacific region (Coates et al.1988) included a large sample from a wider distributional range in the region. The results of all these studies have yielded evidence suggesting that major alterations of chromosome structure have played a significant role in taro karyotype variation. Previous studies on the South Indian taros and those on the South Pacific region have led to the identification of certain karyotypically distinct cytotypes, one in the former group (Sreekumari & Mathew 1991b) and 5 in the latter (Coates et al. 1988). The present study which covered very large number of accessions from all over the Indian subcontinent revealed instances of a large scale chromosome structural repatterning occured during the course of evolution of this polymorphic species. Deletion of parts of chromosome arms, pericentric inversion and unequal translocations are known to be the major cytological processes which bring about karyotype variation (Stebbins 1971). It is generally considered that in plants the original karyotype form is relatively symmetrical and unspecialised, and during the course of evolution, differential diminution of chromosome size and changes in the relative position of the centromere occur leading to karyotype asymmetry and specialization. In the taro types studied here, most of the morphotypes in both ploidy groups possessed karyotypes predominated by metacentric chromosomes. In the others, chromosome structural repatterning has resulted in increased frequency of submetacentric and acrocentric chromosome at the expense of reduction in the frequency of metacentric ones.

Of the 12 cytotypes reported here (2-a to 2-f in the diploid and 3-a to 3-f in the triploid) the 2-a in the diploid and 3-a in the triploid groups constituted the majority, having all the 4 marker chromosome homomorphic metacentric chromosomes. In the diploids, the cytotypes 2-b and 2-c had one marker chromosome, 2-d had 3 marker chromosomes while in 2-e all the 4 marker chromosomes subtelocentric. However, the 2-f cytotype in this ploidy group displayed heteromorphic constitution with one member in each of the 4 marker chromosome pairs metacentric and the other subtelocentric corresponding in morphology with the respective pairs in 2-a and 2-e. It seems that this heteromorphic cytotype could be of hybrid origin involving 2-a and 2-e as putative parents. Since the respective pairs in all the 6 diploid cytotypes remained appreciably same in size irrespective of the difference in position of their centromeres, the change in position of their centromeres could be the result of pericentric inversions rather than deletion of sizeable segment of their short arms.

The karyomorphology of the triploid cytotypes characterised by each chromosome present in 3 doses is very much suggestive of their autoploid nature. Meiotic behaviour in the triploids, exhibiting incidence of trivalents in appreciable frequencies (Sreekumari & Mathew 1993) supports this possibility. Since the morphology of the marker chromosomes in the cytotypes of both ploidy groups is appreciably similar, it may be rationalized that the transformation of these chromosomes from the metacentric to subtelocentric and acrocentric states must have occurred initially in the diploid group, and from them they were carried over to the triploids along with their formation by autopolyploidy. The triploid cytotypes 3-a and 3-b appear to be direct derivatives from the diploid cytotypes 2-a and 2-b respectively. Similarly, the triploid cytotypes 3-c and 3-d must have originated from diploid cytotypes 2-d and 2-e respectively. The karyotype of the triploid cytotypes 3-e and 3-f which showed all the marker chromosomes in heteromorphic state, the former showing 2 doses and latter one dose of subtelocentric chromosomes and their homologue metacentric, appear to be suggestive of their hybrid origin with one of the putative parents possibly being the diploid cytotype 2-e and the other 2-a.

Among the 5 cytotypes identified by Coates et al. (1988) from the South Pacific region based on the morphology of 3 marker chromosomes (3, 7 and 9), one diploid and triploid had all the 3 marker chromosomes metacentric. These are similar to the types denoted herein as diploid 2-a and triploid 3-a respectively. The triploid cytotype identified by Coates et al. (1988) as having 3 acrocentric chromosomes is comparable to the cytotype identified herein as 3-e. They predicted that a progenitor of their 3AAA 7AAA 9AAA type might be found from karyotype studies of Indian taros, the region pressumed to be the original home of the plant (Onwueme 1978, Coates et al. 1988). Cytotype 2-d in which all 3 marker chromosome pairs are subtelocentric seems to be very close to their predicted diploid cytotype. They envisaged 2 karyotypes in taro, i.e., one with all 3 marker chromosome pairs being metacentric and the other with all 3 marker chromosome pairs being acrocentric. The striking difference in chromosome morphology between the cytotypes 2-a (1mm, 3mm, 7mm, 9mm) and 2-e (1stst, 3stst, 7stst, 9stst) involve more chromosome structural alterations than would seem likely to have occurred in the recent evolutionary past. Moreover, the plants belonging to these 2 cytotypes are significantly different in certain obvious characters. Those of cytotype 2-a have all green petioles, broad leaves with an entire margin, cylindrical corms and linear or club shaped cormels, whereas plants of the 2-e cytotype have purple petioles, narrow leaves with an undulate margin, globular and stout corm and cormels. It may be that the 2-a and 2-e types have evolved from the former during the early evolutionary history of the species.

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CYTOLOGY OF SOME SPECIES OF *BIOPHYTUM* (OXALIDACEAE)

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SUMMARY

Chromosome numbers have been reported for the first time in *Biophytum reinwardtii* Klotzsch (n=9), *B. nudum* Edgew. & Hook. (n=8), *B. intermedium* Wight (n=16), *B. candolleanum* Wight (n=7) and the Tirunelveli collection of *B. sensitivum* DC.(n=10). The basic number 7 was suggested as original base number of the genus from which other base numbers such as, 8, 9 and 10 might have originated by stepwise aneuploid increase. On the basis of branching nature of stem and cytology, *B. intermedium* is inferred as a tetraploid, very close to *B. nudum* phylogenetically. The plants of *B. sensitivum* collected from Tirunelveli differed from typical *B. sensitivum* in morphological characters, chromosome number and in having spontaneous cytomixis during the course of meiosis. Hence, the necessity to treat it as a new species is suggested.

Key Words: *Biophytum*, cytotaxonomy.

INTRODUCTION

The species of *Biophytum* are mostly shade-loving herbs, occurring in plains, hills and forests. Hooker (1875) described 8 species of *Biophytum* from peninsular India. Gamble (1957) recorded 2 more species viz., *B. insignis* and *B. longibracteatum* and also raised *B. sensitivum* var. *condolleanum* of Hooker to species status with the name *B. candolleanum*. Nair & Henry (1983) also given a separate species status to *B. sensitivum* var. *nervifolium* of Hooker as *B. nervifolium*. Since the species determination of some of the taxa is obscure, Mathew (1983) held the view that the genus badly needs a revision, at least for India. Cytological studies have a very important role in resolving problems in systematics. Data on chromosome numbers provide a valuable adjunct to taxonomy normally based on plant morphology. As the chromosome number report in the genus so far is confined to *B. sensitivum* (Raghavan & Arora 1958, Mathew 1958, Chatterjee & Sharma 1970, Sarkar et al. 1982), a cytological study of the South Indian species of the genus was undertaken to gather supplementary evidence towards taxonomic interest and the results are reported here.

MATERIALS AND METHODS

The plants were collected from different places in South India. Materials of *B. sensitivum* were collected from Trivandrum, Upper Kodayar, Vadakkankulam and Tirunelveli, *B. reinwardtii* from Trivandrum, Palode, Ponmudi hills, Calicut and Peechi dam site, *B. nudum* from Agasthyar malai, *B. intermedium* from Upper Kodayar and *B. candolleanum* from Idukki.

For meiotic studies, flower buds were fixed in 3:1 ethanol-acetic acid mixture. In order to obtain satisfactory results with the material, a few drops of ferric acetate were added to the fixative. The anthers were smeared in 2%

acetocarmine and the PMCs and tapetal cells photographed from temporarily sealed preparations using Olympus BH-2 research microscope.

OBSERVATIONS

B. sensitivum

Two morphologically distinct accessions of the species were studied.

Accession 1

The plants were collected from Trivandrum, Upper Kodayar and Vadakkankulam. Leaflet tip obtuse, apex apiculate, length/breadth ratio 3.6. About 30-40 bracts present in each umbel; bract, ovate with tapering apex. Sepal length 5 mm, Flower colour yellow. Seeds seven - ridged, tubercled. Chromosome determination from pollen mother cells (PMCs) in all the collections of this morphological form revealed 7 bivalents (Fig.1). Tapetal mitosis in the material from Vadakkankulam showed 14 chromosomes (Fig.2).

Accession 2

The plants collected from Tirunelveli were found to grow in black cotton soil only. They could be distinguished from Acc. 1 by their brick-red flowers. Leaflet-tip rounded with comparatively shorter apiculate apex. The length/breadth ratio of leaflets is 2.6. Number of bracts varied from 10-20 in each inflorescence with broader base and shorter apex when compared to the collections of Acc.1. Sepal length 3.5 mm. Seeds 9-ridged and non-tubercled.

PMCs of this material revealed n=10 (Fig.3). Cytomixis was observed during the different stages of meiosis. Cytoplasmic connections could be clearly discerned. In some of the cells, the chromatin material as well as the nucleolus were seen to migrate to the adjacent cell (Fig.4). The participating cells showed variation in the chromatin content due to partial migration (Fig.5). However, 98% of the pollen grains were stainable in acetocarmine.

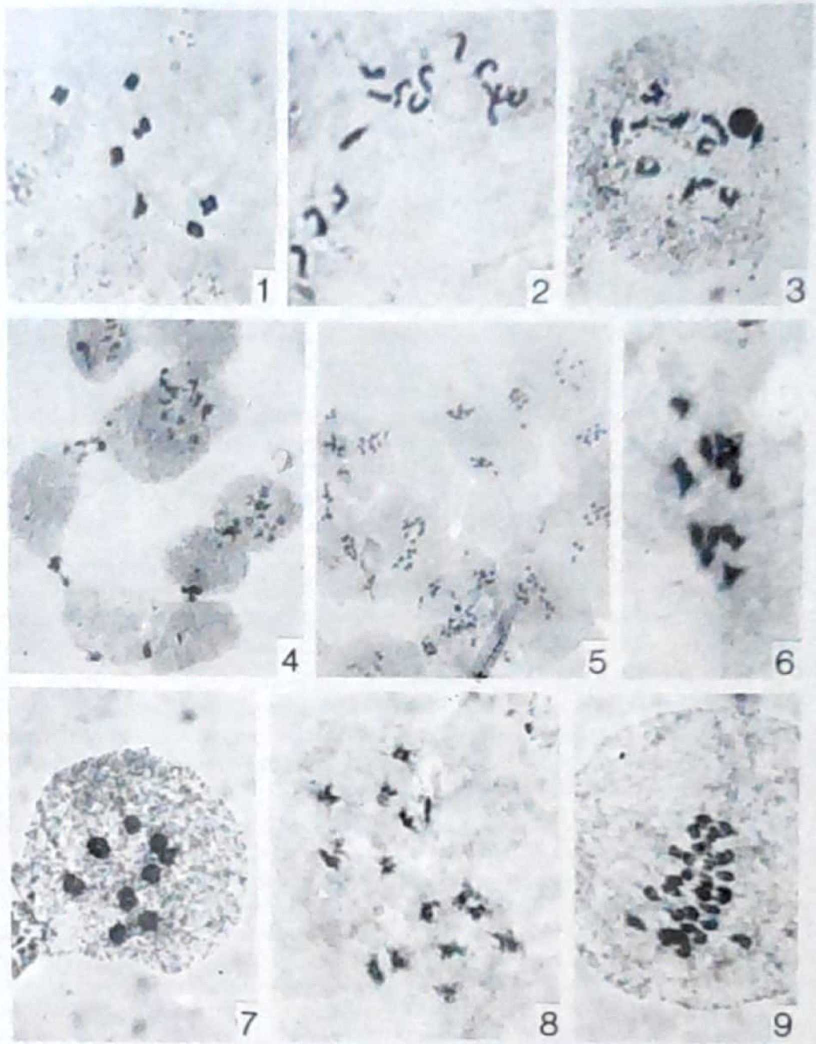
B. candolleanum

It is a tall plant with a stout stem reaching the height of 20-30 cm. The plant shows branching tendency. Leaves are 10-12 cm long and clustered at the top. There are 20-25 leaflets which are closely appressed. The peduncles are also long with 10-15 cm. Flowers, in umbels surrounded by tufts of long bracts. All the plant parts are strigose. Meiosis showed gametic number of 7 (Fig.6).Percentage of stainable pollen grains was 82.

B. reinwaratu

B. reinwardtii resembled *B. sensitivum* in several morphological characters in general. The long pedicels and short sepals give a characteristic appearance to the inflorescence of *B. reinwardtii*. Corolla lobes yellow with reddish streaks in 4 collections but in the collection made from Palode, the reddish streaks were lacking.

Meiotic studies conducted in materials of the species collected from all the 5 different localities showed n=9 as the gametic number (Fig.7). Pollen sterility was found to be about 17%.



Figs. 1-9: Chromosome numbers in *Biophytum* (all x 1500). 1. *B. sensitivum* (Trivanorum), metaphase I (n = 7). 2. *B. sensitivum*, Tapetal mitosis (2n =14). 3. *B. sensitivum*, (Tirunelveli), diakinesis (n = 10). 4. *B. sensitivum*, (Tirunelveli), Cytomixis at prophase I. 5. *B. sensitivum*, (Tirunelveli), Cytomixis at anaphase I. 6. *B. candolleanum*, metaphase I (late) (n = 9). 8. *B. nudum*, metaphase II (n = 8). 9. *B. intermedium*, early anaphase (n = 16).

B. nudum

The plants are slender and dichotomously branching. Leaves consist of 20-25 leaflets. The haploid number was determined as n=8, (Fig.8). Percentage of stainable pollen grains was found to be 50.

B. intermedium

The species has a prostrate habit and an umbellate branching pattern. The leaves have 10-15 leaflets which are comparatively larger in size than that of *N. nudum* with 6 x 3 mm. Sixteen bivalents were observed in each PMC (Fig.9). The pollen sterility was determined as 40.8%.

DISCUSSION

Chromosome numbers in *Biophytum reinwardtii* (n=9), *B. nudum* (n=8), *B. intermedium* (n=16), *B. candolleanum* (n=7) and Tirunelveli collection of *B. sensitivum* (n=10) have been determined for the first time. An extensive chromosome study conducted in Geraniales by Chatterjee & Sharma (1970) indicated x=7 as the basic stock from which different chromosome numbers and others exhibiting numerical alterations. From the chromosomal information on the genus, it is inferred that x=7 is the basic number of *Biophytum* also. The data of chromosome counts determined presently revealed n=7 in 2 species, *B. candolleanum* and *B. sensitivum*. Other numbers such as, n=8 in *B. nudum*, n=9 in *B. reinwardtii* and n=10 in *B. senstitivum* from Tirunelveli might have originated from x=7 through stepwise aneuploid increase.

B. nudum and *B. intermedium* are unique in having slender and branched prostrate stems. Gametic number of *B. nudum* (n = 8) and that of *B. intermedium* (n = 16) suggest that *B. intermedium* is a tetraploid based on x = 8 and phylogenetically closer to *B. nudum* than any other species reported here.

The earlier report of n = 9 in *B. sensitivum* by Raghavan & Arora (1958), Mathew (1958) and Chatterjee & Sharma (1970) could not be confirmed during this study. It is found that *B. sensitivum* and *B. candolleanum* share a common gametic chromosome number, n = 7. Morphologically, they do not differ much from each other. Hence, a varietal status for *B. candolleanum* as done by Hooker (1875) is favoured on the basis of cytological studies.

The plants of *B. sensitivum* collected from Tirunelveli were restricted to black cotton soil areas. They are distinct from other collections of the species in having brick-red flowers and in number, shape and size of bracts in the inflorescence. Nine-ridged and nontubercled seeds also mark its distinction as against the 7-ridged tubercled seeds of other collections. Besides, these 2 forms of *B. sensitivum* were found to have 2 unrelated gametic numbers, viz., n = 10 in the Tirunvelveli material and n = 7 in others. Spontaneous cytomixis observed in Acc. 2 seems to be the characteristic of it. A positive correlation between the frequency of cytomixis and temperature has been noted by Basaviah & Murthy (1987). The hot and dry conditions of the region of collection could have played a role in bringing about the phenomenon. The theroy that the process is under genetic control (Omara 1976, Banerjee & Sharma 1988, Geethamma, 1993) seems more feasible. The low pollen sterility in Acc. 2 can be attributed to the degeneration of abnormal pollen grains produced as a result of cytomixis as suggested by earlier workers (Bhandari et al. 1969, Morriset 1978, Kopul 1990). The distinct morphological characters, chromosome number and the occurence of cytomixis during the course of meiosis in the Tirunelveli material may warrant its treatment as a species distinct from *B. sensitivum*.

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FREQUENCY AND GENETIC EFFECTS OF CONSANGUINITY IN THE NAYARS OF TRIVANDRUM DISTRICT, SOUTH INDIA

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SUMMARY

Consanguinity study carried out in the Nayars of Trivandrum District, Kerala, revealed a fairly high rate of inbreeding (14.71%) in the group. First cousin, first cousin once removed and second cousin were the common types of related marriages with first cousin type predominating and matrilateral cross type showing an edge over patrilateral. Genetic effects of consanguinity scored in terms of mortality (pre- and postnatal) and morbidity showed the frequency to be significantly high in the consanguineous group than in the nonconsanguineous control group. The genetic load due to inbreeding in the community evaluated in terms of A and B statistics showed the load lying between 1 and 2 lethal equivalents per gamete. The high value of B/A ratio was suggestive of the mutational component being appreciable in the group.

Key Words : Nayars, consanguinity, genetic effects.

INTRODUCTION

In consanguineous matings the likelihood of spouses possessing the same gene is considerably increased and hence such marriages are prone to produce offspring who inherit 2 identical alleles in higher frequency than those of unrelated parents. Many harmful traits in man are recessives and therefore inbreeding tends to bring into open such recessive alleles present in heterozygous carrier parents resulting in affected children being born more in consanguineous families. Data of consanguinity effect provide useful clues for estimation of the magnitude of genetic load in the population concerned, and in addition, yield information relevant to genetic counselling.

Related marriages are preferred and encouraged in many castes and communities in Kerala because of the strong caste bind, social customs and traditions. A systematic study of consanguinity and its genetic effects is very much lacking in the inbreeding populations of the state barring a few isolated studies in small groups (Kumar et al.1967, Mathew 1987). The present paper concerns the results of consanguinity studies carried out in one sueh caste, the Nayars, from Trivandrum District, with a view to assessing the frequency of inbreeding and evaluating the genetic effects of the phenomenon, and also to estimate the component of genetic load due to inbreeding in the group.

MATERIALS AND METHODS

In Kerala, Nayars constitute a major Hindu caste almost evenly distributed mostly in the Central and Southern districts including Trivandrum, among whom the practice of related marriages has long been favoured and encouraged.

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Among the rural groups with low literacy level and socio-economic status this custom is still prevalent in appreciable degree. The consanguinity data were collected from a random sample of 1802 families belonging to urban, suburban and rural regions of the district by interviewing the spouses and using an elaborate questionnaire. Genetic effects of consanguinity on mortality, both prenatal (abortion and still birth) and postnatal (infant, child and juvenile death), and morbidity (congenital defects and diseases) were evaluated using Chi-square and tests for proportion, while the data in the non-consanguineous group was taken as control. The mean coefficient of inbreeding (F) was calculated following Wright (1922). The genetic load due to inbreeding was determined in terms of two vital statistics, A and B, using the regression equation: $S = e^{-(A+BF)}$ where S is the proportion of survivors and F is the coefficient of inbreeding (Morton et al. 1956).

OBSERVATIONS

The data regarding the type of marriage and frequency of consanguinity in the three regions (urban, suburban and rural) are furnished in Table 1. Among the related marriages, first cousin type was the most common (12.71%) with a high preference for matrilateral cross types (9.77%). Others were second cousin type (1.33%) and first cousin once removed (0.67%). The total consanguinity rate in the community was 14.71%, and mean coefficient of inbreeding 0.0092. The rate of consanguinity was highest in the rural areas (17.80%), intermediate in the suburban (14.39%) and lowest in the urban (10.22%). The relationship between consanguinity rate and region was highly significant ($P < 0.01$).

The effects of consanguity in terms of mortality and morbidity are presented in Table 2. Prenatal mortality was 5.46% and postnatal mortality was 5.42% in the consanguineous group as against 1.56% and 2.64% respectively in the control group. Morbidity rate in terms of congenital defects and diseases was also higher (11.07%) in the consanguineous group than in the control (2.76%). Both mortality and morbidity rates were highly significant($P < 0.01$).

Estimates of genetic load in terms of A (the measure of the amount of expressed damage in a random-mating population (F=0) and B (the measure of hidden genetic damage that would be expressed fully in a complete homozygote (F=1) statistics computed separately for pre- and postnatal mortality and collectively for total mortality are shown in Table 3. In all cases, B values were higher than A values. The B/A ratio was relatively high for prenatal mortality (62.045) as against a considerably low value (9.212) for postnatal mortality, and for total mortality 31.387.

TABLE 1 : Frequency of consanguinity by type and region in the Nayars of Trivandrum district.

Type of marriage	Frequency Urban %	Suburban %	Rural %	Total No.	Total %
First cousin (IC)	8.76	12.23	15.53	229	12.71
a) Matrilateral cross	7.30	9.11	11.71	176	9.77
b) Patrilateral cross	1.46	3.12	3.82	53	2.94
First cousin once removed (1.5C)	0.55	0.72	0.72	12	0.67
Second cousin (2C)	0.91	1.44	1.55	24	1.33
Consanguineous*	10.22	14.39	17.80	•265	14.71
Non-consanguineous (NC)	89.78	85.61	82.20	1,537	85.29
Mean coefficient of inbreeding (F)	0.00611	0.00835	0.01131	0.00920	

* Consanguinity in relation to region : Significant ($P < 0.01$).

TABLE 2 : Effect of consanguinity on mortality and morbidity in the Nayars of Triavandrum District.

Parameters	No. of cases	Frequency Non-consanguineous	Consanguineous
Prenatal mortality	116	1.56	5.46*
Postnatal mortality	156	2.64	5.42*
Total mortality	274	4.16	10.58*
Morbidity	210	2.76	11.07*

* Significant ($P < 0.01$).

TABLE 3 : Estimates of genetic load in terms of A and B statistics for prenatal, postnatal and total mortality in the Nayars of Trivandrum District.

Mortality	A	B	A+B	B/A
Prenatal	0.0124	0.7693	0.7817	62.045
Postnatal	0.0303	0.2793	0.3096	9.212
Total	0.0402	1.2602	1.3004	31.387

DISCUSSION

The patterns of related marriages in the order of degree of relationship between spouses known in inbreeding groups are uncle-niece/Aunt-nephew (UN/AN), Double first cousins (DIC), first cousins (1C), first cousins once removed (1.5C) and more distant relationship, mostly second cousins (2C) of which 1C is the most common. In the case of 1C, the second level of classification comprises 4 sub-groups, the patrilateral and matrilateral parallel, and the patrilateral and matrilateral cross. In the Nayars of this region 1C type was the most common (12.71%) followed by 1.5C and 2C (Table 1), the total frequency being 14.7%. Within the 1C, the cross type is the most popular with the matrilateral cross type showing an edge over the other. This preferential type is reported to be the most common practice in most countries including India except the Muslim countries in the Middle East where patrilateral parallel type is preferred as part of the Arab culture (Bittles 1992).

Consanguinity rate varies from population to population due to variation in population structure and socio economic and religious factors. In majority of world populations the level of inbreeding is very low, rarely exceeding 5% with F=0.0001 especially in most Western Countries (Cavalli-Sforza & Bodmer 1971) while medium (5-15%) and high (above 15%) inbreeding rates are known in populations of Japan (Fujiki 1987), Brazil (Freire-Maia 1990), India (Rao & Inbaraj 1977) and Middle East Countries (Khlat & Khudr 1984), the highest ever known being 87.6% in the Mudugars of Attappady in Kerala (Joseph & Mathew 1991). The frequency in the Nayars (14.7% with F=0.0092) apparently comes under medium frequency level. In this group, significant urban-rural difference was noticed in the consanguinity rate, the urban section with higher socio-economic and literacy level registering lowest, and rural group the highest frequency. Similar negative correlation between consanguinity rate and socio-economic levels has been reported in most studies in all parts of the world (Freire-Maia 1982, Bittles 1992, Rao & Inbaraj 1977).

According to the genetic theory, parental consanguinity results in increased homozygosity and decreased heterozygosity at each locus depending on the duration and level of inbreeding (Mc Kusick 1983). Homozygosity of lethal or sublethal genes results in offspring that become non-viable in the pre-and postnatal stages leading to mortality and morbidity. Most studies have brought out evidence of increased rates of prenatal mortality, postnatal mortality and morbidity. The data of these effects in the Nayars are significantly higher in the consanguineous group of families, the total mortality being 10.58% in the consanguineous group as against 4.16% in the control (Table 2). The morbidity rate is also significantly high in the consanguineous (11.07%) than in the control (2.76%). This is indicative of alarming harmful effect of consanguinity in the group.

Morton et al. (1956) suggested that inbreeding effects can provide a measure of total genetic burden in different populations in terms of lethal equivalents, a lethal equivalent being defined as a group of mutant genes which would cause on the average, one death if dispersed in different individuals and made homozygous. The lethal equivalents are estimated in terms of A and B statistics. The measure of total genetic damage is a quantity which is equal to the sum of A and B. The lethal equivalent per gamete lies in between B and A+B. In the Nayars of Trivandrum District, the B values are higher than those of A. The lethal equivalent lies between 1 and 2 per gamete.

The ratio of the load in a fully inbred population to that of a random-mating population is estimated by the B/A values, the magnitude of which provides, according to the load theory, critical information on the relative importance of the mutational and segregational loads. According to Crow's (1958) concept, high value of B/A is suggestive of mutational load. In the present study the B/A value for mortality is relatively high (31.39) which is suggestive of the load in the present population being mostly mutational.

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EFFECT OF GAMMA RAYS, EMS AND SODIUM AZIDE ON PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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SUMMARY

Dry seeds of groundnut were subjected to mutagenic treatments of gamma rays, EMS and sodium azide. Seedling height, contents of chlorophyll, free amino acids, proteins and nucleic acids (DNA and RNA) were increased in lower doses/concentrations up to 300 Gy/30 mM but were decreased in higher dose/concentration (500 Gy/50 mM) of mutagens. The stimulation was a dose dependent response. The cultivar VRI-2 was found to be better for mutagenic treatment than TMV-7 cultivar. It is suggested that the plant height, contents of chlorophyll, free amino acids, proteins, DNA and RNA can be enhanced in lower doses/concentrations of mutagenic treatment in groundnut.

Key Words: *Arachis hypogaea*, mutagens, biochemical analysis.

INTRODUCTION

The methods of experimental mutation research are utilized in plant breeding since about 40 years. Induced mutations in crop plants contribute by increasing genetic variability. Mutagenic effects on plants generally have been associated with disturbances of chromosomal and extrachromosomal origin and finally detected as growth retardation or death (Gaul 1970). Mutagens affect the metabolism of the individuals and influence the activity or synthesis of enzyme and growth regulators (Jain & Khanna 1987). Such harmful effects of mutagens lead to various forms of physiological expression of damage such as retarded plant growth, induction of mutations, sterility and death. Mutagens induced biochemical and physiological changes during seed germination in rice (Inoue et al. 1975) and cowpea (Khanna 1988, 1991). Low doses of radiations have been found to have a stimulatory effect in different crops (Sparrow 1966, Khanna 1988).

Hence, the present work was carried out to examine the effect of gamma rays, (GR), EMS and sodium azide (SA) on the physiological and biochemical changes in M_1 seedlings of 2 groundnut cultivars.

MATERIALS AND METHODS

Two non-dormant, local, groundnut (*Arachis hypogaea* L.) varieties belonging to the spanish type were tested: (1) Virdhachalam 2 (VRI-2), with 48% oil content, and (2) Tindivanam 7 (TMV-7), with 49.6% oil content. All seeds were hand-shelled and only fully mature, undamaged kernels were used in the present study.

Dry seeds of groundnut with a water content of 13%, were treated with gamma rays, EMS and SA. Different doses of gamma rays from 100 Gy to 500 Gy were administered. EMS dissolved in phosphate buffer at pH 6.0 was applied at concentrations of 10 mM to 50mM for 2h. SA treatment was conducted at concentrations of 10 mM to 50 mM for 2 h in phosphate buffer at pH 3.0. All chemical mutagenic treatments were performed at 20±2°C and were immediately follwed by a 2 to 4 h post-treatment washing in running tap water.

Fifty seeds of each treatment were allowed to grow in red soil in the laboratory at 25±2°C and seedling height was measured on the thirteenth day after planting. Fresh leaves were harvested from the plants and chlorophyll extracted and estimated by the method of Arnon (1949). Proteins were estimated quantitatively by the method of Lowry et al. (1951). Free amino acids were analysed quantitatively by the method of Troll & Canan (1953). Nucleic acids were extracted and estimated quantitatively according to the method of Jayaraman (1988). The experiment was repeated twice with duplicate samples.

OBSERVATIONS

The effect of mutagenic treatments of gamma rays, EMS and SA on seedling height, chlorophyll contents, proteins, free amino acids and nucleic acids of M_1 generation was studied. Changes in seedling height during M_1 generation are presented in Table 1, which indicates that there is a gradual increase up to 300 Gy gamma rays and 30 mM EMS and SA treatments as compared to the control. In contrast to this, seedling height was decreased with the increase in the dose/concentration of mutagens. Gamma rays were found to be more effective for increase in seedling height than EMS and SA treatments. The highest mean value of seedling height was 13.6 cm in gamma rays

TABLE 1 : Effect of gamma rays, EMS and SA on seedling height, chlorophyll content and free amino during M_1 generation in two groundnut cultivars (Values are Mean±SD).

Mutagens	Seedling height (cm)		Chlorophyll content		Free aminoacids (mg / g FW)	
	VRI-2	TMV-7	VRI-2	TMV-7	VRI-2	TMV-7
Control	7.8±1.2	7.3±1.3	11.1±2.3	10.5±2.0	11.2±2.5	10.5±1.8
GR (Gy)						
100	8.9±1.6	8.5±1.5	13.0±2.9	12.2±2.6	12.0±2.7	11.5±2.6
200	10.8±2.3	10.5±2.5	14.9±3.5	14.2±3.2	15.4±3.8	14.2±3.0
300	13.6±2.7	12.5±1.9	17.9±3.7	17.5±2.9	16.7±2.5	15.0±2.1
400	7.9±1.2	7.4±1.1	16.4±2.4	16.0±2.2	15.3±1.9	12.1±1.8
500	6.6±1.3	6.5±1.2	15.4±2.5	15.0±2.3	13.2±2.3	10.0±2.0
EMS (mM)						
10	8.1±1.7	7.4±1.4	13.7±2.6	12.5±2.7	11.5±1.9	10.6±2.1
20	9.6±1.8	9.3±1.9	15.7±3.0	14.8±2.6	13.2±2.4	12.0±1.8
30	11.5±2.1	10.8±1.8	18.8±3.9	18.2±3.5	15.4±2.8	14.2±2.6
40	6.2±1.7	5.4±1.5	16.8±2.9	16.4±2.4	13.2±2.2	12.1±2.1
50	5.0±1.3	4.9±1.3	16.1±2.6	15.7±2.3	11.0±2.1	10.0±1.9
SA (mM)						
10	8.8±1.7	8.2±1.5	16.6±2.8	11.0±1.8	11.5±1.9	10.8±1.7
20	10.8±2.0	10.3±2.1	15.8±2.6	15.2±2.9	12.4±2.2	12.2±2.0
30	12.2±2.3	11.6±2.1	19.3±3.5	18.9±3.2	15.6±2.9	14.2±2.3
40	7.5±1.8	7.1±1.5	17.6±3.0	17.3±2.6	14.2±2.3	13.5±2.1
50	6.7±1.5	6.5±1.4	16.3±2.7	15.8±2.3	12.2±2.1	11.3±1.9

(VRI-2) whereas the mean values were 12.2 cm and 11.5 cm in SA and EMS treatment (VRI-2) respectively, as compared to the mean value of 7.8 cm in the untreated.

The chlorophyll content was increased in all the 3 mutagenic treatments. While the chlorophyll level was increased up to 300 Gy and 30 mM mutagenic treatment and later slightly decreased. The chlorophyll content was more in SA treatment 19.3 followed by EMS treatment 18.8 and gamma ray treatment 17.9 in VRI-2 cultivar whereas it was 11.1 in control (Table 1).

The results obtained for free amino acid content, are given in Table 1. Free amino acid content steadily increased up to 300 Gy/30 mM in both cultivars, while the content was slightly decreased in higher doses/concentrations. Free amino acid content was high in vRI-2 cultivar in gamma irradiation treatment (16.7 mg/g FW) at 300 Gy dose whereas the content was low in EMS treatment (15.4 mg/g FW) at 30 mM concentration as compared to the control (11.2 mg/g FW).

The results depicted in Table 2 indicate the protein content in both cultivars. Protein content was maximum at 300 Gy/30 mM mutagen treatment, and thereafter slightly, decreased in both cultivars. Protein content was more in VRI-2 cultivar with gamma rays treatment 20.6 mg/g FW at 300 Gy followed by SA treatment 20.1 mg/g FW at 30 mM and EMS treatment 19.7 mg/g FW at 30 mM whereas it was 14.3 mg/g FW in control.

In order to analyse the effect of mutagenic treatment on nucleic acid contents, DNA and RNA were estimated in leaves. The results are shown in Table 2, where it is seen that DNA and RNA

TABLE 2 : Effect of physical and chemical mutagens on proteins and nucleic acid (DNA and RNA) contents in seedlings of two groundnut cultivars (Values are Mean±SD).

Mutagens	Protein content (mg / g FW)		DNA content (mg / g FW)		RNA content (mg / g FW)	
	VRI-2	TMV-7	VRI-2	TMV-7	VRI-2	TMV-7
Control	14.3±2.1	13.9±2.0	2.0±0.6	1.8±0.5	0.8±0.3	0.7±0.2
GR (Gy)						
100	15.2±3.2	14.0±2.5	2.2±0.4	1.8±0.4	0.9±0.2	0.8±0.3
200	18.9±3.8	16.3±3.4	2.8±0.6	2.5±0.8	1.3±0.6	1.2±0.5
300	20.6±3.9	18.7±3.7	3.5±1.0	3.2±1.1	1.9±0.7	1.8±0.3
400	14.3±2.1	12.9±1.8	1.8±0.6	1.6±0.4	1.2±0.3	1.0±0.3
500	12.7±1.9	12.1±2.0	1.2±0.3	0.9±0.2	0.8±0.3	0.6±0.2
EMS (mM)						
10	15.4±3.2	13.6±2.7	1.9±0.6	1.8±0.6	0.9±0.4	0.8±0.2
20	17.4±3.9	15.5±3.2	2.5±0.9	2.3±0.7	1.5±0.6	1.2±0.4
30	19.7±3.8	17.8±3.5	3.9±0.8	3.8±1.1	2.2±0.8	2.2±0.9
40	13.4±2.4	12.1±2.1	1.8±0.8	1.7±0.6	1.8±0.7	1.6±0.5
50	11.8±1.9	10.2±1.8	1.0±0.3	0.9±0.2	1.0±0.3	0.9±0.2
SA (mM)						
10	16.3±2.9	15.4±3.2	1.9±0.5	1.9±0.5	0.9±0.3	0.7±0.2
20	19.0±3.8	18.0±3.6	2.8±0.6	2.3±0.4	1.8±0.3	1.3±0.4
30	20.1±4.1	18.3±3.5	3.6±1.1	3.5±1.0	2.1±0.7	1.9±0.4
40	15.2±3.2	14.3±2.3	2.8±0.8	2.7±0.5	1.2±0.6	1.1±0.5
50	13.4±2.1	12.5±1.8	1.8±0.6	1.5±0.7	0.8±0.2	0.8±0.1

contents increased up to the 300 Gy/30 mM of mutagenic treatment thereafter, decreased with an increase in mutagens dose/concentration. The nucleic acid content was more in VRI-2 cultivar than TMV-7. Both DNA and RNA contents were found to be high at 30 mM of EMS treatment (DNA 3.9 mg/g FW and RNA 2.2 mg/g FW) followed by SA and gamma ray treatment as compared to the control value (DNA: 2.0 mg/g FW and RNA: 0.8 mg/g FW).

DISCUSSION

In the present study, the seedling growth measure on thirteenth day indicated that the reduction in seedling height was proportional to dose but a stimulation effect was observed up to 300 GY/30 mM dose/concentration. Reduced growth has been explained on the basis of auxin destruction, changes in ascorbic acid content, and physiological and biochemical disturbances (Gordon 1957, Gunckel & Sparrow 1961, Singh 1974, Usuf & Nair 1974). Chromosome breakage during mitotic inhibition (Sparrow & Evans 1961) and inhibition of DNA synthesis (Mikaelsen 1968) have also been implicated as causes of reduced plant growth. Stimulation of growth observed at lower doses might be caused by elevated auxin level (Gordon 1957, Gowda 1977). A similar response was noticed by Krishna et al. (1984) in Rhodes grass, Ashri & Herzog (1972) in peanut and Cheng & Gao (1988) in barley.

In our study, the chlorophyll content was increased in lower doses/concetrations but decreased in higher dose/concentration. Roy & Clark (1970) observed significant changes in chlorophyll content due to mutagenic treatment in the seed"ings of *Vicia faba*. The total chlorophyll content was increased in lower doses/concentrations whereas it was slightly decreased in higher doses/concentrations of mutagens. Similar result was observed in barley seedlings and reported by Giacomelli et al. (1967).

Our observations clearly indicate that the free amino acids, proteins, and nucleic acids were increased gradually in lower doses/concentrations but they were slightly decreased in higher doses/concentrations of mutagens. In corn seedlings, Cherry (1968) noted that the production of proteins; soluble nucleotides and RNA was reduced by X-irradiation and indicated that this reduction was roughly parallel to growth reduction (Sparrow & Evans 1961). Similar results also were observed in peanut by Van Huystee et al. (1968). Both DNA and RNA contents were increased in lower dose and decreased in higher doses in cowpea (Khanna 1991). Similar results were obtained in our study. In the present study, the mean values of plant height and contents of chlorophyll, free amino acids, proteins, and nucleic acids were increased up to LD_{50} dose/concentration, thereafter; they were slightly decreased than of the control. This may be due to the saturation effect of mutagens. Similar observations have been made in rice (Inoue et al. 1975) and chickpea (Khanna 1991).

The results reported here are consistent with earlier studies in peanut (Van Huystee et al. 1968), rice (Inoue et al. 1975), kidney bean (Nirale & Gaur 1988) and chickpea (Khanna 1988, 1991). In general, gamma rays, EMS and SA treatments showed a similar response on both cultivars and lower doses/concentrations increased the mean values of all the parameters. Therefore, the lower doses/concentrations of mutagens could enhance the biochemical products in groundnut.

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DOMINANT TRAITS IN WINGED BEAN MUTANTS

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SUMMARY

Winged bean is a perennial climbing system having trifoliate leaves, tuberous roots and quadrangular pods. Inheritance of 10 morphological characters has been studied by crossing true breeding mutant lines. Mutant characters such as, long inflorescence axis, early maturity, flowering at lower nodes and tallness were found to be dominant. While linear leaves, flat pod shape and wingless pods displayed recessive features. Presence of anthocyanin on different plant parts also revealed a dominant nature.

Key Words: Winged bean, dominant traits, intermutant crosses.

INTRODUCTION

Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) has been described as a wonder legume for the tropics. It is a climbing system and comprises a fine source of good quality protein and oil. Virtually all parts of the plant are edible and immensely nutritious. A few crops have risen so quickly from total obscurity to the winged bean's current level of prominence. Inspite of the outstanding nutritional potential, the winged bean has failed to gain popularity and acceptance among the farmers. This has been chiefly due to the high cost of production (on account of the need for staking), photosensitivity and presence of antinutritional factors. After carefully screening several hundred germplasm accessions and for overcoming the problems in winged bean, Eagleton et al. (1985) emphasized the utility of mutation breeding in winged bean. Keeping this in view a mutation breeding programme was initiated in our laboratory which yielded several true breeding mutant lines having useful features of high economic value. Attempts were made to cross some of the promising mutant lines with the hope of evolving a desirable recombinant type.

MATERIALS AND METHODS

The winged bean variety, JC 4198, obtained from NBPGR, New Delhi, and its true breeding mutants were grown in botanical garden. Sowing was carried out in 3 batches, each with 15 d interval so as to ensure availability of receptive flowers for an extended period.

In a mature flower bud (corolla extending about 8 to 12 mm beyond the calyx and having changed colour from green to whitish-green), the anthesis usually occurs during the night and pollination takes place while the flower remains closed. For the cross pollination, the flowers were emasculated in the evening before anthesis. The flower buds were opened through a slit along the base of keel petal with pointed forceps and all the anthers were removed. Such buds were tagged and pollinated the following morning. The pollen was transferred from a pollen parent using the heavily pollen loaded hairy style as a brush (Erskine & Bala 1976). The mutants were compared for their abilities to accept foreign pollen and the successful development of pods/seeds.

RESULTS AND DISCUSSION

The parental characters of winged bean mutants are presented in Table 1. The mutants varied markedly in their abilities to accept foreign pollen when used as female parents. The dwarf mutant showed maximum percentage of successful crosses (22.70%) followed by long pod mutant (21.73%), as compared with the control JC 4198, which showed 17.39% success in crosses, the lowest, 6.97% was shown by linear leaf mutant. In case of triangular leaf and antho bud the success rate was 11.11% and 10.69% respectively.

TABLE 1 : Characters of winged bean mutants.

Mutant	Stem colour	Leaf shape	Inflorescence axis	Calyx colour	Pod shape	Pod wing	Pod wing colour	Pod speck colour
Control	Green	Obovate	Short	Green	Rectang.	Winged	Green	Green
Linear leaf	Green	Linear	Long	Green	Rectang.	Winged	Green	Green
Antho bud	Green	Obovate	Short	Purple	Rectang.	Winged	Purple	Purple
Antho stem	Purple	Obovate	Short	Green	Rectang.	Winged	Green	Green
Flat pod	Green	Obovate	Short	Green	Flat	Wings reduced	Green	-
Wingless pod	Green	Obovate	Short	Green	Squarish	Wingless	-	-
Long pod	Green	Obovate	Short	Green	Rectang.	Winged	Green	Green
Early maturing	Green	Obovate	Short	Green	Rectang.	Winged	Green	Green
Tall	Green	Obovate	Short	Green	Rectang.	Winged	Green	Green
Xantha	Greenish yellow	Obovate	Short	Greenish yellow	Rectang.	Winged	Greenish yellow	Greenish yellow
Triangular leaf	Green	Triang.	Long	Green	Rectang.	Winged	Green	Green

TABLE 2 : The hybrids obtained after crossing winged bean mutants.

Cross	F_1 hybrid
Linear leaf X Normal leaf	Normal leaf
Antho bud X Normal	Antho bud
Antho bud X Antho stem	Antho bud & Antho stem
Flat pod X Normal pod	Normal pod
Early maturing X Linear leaf	Early maturing & normal leaf
Stiff stem x Normal stem	Normal stem
Tall X Dwarf	Tall
Purple flower X Blue flower	Purple flower
Long inflorescence axis X Normal	Long inflorescence axis
Xantha X Normal	*Xantha*
Long pod X Normal pod	Long pod
Flat pod X Normal pod	Normal pod
Linear leaf X Triangular leaf	Linear leaf
Wingless pod X Winged pod	Winged pod

The analysis of F_1 plants after crossing indicates traits of dominant nature (Table 2). Erskine & Khan (1977) demonstrated in winged bean a complete dominance of purple over green stem colour, purple over green calyx colour, purple over green pod-wing colour, purple sepals over green and rectangular over flat pod shape. Our present studies of winged bean mutants are quite in conformity with the foregoing findings, besides indicating the existence of some new dominant traits in that system. Such traits comprise normal leaf shape over linear, normal pod over flat pod, winged pod over wingless pod, long inflorescence axis over short, early maturity over late, tallness over dwarfness and flowering at lower nodes over the normal.

The other mutant characters like linear leaf, flat pod, stiff stem and winglessness have, however, revealed a recessive feature. Further studies are underway to understand the genetics of winged bean mutants.

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THE PRODUCTIVE MUTANTS IN SAFFLOWER

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SUMMARY

In the present investigation different mutagens like gamms rays, ethyl methanesulphonate, N-nitroso-N-ethyl urea and sodium azide were employed for inducing variability in safflower. Several mutants could be seen while screening the M_2 population. Majority of such mutants bred true during the subsequent generation. A critical assessment of the varied mutants revealed the productive potential attained by some of them. These mutants can be successfully exploited in conventional breeding programme for generating a desirable recombinant type.

Key Words: Mutagens, mutants, safflower.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the important oil crops of our country belonging to Asteraceae. At global level, India occupies the first position in terms of acreage and production of safflower followed by Mexico, USA, Ethiopia and Australia. Initially, the safflower oil was used in the preparation of paints, varnishes and surface coatings. But in recent years, it has gained substantial importance as a highly nutritious edible oil. In view of the immense applications of safflower, studies were planned to accomplish its genetic improvement through mutation breeding. It was visualised that this particular approach would help in evolving desirable plant types in case of safflower carrying useful alterations with reference to maturity characters, yield traits and the level of seed oil.

MATERIALS AND METHODS

The seed material of 2 varieties of safflower viz., Sharda (Bsf-168/4) and Annigeri-1 (A-1) obtained from Mahatma Phule Agricultural University, Zonal Research Station, Solapur was used in the present study. Four mutagens namely, gamma rays (GR), ethyl methanesulphonate (EMS), N-nitroso-N-ethyl urea (NEU) and sodium azide (SA) were employed in the present study.

Healthy and dry seeds of safflower having uniform size and equilibrated to a moisture level of 12.5% were exposed to gamma ray doses of 10, 20, and 30 kR. During chemical mutagenic treatments, the seeds were immersed in distilled water for 14 h and later in the mutagenic solution for 6 h with continuous shaking. The seeds soaked in distilled water for 20 h served as control.

The different concentrations used for the chemical mutagenic treatments were 0.05%, 0.10% and 0.15% for EMS, 0.003%, 0.005% and 0.008% for NEU and 0.001%, 0.003% and 0.005% for SA.

Immediately after completion of treatment, the seeds were washed thoroughly in tap water. Later, they were post-soaked in distilled water and sown in field following randomized block design (RBD) with 3 replications along with control for raising the M_1 generation. The seeds of 25 normal looking M_1 plants selected at random were collected on individual plant basis from all treatments and control. They were used for raising the M_2 generation on plant to a row basis.

A critical screening of the M_1 population was carried out and the details of different morphological mutants were noted. The seed oil content in different mutants was estimated on NMR.

OBSERVATIONS

The screening of M_2 generation of safflower revealed the presence of a broad spectrum of mutants with a wide range of morphological variability in both the variety, Sharda and Annigeri-1. The different mutants in M_2 generation were of the following types: (1) robust, (2) luxuriant, (3) tall, (4) extreme dwarf, (5) bent stem, (6) curved stem, (7) curved branch, (8) cymosely branched, (9) divergently branched, (10) dark green leaf, (11) early flowering, (12) late flowering and (13) sterile, in both the varieties of safflower.

The data pertaining to the frequency and spectrum of morphological mutants of safflower are given in Tables 1 and 2. The frequency of mutants showed a declining trend with the gradual increase in dose/concentration of majority of the mutagens. The only exception to this was observed in case of the NEU treatment of variety Sharda where the maximum frequency (8.27%) was induced by the 0.005% concentration of mutagen. The lowest mutation frequency could be seen at the highest dose/concentration of the four mutagens. The relative percentage of individual mutant type was found to be random in different treatments in both the varieties of safflower. The details of morphological characters of the mutants were collected and the pertinent descriptions have been indicated in Tables 3 and 4 respectively.

DISCUSSION

The observations recorded in the present study revealed induction of a broad genetic variability in case of safflower. The promising utility potential of mutation breeding has already been demonstrated by several researchers for increasing productivity of oil seeds like groundnut (Gregory 1956, Emery et al. 1964), soyabean (Rawlings et. al. 1958, Koo 1992), mustard (Jacob 1957, Rai 1957, Zareen 1991) and safflower (Beard et al. 1958, Chatterjee & Prasad 1970, Sahu et al. 1980, Reddy 1991).

The induced genetic variability has been critically evaluated for developing high yielding varieties in groundnut and castor (Ankineedu & Kulkarni 1968). The important aspects like quantity and quality of oil and protein have been successfully improved through mutational techniques (Seetharam 1971, Hakande 1992). It is agreed by different mutation breeders that the desirable mutants in different oil crops would be able to contribute effectively towards the oil and protein production besides providing induced genetic variations for getting the much sought after disease/insect/pest resistance (Labana et al. 1979).

In the present study, the majority of morphological mutants could be observed as breeding true in the subsequent M_3 generation. A perusal of Tables 3 and 4 sufficiently indicates the attainment of positive attributes by some mutants of safflower. In this regard, the early flowering, robust, luxuriant, cymosely branched and large leaf mutants are specially of great value. A good scope exists for exploiting such mutants on a commercial scale. They can be very well incorporated in the cross breeding programme of safflower to help evolve a desirable recombinant type.

TABLE 1 : Effect of mutagens on frequency and spectrum of productive mutants in M_2 generation of safflower var. Sharda.

Mutagen	Dose/ concent-ration	Frequency of productive mutants (%)	Relative percentage													
			Robust	Luxu-riant	Tall	Extreme dwarf	Bent stem	Curved stem	Curved branch	Cymosely branched	Diver-gently branched	Large leaf	Dark green leaf	Early flower-ing	Late flower-ing	Sterile
GR	10kR	9.4	7.7	7.7	-	7.7	7.7	15.3	-	14.2	14.2	7.1	7.1	7.1	-	7.1
	20kR	7.8	18.1	9.0	9.0	9.0	9.0	-	-	15.3	7.7	7.7	7.7	7.7	7.7	-
	30kR	6.9	10.0	10.0	-	-	20.0	10.0	-	-	8.3	8.3	-	8.3	8.3	8.3
EMS	0.05%	9.7	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	-	7.1	-	7.1	21.4	7.1
	0.10%	7.8	9.0	9.0	9.0	27.2	-	9.0	9.0	9.0	-	9.0	-	9.0	-	-
	0.15%	7.6	9.0	-	9.0	9.0	-	-	-	9.0	9.0	-	18.1	-	18.1	18.1
NEU	0.003%	7.0	10.0	10.0	10.0	10.0	10.0	10.0	-	10.0	-	10.0	-	10.0	10.0	-
	0.005%	8.2	8.3	8.3	8.3	16.0	-	8.3	8.3	8.3	-	-	8.3	8.3	-	-
	0.008%	6.8	10.0	10.0	-	-	10.0	10.0	-	10.0	10.0	10.0	10.0	-	10.0	10.0
SA	0.001%	9.8	14.2	7.1	-	14.2	-	-	7.1	14.2	14.2	7.1	7.1	7.1	-	7.1
	0.003%	9.0	15.3	7.7	7.7	-	7.7	-	7.7	15.3	7.7	7.7	7.7	7.7	7.6	-
	0.005%	8.2	16.6	8.3	-	25.0	8.3	8.3	-	-	8.3	8.3	-	8.3	8.3	8.3

TABLE 2 : Effect of mutagens on frequency and spectrum of productive mutants in M_2 generation of safflower var. Annigeri-1.

Mutagen	Dose/ concent-ration	Frequency of productive mutants (%)	Relative percentage													
			Robust	Luxu-riant	Tall	Extreme dwarf	Bent stem	Curved stem	Curved branch	Cymosely branched	Diver-gently branched	Large leaf	Dark green leaf	Early flower-ing	Late flower-ing	Sterile
GR	10kR	8.0	9.0	9.0	-	18.1	9.0	9.0	-	-	9.0	9.0	-	18.1	9.0	9.0
	20kR	7.8	10.0	10.0	-	20.0	10.0	10.0	-	-	-	10.0	-	20.0	-	10.0
	30kR	6.7	22.2	-	11.1	22.2	11.1	11.1	-	-	-	-	22.2	-	11.1	11.1
EMS	0.05%	9.1	15.3	7.7	7.7	-	7.7	7.7	7.7	15.3	7.7	-	-	15.3	7.7	-
	0.10%	8.4	8.3	-	16.6	8.3	8.3	8.3	8.3	-	16.6	8.3	-	8.3	8.3	-
	0.15%	7.8	18.1	9.0	-	-	9.0	9.0	9.0	9.0	9.0	-	-	9.0	9.0	9.0
NEU	0.003%	9.2	8.3	16.6	8.3	8.3	-	-	8.3	16.6	8.3	-	8.3	8.3	8.3	-
	0.005%	8.9	15.3	-	7.7	7.7	7.7	-	7.7	7.7	7.7	7.7	-	15.3	7.7	7.7
	0.008%	7.3	10.0	10.0	-	10.0	20.0	-	10.0	-	10.0	10.0	-	-	10.0	10.0
SA	0.001%	8.2	22.2	11.1	11.1	11.1	-	-	-	11.1	-	11.1	-	11.1	-	-
	0.003%	7.8	9.0	-	-	9.0	18.1	-	9.0	9.0	-	9.0	-	9.0	9.0	-
	0.005%	7.0	10.0	-	10.0	10.0	-	-	20.0	10.0	-	10.0	10.0	10.0	10.0	10.0

TABLE 3 : Morphological characters of productive mutants in M_2 generation of safflower var. Sharda.

Characters	Control	Robust	Luxu-riant	Tall	Extreme dwarf	Bent stem	Curved stem	Curved branch	Cymosely branched	Diver-gently branched	Large leaf	Dark green leaf	Early flower-ing	Late flower-ing	Sterile
Plant height (cm)	106	116	110	125	65	98	95	100	96	97	97	100	92	87	68
No. of branches	6	10	10	7	5	5	5	11	7	9	6	7	10	7	2
Days to flowering	84	80	79	82	82	88	87	82	87	79	83	86	74	88	85
Days to maturity	115	108	108	110	111	111	114	110	116	107	112	116	102	113	114
Total duration	139	136	136	135	137	137	137	138	140	137	137	141	130	142	138
No. of capitula per plant	8	19	17	11	10	6	6	15	13	17	9	10	15	12	1
No. of seeds per capitulum	20	22	22	20	17	18	13	21	19	18	23	21	20	20	-
Weight of 100 seeds (g)	6	6	6	6	5	5	5	6	6	6	5	6	5	5	-
Seed yield per plant (g)	9	24	22	14	10	6	4	19	15	17	10	14	17	12	-
Seed oil (%)	29	31	30	25	25	26	25	26	32	32	28	24	28	26	-

TABLE 4 : Morphological characters of productive mutants in M_2 generation of safflower var. Annigeri-1.

Characters	Control	Robust	Luxuriant	Tall	Extreme dwarf	Bent stem	Curved stem	Curved branch	Cymosely branched	Divergently branched	Large leaf	Dark green leaf	Early flowering	Late flowering	Sterile
Plant height (cm)	93	105	97	126	63	96	94	105	98	95	100	95	98	92	62
No. of branches	6	11	11	6	6	6	4	12	9	8	7	8	10	8	1
Days to flowering	80	81	80	84	81	90	88	81	85	81	82	88	72	84	86
Days to maturity	115	110	110	112	110	113	116	112	115	112	114	118	104	116	118
Total duration	139	136	135	136	136	136	140	136	138	134	137	139	130	140	140
No. of capitula per plant	8	20	18	10	9	4	4	17	19	19	12	11	14	11	1
No. of seeds per capitulum	20	21	20	19	18	14	10	22	23	19	22	20	19	18	-
Weight of 100 seeds (g)	6	7	7	6	5	4	5	6	7	7	6	6	5	6	-
Seed yield per plant (g)	9	21	23	13	9	6	5	21	20	19	12	16	18	11	-
Seed oil (%)	28	29	30	26	24	25	23	27	31	35	25	22	29	25	-

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J. Cytol. Genet. 30 (2) :181-188 (1995)

HIGH RESOLUTION G- AND R-BANDING PATTERNS OF THE PROMETAPHASE CHROMOSOMES OF THE MURRAH BUFFALO (*BUBALUS BUBALIS* L.)

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SUMMARY

The Bovine chromosomes have been evaluated primarily after C-banding (CBG) and G-banding (GTC). Two types of R-banding have been described; those produced by heat denaturation (RHG) and those by fluorescent acridine orange staining after BrdU incorporation (RBA). However, these have failed to give sufficient details (GTC, CBG), are difficult to reproduce (RHG), or require fluorescent microscopy (RBA). We have successfully applied to buffalo chromosomes the simple R-banding method using BrdU, the fluorescent 33258 Hoechst stain followed by blue light exposure and Giemsa staining (RB-FPG). As compared to other R-banding techniques, the RB-FPG method produces a very precise banding pattern. It enables definite identification and easy pairing. We consider this as most reliable and useful technique for studying Bovine chromosomes.

Key Words : Chromosomes, banding, idiogram, karyotype, Murrah buffalo.

INTRODUCTION

The discovery of the RB-FPG banding procedure by Romangnano & Richer (1984) has provided a remarkable improvement in the identification and description of individual chromosomes of mammals. The benefits of this technique are quite evident in the family Bovidae, whose chromosomes, especially the smaller elements of the karyotype, are not easily distinguishable when G- or R-banded. The difficulty lies in the fact that in Bovidae, both centromeres and telomeres in the chromosomes are mostly G-negative and, therefore, the smaller autosomes have to be identified by relying upon a very few and often undefined bands.

The various workers have been using RBS technique for definite identification of chromosomes in Bovidae (Popescu 1975, Gustavsson & Hagelthorn 1976, Di Berardino et al. 1981, 1987, Hayes et al. 1991). Iannuzzi et al. (1990) compared G- and R-banded chromosomes of cattle and river buffalo at prometaphase. The karyotype of heifer with 2n = 59 was studied using C- and high resolution G-banding techniques by Sharshov & Grafodatskii (1990).

As a contribution to the establishment of the standard RB- FPG-banded karyotype of Murrah buffalo *(Bubalus bubalis)*, this paper presents karyotype and idiograms of the RB- FPG- banding patterns of prometaphase chromosomes.

MATERIALS AND METHODS

Short-term whole blood culture method originally developed by Basrur & Gilman (1964) and modified by Yadav & Balakrishnan (1985) was used. The technique involves the following steps : peripheral blood drawn from the jugular vein of 4 male individuals of Murrah breed was cultured for 72 h in TC-199 medium supplemented with 10% adult cattle serum, pencillin and streptomycin and pokeweed mitogen. Seven h before harvest, 5-bromodeoxyuridine was added to cultures at a concentration of 50 μg/ml of medium. Thirty min before harvest, colchicine was added to a final concentration of 0.1 μg/ml. The cells were treated with hypotonic KCl and fixed in 1:3 acetic-methanol and the slides were prepared and air-dried.

The resulting chromosome preparations were R-banded by the FPG method (Perry & Wolf 1974, Gagne 1980) consisting of following steps: The prepared slides are stained for 15 min in 0.5% solution of 33258 Hoechst, a benzimidol derivative (Misawa et al. 1977). After rinsing thoroughly in tap water the slides were first immersed and then mounted in 2 x SSC solution. Next, they were placed at a distance of 2 or 3 cm from a fluorescent blue light tube for approximately 2 h (110-130 min). After exposure, the preparations were thoroughly rinsed to wash off both the coverslip and the salt solution. Slides were transferred to a 2% Giemsa solution for 5 min and the resulting pattern was analysed under a light microscope. Karyotypes were prepared according to Tenth European Colloquium on Cytogenetics of Domestic Animals (Iannuzzi 1994). The complementary G-bands were prepared on another slide, according to the technique of Seabright (1971). The C-bands were obtained using barium hydroxide method of Sumner (1972).

OBSERVATIONS

The majority of cells in the RB-FPG banded preparations showed chromosomes exhibiting clear and definite bands. In order to compare the bands of each chromosome with a complementary

TABLE 1 : Morphometric data of somatic karyotype of Murrah.

Chrom. Pair	S. Arm	L. Arm	Mean Total Length	Relative Length (%)	Arm Ratio	Centromeric Index	Chromosome Type
1	4.10	10.09	14.00	7.09	2.49	28.61	Submetacentric
2	4.00	9.99	14.00	6.91	2.49	28.62	"
3	3.57	9.62	13.19	6.51	2.68	27.10	"
4	3.41	8.40	11.81	5.83	2.46	28.90	"
5	2.74	6.48	9.22	4.55	1.46	40.55	"
6	0.00	9.47	9.47	4.42	0.00	0.00	Acrocentric
7	0.00	9.01	9.01	4.40	0.00	0.00	"
8	0.00	8.67	8.67	4.28	0.00	0.00	"
9	0.00	8.17	8.17	4.04	0.00	0.00	"
10	0.00	8.02	8.02	3.96	0.00	0.00	"
11	0.00	8.00	8.00	3.95	0.00	0.00	"
12	0.00	7.37	7.37	3.64	0.00	0.00	"
13	0.00	7.12	7.12	3.51	0.00	0.00	"
14	0.00	6.76	6.76	3.34	0.00	0.00	"
15	0.00	6.34	6.34	3.31	0.00	0.00	"
16	0.00	6.27	6.27	3.09	0.00	0.00	"
17	0.00	5.57	5.57	2.84	0.00	0.00	"
18	0.00	5.67	5.67	2.80	0.00	0.00	"
19	0.00	5.56	5.56	2.74	0.00	0.00	"
20	0.00	5.46	5.46	2.69	0.00	0.00	"
21	0.00	4.96	4.96	2.45	0.00	0.00	"
22	0.00	4.65	4.65	2.29	0.00	0.00	"
23	0.00	4.47	4.47	2.21	0.00	0.00	"
24	0.00	3.98	3.98	1.96	0.00	0.00	"
X	0.00	10.60	10.60	5.06	0.00	0.00	"
Y	0.00	3.19	3.19	1.57	0.00	0.00	"

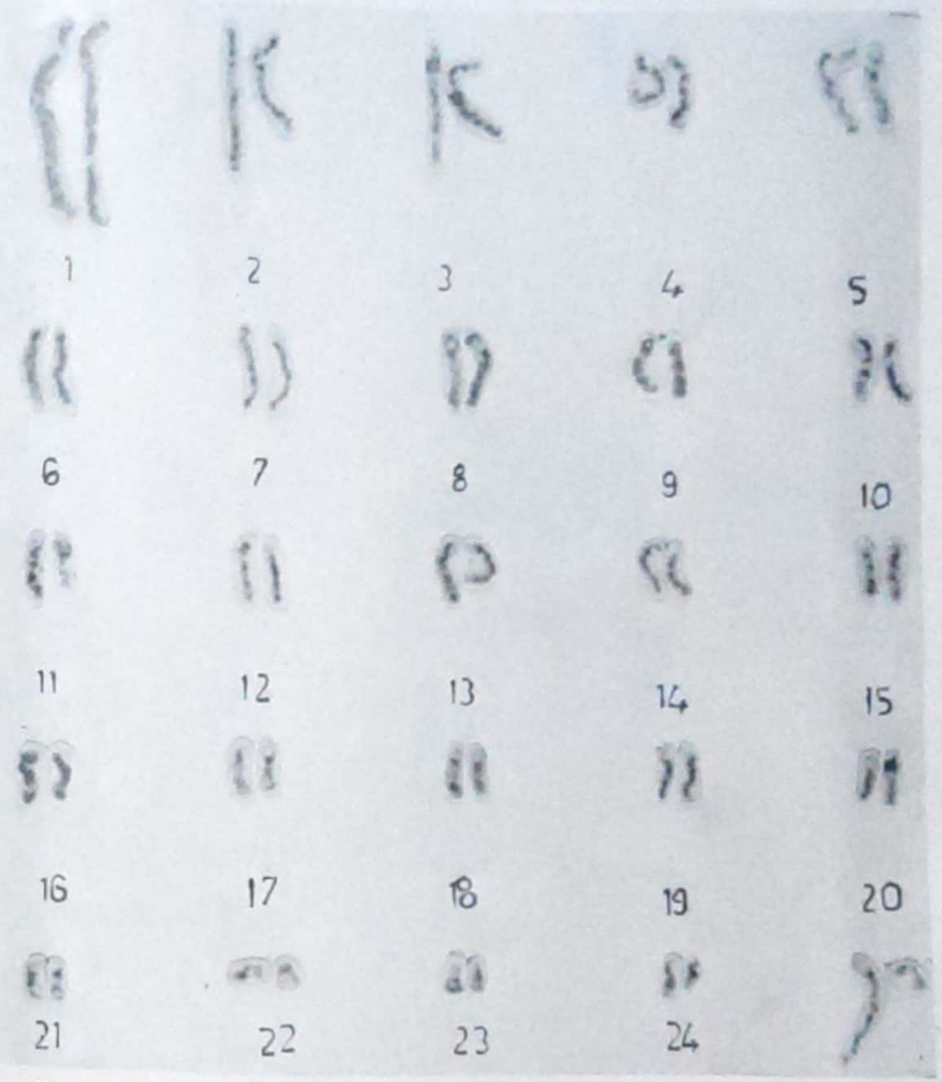

Fig. 1: G-banded karyotype of Murrah buffalo.

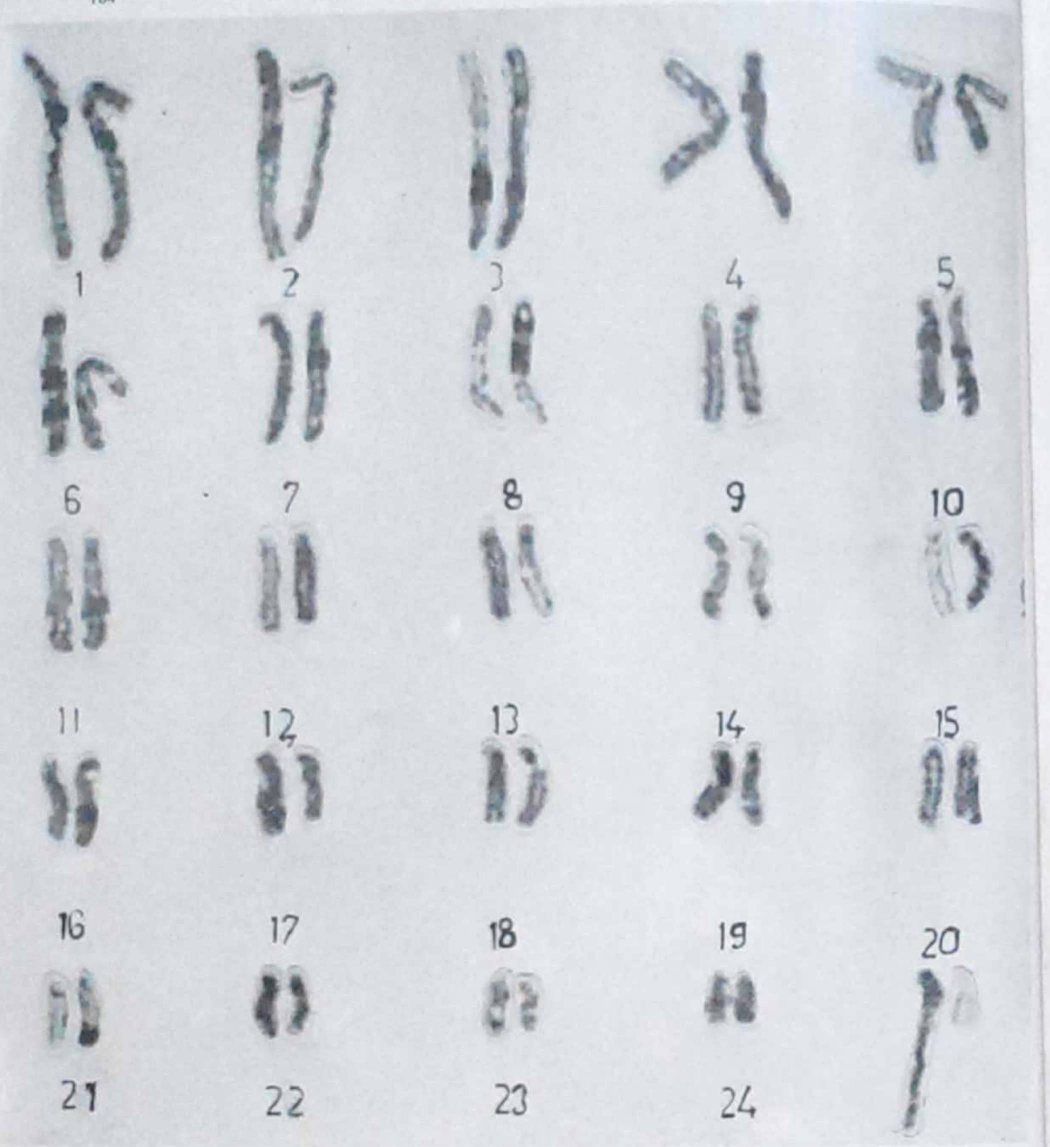

Fig. 2: R-banded karyotype of Murrah buffalo.

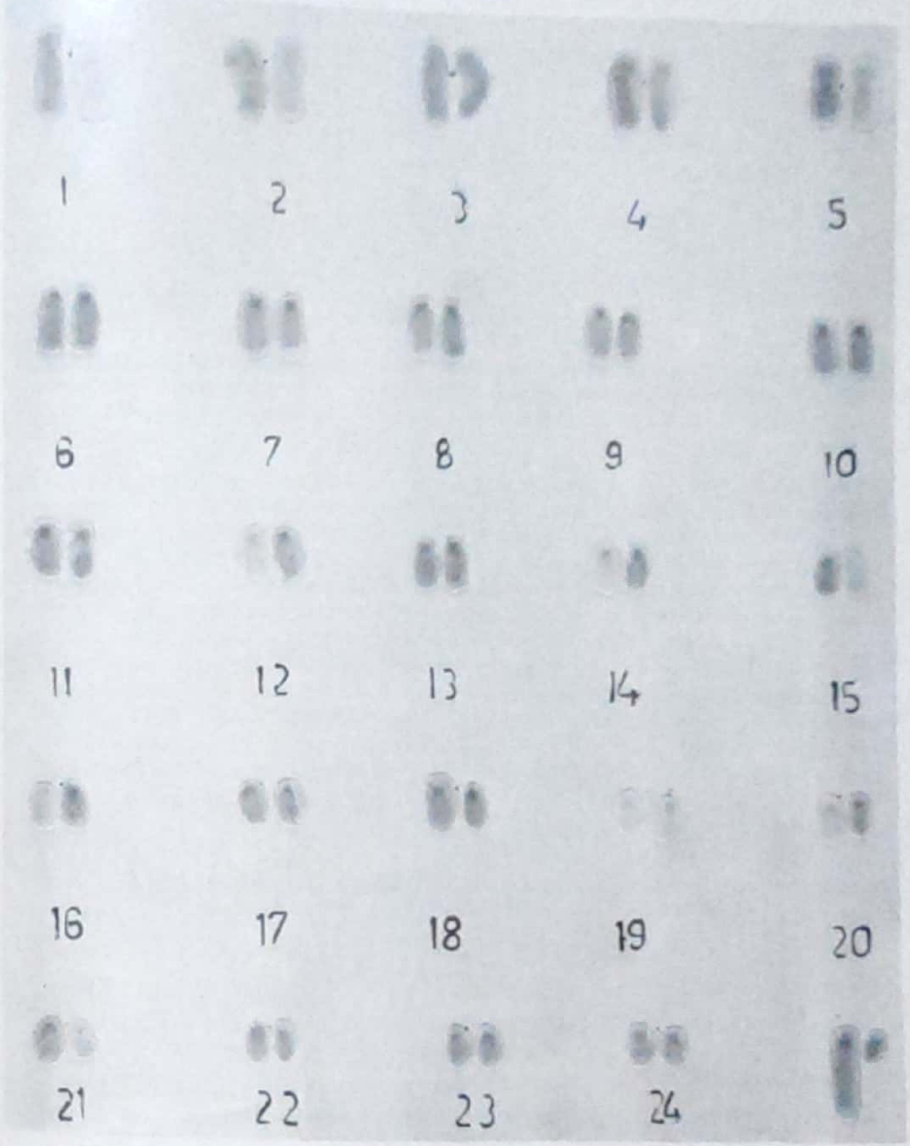

Fig. 3: C-banded karyotype of Murrah buffalo.

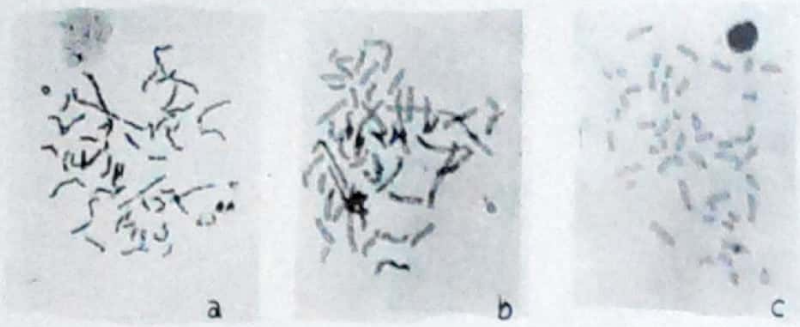

Fig. 4: a. Prometaphase showing G-banded chromosomes. b. Prometaphase showing R-banded chromosomes. c. Metaphase showing C-banded chromosomes.

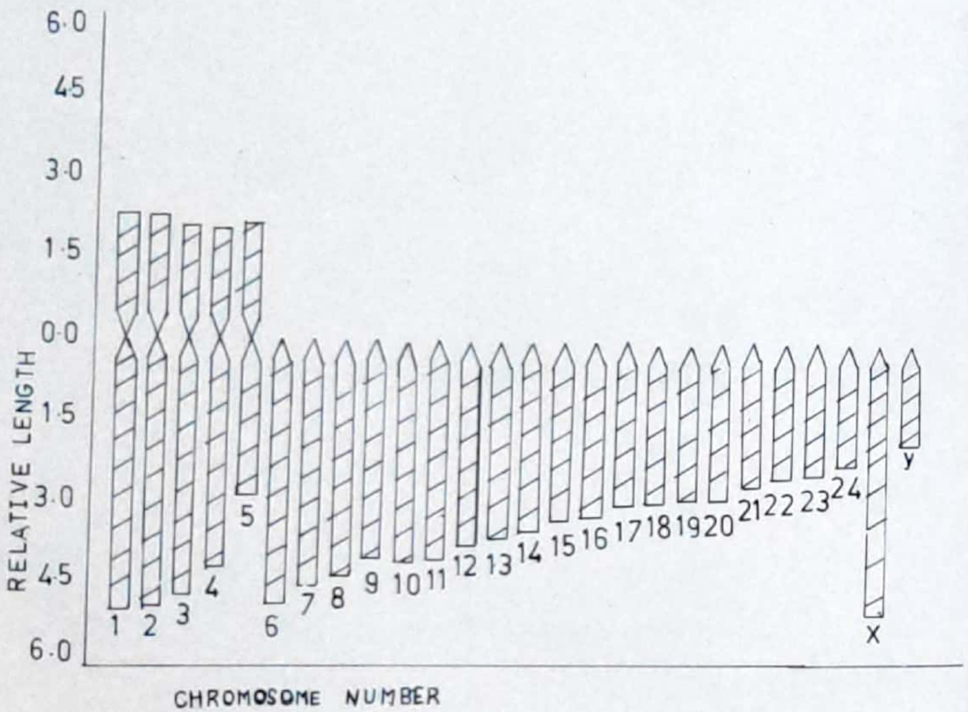

Fig. 5: Idiogram of chromosomes of Murrah buffalo.

pattern, both GTH and RB-FPG banded karyotypes were prepared (Figs. 1, 2). After RB-FPG the chromosomes are more clearly banded than after other R-banding methods. The C-banded karyotype and the whole idiogram of the buffalo chromosomes are presented in Figs. 3 and 5 respectively. Morphometric data of somatic karyotype have been presented in Table 1.

The first five pairs of autosomes of Murrah buffalo are submetacentric; pairs 1,2 and 4 are submetacentric while pairs 3 and 5 are almost metacentric. Rest of the autosomes, pairs 6 - 24 are all acrocentrics of decreasing size. The X-chromosome is the largest acrocentric while the Y-chromosome is among the smaller acrocentrics.

DISCUSSION

The present paper has to be considered as a contribution to the establishment of the standard RB- FPG-banded karyotype of Murrah breed of buffalo.

The remarkable increase in the R-band resolution was achieved by combining with BrdU, Hoechst 33258 which links to DNA by hydrophobic bonds (Bontemps et al. 1975, Comings 1975) with a 4-fold strong affinity to poly (dA-dBrdU) than a poly (dA-dT) segments (Latt & Wohlleb 1975). The combined incorporation delays chromosome contraction and enhances band contrast (Ronne 1983).

The present RB- FPG-banded karyotype can be utilized for further studies on detection of numerical as well as structural chromosomal abnormalities, evolutionary relationship among the members of the family Bovidae, and gene mapping by using in situ hybridization procedure (Geffrotin et al. 1984). It is known that after BrdU addition at the mid-S-phase of the cell cycle, the late replicating regions become elongated (Zakhrov & Egolina 1972) and sensitive to light (Hutchinson 1973). In comparision to the normal chromosomes, the BrdU substituted chromosomes become extended along the regions that have made their replication in the presence of the thymidine analogue. In our second half of the S-phase, it is incorporated in the G-bands and also in the late replicating R-bands of the inactive X-chromosome. These regions will break readily when exposed to blue light in the presence of 33258 Hoechst (Comings 1975, Galley & Purkey, 1972, Hutchinson 1973, Misawa et al. 1977). Subsequently, Giemsa will not stain the damaged chromatin that will stain darkly (Gonzalez-Gil & Navarrete 1982). Thus, the early replicating R-bands are visible on otherwise uncoloured chromosomes.

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ADH POLYMORPHISM AND UTILISATION OF ALCOHOLIC RESOURCES IN THREE DROSOPHILIDS

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SUMMARY

Gel electrophoretic analysis of 7 Indian geographical populations of 2 colonising drosophilids (*Drosophila melanogaster* and *Zaprionus indianus*) revealed clinal variation of alcohol dehydrogenase-fast (Adh^F) (3% for 1° latitude in *D.melanogaster*, > 1% for 1° latitude in *Z. indianus*). *D. melanogaster* and *Z. indianus* revealed significantly higher ethanol and acetic acid tolerance levels as compared to *D. immigrans*. The parallel patterns of utilisation of primary and secondary alcohols seem to be correlated with the concentrations of these metabolites found in natural food resources. The longevity effects of n-butanol (0.5 - 1.5%) were found to be significantly higher in all the 3 drosophilids as compared to n-propanol and that of secondary alcohols. The interspecific differences for resource utilisation are in agreement with niche-width hypothesis and seem to be adaptively maintained by natural selection mechanisms.

Key Words : Gel electrophoresis, alcohol dehydrogenase, primary and secondary alcohols, drosophilids.

INTRODUCTION

Ethanol stress is a major environmental constraint for fruit breeding species, and ethanol tolerance is a significant trait in their ecology (Parsons 1983, David 1988). Ethanol adaptation in *Drosophila melanogaster* is related to the occurrence of a high alcohol dehydrogenase (*Adh*) activity and to a widespred polymorphism of 2 major alleles at the *Adh* locus (Van Delden 1982, David et al. 1989). Since gel electrophoretic analysis has helped in elucidating the genetic structure of geographical populations of diverse taxa, it was considered worthwhile to characterise the extent of genic divergence at *Adh* locus in some latitudinally varying Indian natural populations of *D. melanogaster* and *Zaprionus indianus*.

The phenomenon of ethanol tolerance has been studied from the ecological and genetic points of view in *D. melanogaster* (Chambers 1988). Alcohol dehydrogenase (ADH) is known to be involved in the utilisation and detoxification of exogenous alcohols. The fermentation byproducts produced in the environment depend on the type of microflora (yeasts and other microbes) involved and the types of organic matter undergoing decomposition. Thus, it can be predicted that diverse types of drosophilids could reflect interspecific differences in tolerance to different alcoholic resources (David & Van Herrewege 1983). Alcoholic fermentation of sweet resources is followed by a bacteria-mediated acetic acid fermentation, so that acetic acid is released in the feeding substrate. Although *Drosophila* flies are called vinegar flies, little attention has been paid to the effect of acetic acid in these species.

Isopropanol, a secondary alcohol has also depicted higher metabolic utilisation in *D. melanogaster* while reports on its selection effect on alcohol dehydrogenase-fast (Adh^F) frequency seem to be contradictory (Van Delden et al. 1975, 1978). However, other secondary alcohols have revealed lower metabolic utilisation and showed even toxic effects in *D. melanogaster* (David & Bocquet 1976, David et al. 1976). Thus, studies on utilisation of primary and secondary alcohols have only been attempted in *D. melanogaster* which is mainly adapted to metabolism of manmade alcoholic fermentations (Chambers 1988). Thus, it was considered worthwhile to find out utilisation patterns of primary and secondary alcohols as well as acetic acid in *D. melanogaster, Z. indianus* and *D. immigrans*.

MATERIALS AND METHODS

D. melanogaster, D. immigrans and *Z. indianus* are successful colonising species. *Zaprionus* is a related genus that evolved from close to the *immigrans* species group radiation. Isofemale lines were established from population samples of *D. melanogaster* and *Z. indianus* from seven geographical sites (Cochin to Kullu: 10° N to 31°.85'N). Single individual homogenates were subjected to electrophoresis at 250 V and 25 mA at 4°C for 4 h. The gel slices were stained for alcohol dehydrogenase by standard staining procedure (Harris & Hopkinson 1976). Genetic control of alcohol dehydrogenase (ADH) banding patterns was interpreted from the segregation patterns of enzyme electromorphs of parents, F_1, F_2 progeny of several single-pair matings. The genetic indices were calculated by following standard statistical formulae (Ferguson 1980). The adult utilisation of primary and secondary alcohols and acetic acid was assessed following the procedure of Starmer et al. (1977) and David & Van Herrewege (1983). Adult survivorship was expressed as the number of adults alive after various time intervals. The LT_{50} values were calculated as the number of hours at which 50 % of flies had died and were estimated by linear interpolation. The ethanol concentration was obtained at LT_{50} maximum/LT_{50} control = 1. LC_{50} denotes ethanol concentration killing 50% flies.

OBSERVATIONS

ADH polymorphism

The ADH electrophoretic phenotypes included segregating 2-banded patterns (of either faster or slower mobilities) and 3-banded patterns at a single polymorphic zone of ADH activity in *D. melanogaster* and *Z. indianus*. Species-specfic genetic crosses between individuals having triple/4-banded ADH patterns produced 1:2:1 proportions of offsprings with alternating 2-banded variants and triple/4-banded patterns in accordance with monogenic control of ADH electrophoretic phenotypes. Thus, the observed ADH electromorphs were represented by post-translational or conformational isozymes i.e. homozygous genotypes depicted 2-banded patterns. *D.melanogaster* and *Z. indianus* revealed only 2 types of *Adh* electromorphs (Adh^S and Adh^F) and no rare variant was observed in any species.

Population genetic structure

The data on sample size, allelic frequencies, observed and expected heterozygosity at the polymorphic *Adh* locus in 7 Indian populations of *D. melanogaster* and *Z. indianus* are given in Table 1. The data on Wright's Fixation Index (F_{ST}) and correlation coefficient of Adh^F allelic frequency with latitude are given in Table 1. The allelic frequency changes at *Adh* locus in *D.melanogaster* populations were found to be significantly higher (3% with 1° latitude). In *Z. indianus*, the Adh^F frequency increased significantly with increasing latitude (>1% with 1° latitude). The data on Wright's Fixation Index (F_{ST}) at *Adh* locus revealed significant genic differentiation in *D. melanogaster* and moderate in *Z. indianus* populations.

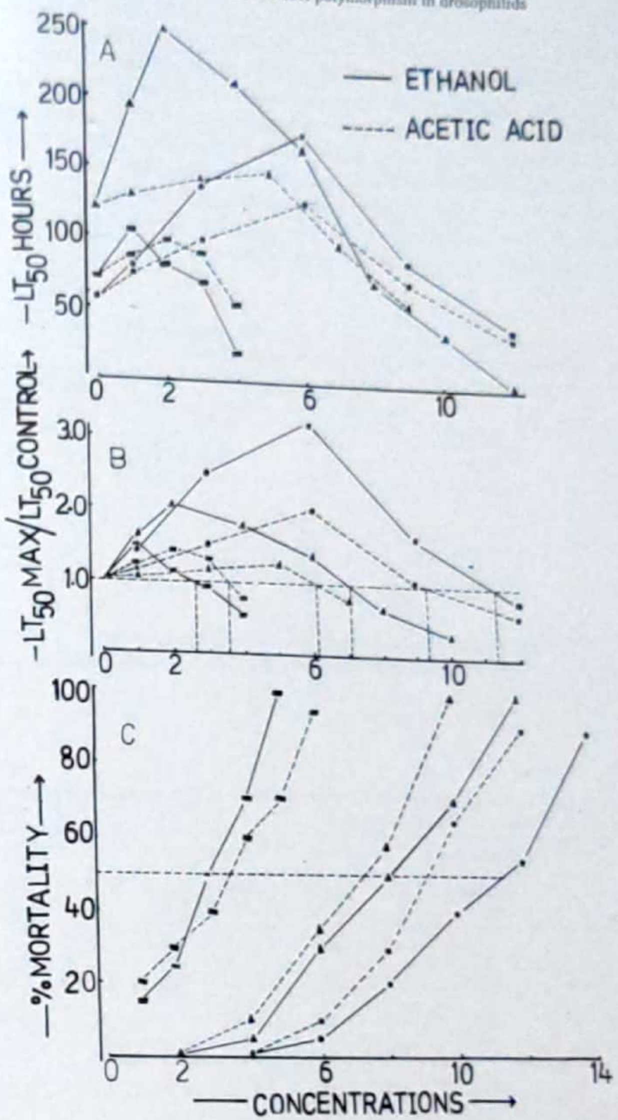

Fig. 1: Comparative profiles of (A) adult survivorship expressed as LT_{50} h (B) LT_{50} max/LT_{50} control (C) percent mortality relationships at different concentrations of ethanol and acetic acid in *D. melanogaster* (● — ●); *Z. indianus* (▲ — ▲) and *D. immigrans* (■ — ■).

The increased longevity data due to ethanol revealed lesser effects in *D. immigrans* (105 h) as compared with *D. melanogaster* (180 h) and *Z.indianus* (250 h) (Fig.1). The adult threshold values were found to be lesser in *D. immigrans* (2.7%) and *Z. indianus* (7.10%) but higher for *D. melanogaster* (11.3%). The LC_{50} ethanol concentrations were calculated from mortality data of adults after 4 d in all the three species. The LC_{50} values were found as 3.0% in *D. immigrans*, 8.0% *in Z. indianus* and 11.30% in *D. melanogaster* (Fig.1). The increased longevity data revealed parallel but lesser effect of acetic acid utilisation. The data on acetic acid threshold values, LC_{50} values, mortality and longevity responses further supported that acetic acid was utilised as resource in the 3 species (Fig.1, Table 2). The ranking of 3 *Drosophila* species on the basis of ethanol and acetic acid tolerance is *D. melanogaster* > *Z. indianus* > *D. immigrans*.

TABLE 1: Distribution of Adh allelic frequencies, observed/expected heterozygosity, F_{ST} values and correlation (r) with latitude in natural populations of *D. melanogaster* and *Z. indianus*.

		D. melanogaster			*Z. indianus*		
Populations	Latitude	F	S	Ho/He	F	S	Ho/He
Cochin	10°N	.11	.89	.15/.20	.25	.75	.35/.37
Tirumala	13°.40'N	.16	.84	.15/.26	.36	.64	.47/.46
Nagpur	21°.16'N	.30	.70	.21/.42	.43	.57	.44/.49
Bhopal	23°.16'N	.56	.44	.33/.49	.45	.55	.46/.49
Rohtak	28°.94'N	.74	.26	.27/.38	.49	.51	.49/.50
Dehradun	30°.19'N	.80	.20	.22/.32	.48	.52	.52/.50
Kullu	31°.85'N	.82	.18	.20/.29	.55	.45	.46/.49
F_{ST} value			.329			.037	
Correlation (*Adh* Vs latitude)			+.97**			+.93**	

**Significant at 5% level; F & S represent fast and slow allelic variants; Ho/He refer to observed/expected heterozygosity; F_{st} indicates Wright's Coefficient.

TABLE 2: Data on acetic acid, primary and secondary alcohols utilisation (LT_{50} h, threshold values and LC_{50} values) in *D. melanogaster, D. immigrans* and *Z. indianus*.

	D. melanogaster			*Z. indianus*			*D. immigrans*		
	LT_{50} hrs	Threshold value (%)	LC_{50} (%)	LT_{50}hrs	Threshold value (%)	LC_{50} (%)	LT_{50}hrs	Threshold value (%)	LC_{50} (%)
Acetic acid	130	9.3	9.2	153	6.1	7.3	98	3.5	3.5
Ethanol	180	11.30	11.30	250	7.10	8.00	105	2.70	3.00
n-propanol	188	3.00	3.10	156	1.30	1.80	90	0.80	0.80
n-butanol	110	2.51	2.70	186	1.45	·1.70	84	0.65	0.60
Iso-propanol	74	2.60	2.50	137	0.70	1.20	72	0.50	0.65
Iso-butanol	68	0.82	0.90	127	0.58	0.68	71	0.40	0.42

p=primary; s= secondary alcohols.

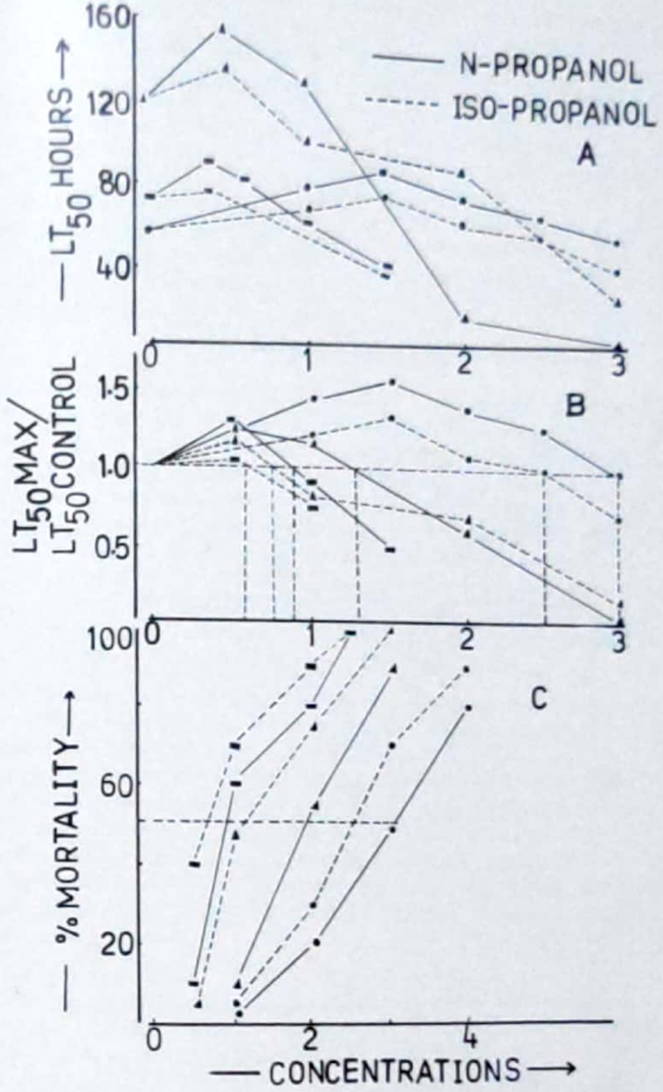

Fig. 2: Data on (A) LT_{50} hrs, (B) LT_{50} max/LT_{50} control and (C) percent mortality at different concentrations of n-propanol and iso-propanol in *D. melanogaster* (● — ●); *Z. indianus* (▲ — ▲) and *D. immigrans* (■ — ■).

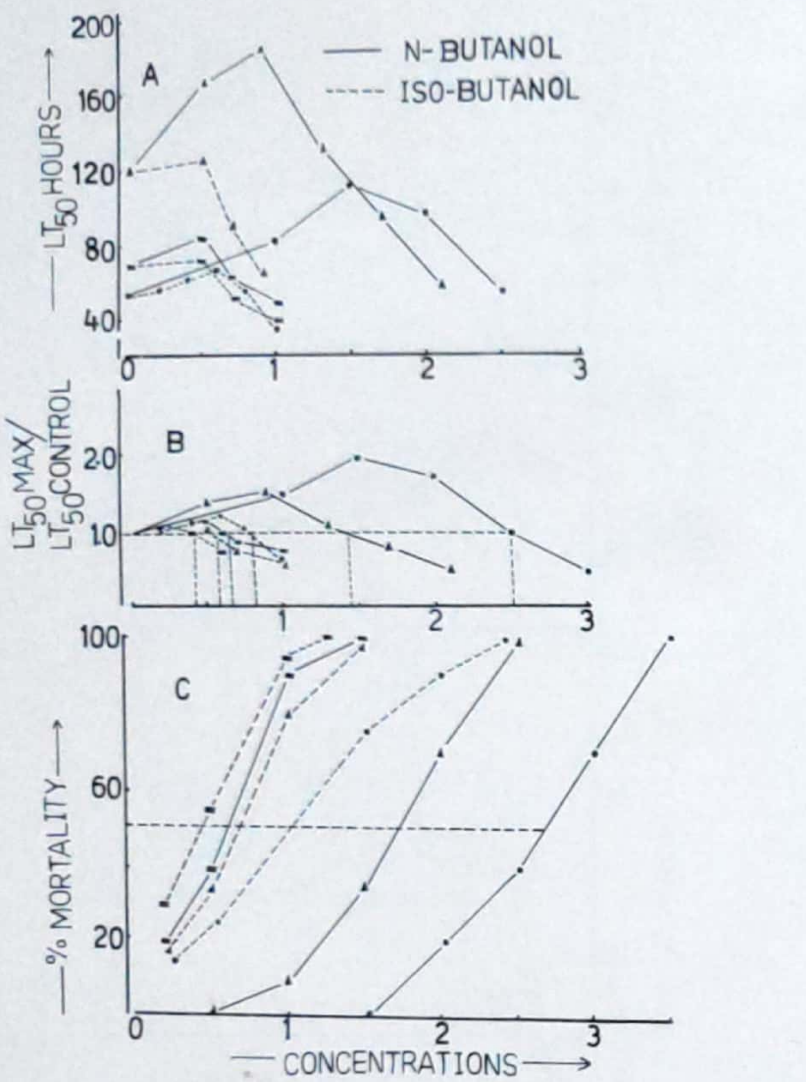

Fig. 3: Comparative data on (A) survivorship, (B) threshold values and (C) per cent mortality relationships at different concentrations of n-butanol and iso-butanol in *D. melanogaster* (● — ●); *Z. indianus* (▲ — ▲) and *D. immigrans* (■ — ■)

The 3 drosophilids utilised ethanol, acetic acid and n-propanol resources in a parallel way. *D. immigrans* populations revealed lesser ethanol and acetic acid threshold indices than that of *D. melanogaster* and *Z. indianus*. The acetic acid and n-propanol threshold values as well as LC_{50} values were found to be lower than that of ethanol utilisation indices. The present results revealed that acetic acid and primary alcohols constitute parallel resources. n-Propanol and 2-propanol are also metabolised by all the three drosophilids (Fig.2). n-Butanol is metabolised more than that of 2-butanol. The LT_{50} h for n-butanol have revealed significant increase in longevity. Interestingly primary alcohols were also utilised as resources by *D. immigrans*. The alcoholic resource utilisation indices of *D. immigrans* might be lower due to simultaneous utilisation of diverse alcoholic resources as compared to *D.melanogaster* which is a ethanol adapted species. Lower concentrations of n-propanol and 2-propanol are nontoxic to *Z. indianus* and *D. melanogaster*. However, 2-butanol (>0.5%) revealed toxic effect for *D. melanogaster*, *Z. indianus* and *D. immigrans* (Fig. 3) in which the longevity hours were equal to that of control.

DISCUSSION

The occurrence of parallel clinal allelic frequencies divergence at *Adh* locus across 2 colonising species (*D. melanogaster* and *Z. indianus*) are in agreement with the variable climatic gradient along North-South axis of the Indian subcontinent. The observed latitudinal variation at *Adh* locus in Indian populations of *D. melanogaster* and *Z. indianus* concur with other reports on *Adh* polymorphism in different continental populations of *D. melanogaster* i.e. Russia (Grossman et al. 1970), U.S.A. (Vigue & Johnson 1973, Singh & Rhomberg 1987), Mexico (Pipkin et al. 1973), Australia (Oakeshott et al. 1982, Knibb 1983), China (Jiang et al. 1988), Japan (Watada et al. 1986). The clinal variation across different species as well as across diverse biogeographical regions cannot be explained on the basis of stochastic process (genetic drift) and/or gene flow. Thus, the occurrrence of parallel clinal allozymic variation at *Adh* locus in Indian populations of *D. melanogaster* and *Z. indianus* can be explained on the basis of natural selection mechanisms.

The present results revealed significant increase of longevity of *D. immigrans* adults at lower ethanol as well as acetic acid concentrations i.e., overall fitness in this species increased by environmental ethanol and acetic acid at lower concentrations and was reduced at higher concentrations. This is in sharp contrast to that of *Z.indianus* and *D. melanogaster* in which fitness increased at ethanol and acetic acid concentration up to 7% or more. Thus, it can be inferred that all the 3 drosophilids utilised ethanol and acetic acid as resources at lower concentrations but slightly higher concentrations became toxic to *D. immigrans* but not to *Z. indianus* and *D. melanogaster*. The 2 primary alcohols (0.65 to 1.45% n-propanol and n-butanol) and secondary alcohols only at significantly lower concentrations(0.4 to 0.7%) were utilised as resources and were not found to be toxic in *Z. indianus* and *D. immigrans*.

In the present studies, *D. melanogaster* and *Z. indianus* were found to be highly tolerant while *D. immigrans* revealed intermediate response for utilisation of alcoholic resources. Since ethanol tolerance is genotypic dependent, the observed inter-specfic differences in ethanol tolerance may be due to regulatory genetic mechanisms rather than structural differences between ADH allozymes (David & Van Herrewege 1983). The 3 colonising drosophilids revealed significant inter-specific divergence in their potential to utilise primary and secondary alcohols i.e. adult threshold values, LC_{50} values were found to be significantly higher in *D. melanogaster* and *Z. indianus* as compared to *D. immigrans*. The lower but almost parallel threshold values for primary and secondary alcohols in *D. immigrans* seem to be correlated with lower levels of different alcohols in diverse types of fruits and vegetable resources. The comparative profiles of alcoholic utilisation in *D. melanogaster*, *D. immigrans* and *Z. indianus* reflect the species-specific adaptive characteristics for alcoholic metabolism.

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KARYOLOGICAL ANALYSIS ON SIX SPECIES OF CASSIDINAE (CHRYSOMELIDAE : COLEOPTERA)

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SUMMARY

Karyological investigations were carried out on 6 species of Cassidinae viz., *Aspidomorpha dorsata* F., *A. nigrovittata* Boh., *Cassida circumdata* Hbst., *C. enervis* Boh., *C. vibex* L. and *Epistictina vividemaculata* Boh. Both the species of *Asipdomorpha* showed 7 autosomal bivalents during metaphase I and Xy type of male sex determining system. All the species of *Cassida* depicted 2n=18 (8+Xy_p) except *C. vibex* (2n=21; 9+Xyy_p). *E. vividemaculata* possessed 2n=18 with Xy male sex chromosome system. Karyotype comprised meta-, submeta- and acrocentric chromosomes. Chiasma frequency varied from 10 to 14 per nucleus.

Key Words: Coleoptera, Chrysomelidae, karyotype, chiasma frequency, sex chromosome system.

INTRODUCTION

Chrysomelidae comprises over 37000 species (Jolivet 1988). Cytological data of 745 species and subspecies have been recorded by Petitpierre et al. (1988). The diploid chromosome number varies from 8 in *Homoschema nigriventre* (Virkki 1965) to 64 in *Disonycha bicarinata* (Vidal 1984) in this family. In the present paper, cytology of 6 species belonging to subfamily Cassidinae has been described. Karyotypic details of 4 species, *Aspidomorpha dorsata* Fab., *A. nigrovittata* Boh., *Cassida enervis* Boh. and *Epistictina vividemaculata* Boh.are new additions to the cytology of Coleoptera, whereas, *C. circumdata* Herb. and *C. vibex* L. were reinvestigated.

MATERIALS AND METHODS

Adult male individuals of *Aspidomorpha dorsata* , *A. nigrovittata* and *Cassida circumdata* were collected from Dehradun (Uttar Pradesh); *C. enervis* and *C. vibex* from Bangalore and *Epistictina vividemaculata* from Paonta Saheb (Himachal Pradesh) during 1987 - 1992. Karyological preparations were made from testes by air-drying technique (Yadav & Lyapunova 1983).

OBSERVATIONS

Aspidomorpha dorsata

Only meoitic stages were observed. Number of bivalents at metaphase I and haploid number of chromosomes at metaphase II revealed 16 as the diploid number in this species. The metaphase II karyotype comprised 6 metacentric and one (7th) acrocentric autosomes and a submetacentric X chromosome (Fig. 2). TCL was 35.74 µm (Table 1). The size of X is close to that of the third autosome. Seven autosomal bivalents and a Xy sex bivalent were observed during diakinesis and at metaphase I (Figs. 13, 14). Chiasma frequency was 14 per nucleus. The cells at metaphase II uniformally carried 7 autosomes. However, only one sex chromosome, either X or y, was present (Fig. 1). Male meioformula for this species is 7AA+Xy.

TABLE 1. Percentage relative lengths of chromosomes and TCL of karyotypes.

Species	1	2	3	4	5	6	7	8	9	X	Y	TCL
A. dorsata	14.55	13.68	13.48	12.33	11.62	10.50	10.40	-	-	13.44	?	35.74
A. nigrovittata	15.14	15.05	13.23	13.11	12.36	10.38	10.11	-	-	10.62	?	49.21
C. circumdata	13.58	13.38	11.96	10.25	10.12	9.37	8.95	8.03	-	11.23	3.13	25.37
C. enervis	15.22	13.95	12.71	11.65	11.47	11.26	10.51	5.17	-	5.23	2.83	52.32
C. vibex	14.27	12.74	12.39	11.89	9.47	8.83	8.55	7.63	7.37	4.83	2.03	60.98
E. vividemaculata	14.92	11.43	10.84	10.23	9.47	9.47	8.17	6.97	-	13.61	4.89	28.18

TCL = Total chrosome length in µm.

A. nigrovittata

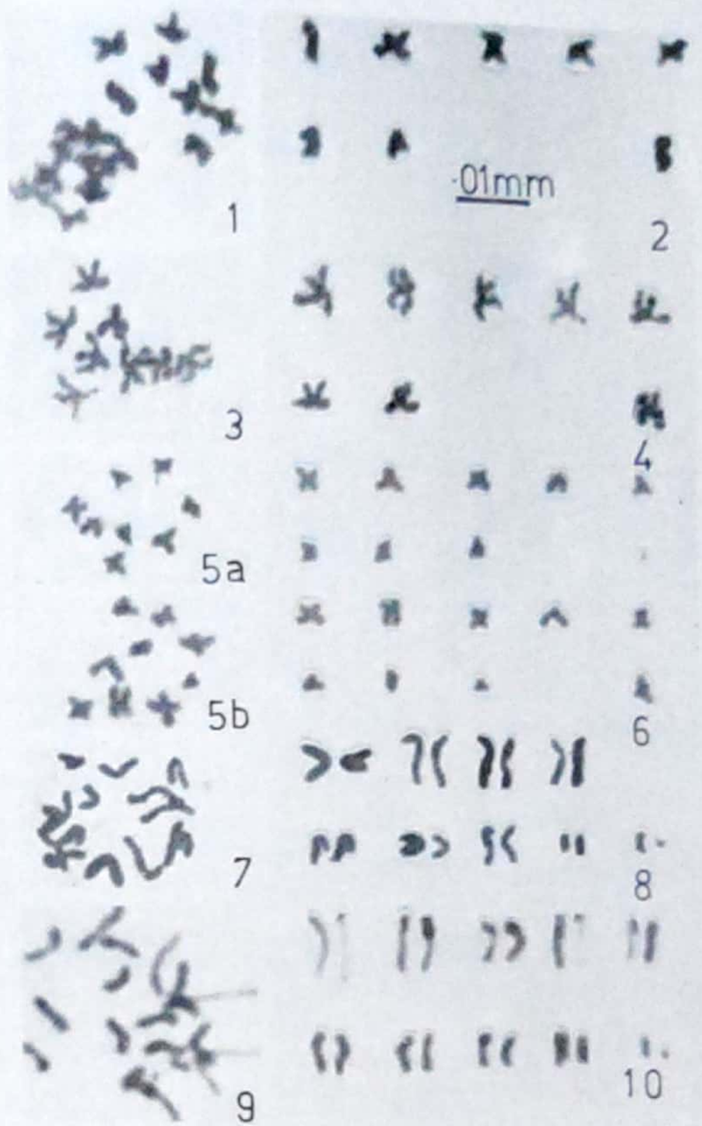
Metaphase II karyotype revealed all the 7 autosomes to be metacentric and the X to be submetacentric in nature (Fig. 4.). TCL was 49.21 µm (Table 1). On the basis of the size, the X lies between fifth and sixth autosomes. Diakinetic and metaphase I cells carried 7 autosomal bivalents and a Xy sex bivalent (Figs. 15, 16). Chias na frequency was 13 per nucleus. Male meioformula is 7AA+Xy.

Cassida circumdata

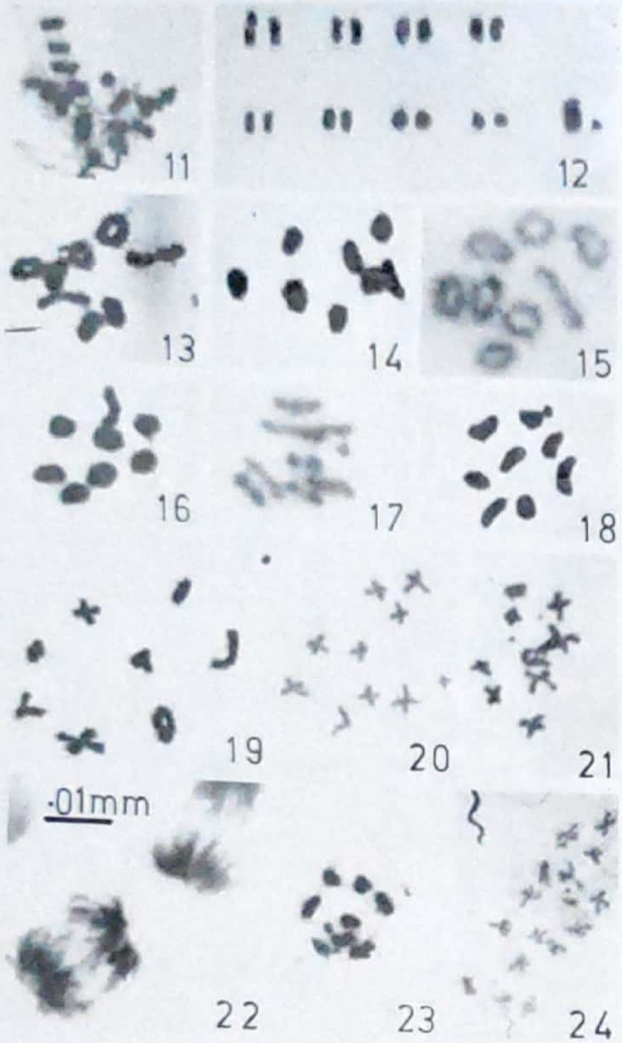
The diploid number of 18 chromosomes is in conformity with earlier reports (Manna & Lahiri 1972, Yadav 1973, Yadav & Pillai 1975). Karyotypes prepared from cells at metaphase II revealed 5 metacentric (1, 3, 5-7), 1 submetacentric (2) and 2 acrocentric autosomes (4, 8); a metacentric X and a dot-shaped y chromosome. TCL was 25.37 µm. The X lies between third and fourth autosomes (Table 1). Metaphase I showed 7 rod-shaped autosomal bivalents and the Xy_p sex pseudobivalent (Fig. 17). Chiasma frequency was 14 per nucleus. Meioformula is $8AA+Xy_p$. Yadav & Pillai (1975) observed 2 y chrosomes in some cells.

C. enervis

Spermatogonial metaphase showed 2n=18 (Fig. 7). This is similar to an earlier report (Sharma & Sood 1978). The karyotype consists of 5 pairs (1, 3, 6-8) of metacentric and 3 pairs (2, 4, 5) of submetacentric autosomes. The X seemed to be metacentric while y is a small dot-shaped element (Fig. 8.). TCL was 52.32 µm. The X was close to eighth pair of autosomes according to the size (Table I). During diakinesis and metaphase I, 8 autosomal bivalents were observed. The sex bivalent was in the form of Xy_p (Figs. 18, 19). Chiasma frequency was 10 per nucleus. Male meioformula is $8AA+Xy_p$.



Figs. 1-10: Karyotypes. 1, 2. Metaphase II with X chromosome and karyotype of *Aspidomorpha dorsata*. 3, 4. Metaphase II with X chromosome and karyotype of *A. nigrovittata*. 5a, b, 6. Metaphase II with y and X chromosome and karyotype of *Cassida circumdata*. 7, 8. Spermatogonial metaphase and karyotype of *C. enervis*. 9, 10. Spermatogonial metaphase and karyotype of *C. vibex*.



Figs. 11-24: Meiosis. 11, 12, 23, 24. *Epistictina vividemaculata*. 11, 12. Spermatogonial metaphase and karyotype. 13, 14. Diakinesis and M I of *A. dorsata*. 15, 16. Diakinesis and M I of *A. nigrovittata*. 17. M I of *C. circumdata*. 18, 19. M I and diakinesis of *C. enervis*. 20-22. *C. vibex*. 20, 21. M II with X and y chromosomes. 22. Gonial anaphase. 23, 24. M I and II with X and y chromosomes.

C. vibex

Spermatogonial metaphase revealed 2n=21 (Fig. 9). The karyotype comprises 4 pairs (3, 5, 7, 9) of metacentric, 4 pairs (1, 2, 6, 8) of submetacentric and 1 pair (4) of acrocentric autosomes (Fig. 10). The X is metacentric and y is dot-shaped. The autosomes showed gradual decrease in size. TCL was 60.98 µm. The size of X was smaller than the last pair of autosomes (Table 1). Spermatogonial anaphase showed normal segregation of chromosomes (Fig. 22). During metaphase I 2 y chromosomes were paired with the X chromosome in the formation of sex pseudobivalent and autosomes represented 9 dumb-bell shaped bivalents. Male meioformula is 9AA+Xyy$_p$. Cells with 2 types of metaphase II configurations were observed. One with the X (Fig. 20) and the other with 2 ys (Fig. 21), in addition to 9 autosomes. However, Petitpierre (1985) recorded 2n=20 with 9AA+Xy$_p$ type of sex chromosome mechanism in this species.

Epistictina vividemaculata

Spermatagonial metaphase showed 2n=18 (Fig. 11). The karyotype contained 8 pairs of autosomes and the sex chromosomes X and y (Fig. 12). The first pair of autosomes is relatively larger, whereas, the remaining autosomes decrease gradually in size. The morphology of chromosomes was not clear owing to over condensation. TCL was 28.17 µm. The X is second in the order of size and y is a dot-shaped element (Table 1). Metaphase I comprised 8 pairs of rod- and ring- shaped autosomal bivalents and Xy sex bivalent (Fig. 23). Chiasma frequency was 11 per nucleus. Male meioformula is 8AA+Xy. Cells with 2 types of metaphase II configurations, one with X and other with y, were observed (Fig. 24). At this stage, most of the chromosomes appeared to be metacentric.

DISCUSSION

Petitpierre et al. (1988) included the data on 55 species of Cassidine in their world list of chromosome numbers and meioformulae of Chrysomelidae. Chromosomes of another 8 species have been investigated by other workers (Bisoi & Patnaik 1990, Dua & Kacker 1976, Gill et al. 1987, Mittal et al. 1984, Pillai 1984). As such cytological data of 63 species belonging to 9 tribes are on record todate.

The diploid chromosome numbers show a wide variation from 16 in notoscanthine *Notoscantha macculipennis* (Sood 1978), aspidomorphine *Aspidomorpha dorsata* and *A. nigrovittata* (present report) and cassidine *Nodostoma haroldi* (Gill et al. 1987, Mittal et al. 1984) to 51 in stolaine *Stolas angulata* (Vaio & Postiglioni 1974) (Fig. 25). However, the distribution is not even. As many as 35 species of 5 tribes possess 18 chromosomes in their diploid complements. However, 2n=20, modal number of Polyphagan Coleoptera is possessed by only 4 species and 2n=24, the most common number in Chrysomelidae, is carried by only 2 species.

Xy$_p$ is the most common male sex chromosome system possessed by 55 species out of 65 reports on 62 species of Cassidinae. Three species of *Stolas* possess multiple sex chromosome systems : X$_p$neoXneoY$_p$ by *S. angulata* (Vaio & Postiglioni 1974) and $X^1_pX^2_p$ neo X neo Y by *S. bonariensis* and *Stolas* sp. (Panzera et al. 1983). *S. duodecimverrucata* shows XO (Vidal 1984). *Cassida subferruginea* has neo-XY (Petitpierre 1985), *Laccoptera guadrimaculata* (Sharma & Sood 1978), *C. vibex* (present report) and *Chiridopsis* sp. (Yadav & Pillai 1975) besides Xyp, have Xyyp also. Lanier (1972) recorded Xy system in *Plagiometriona clavata*. Xyy$_p$ and the multiple systems

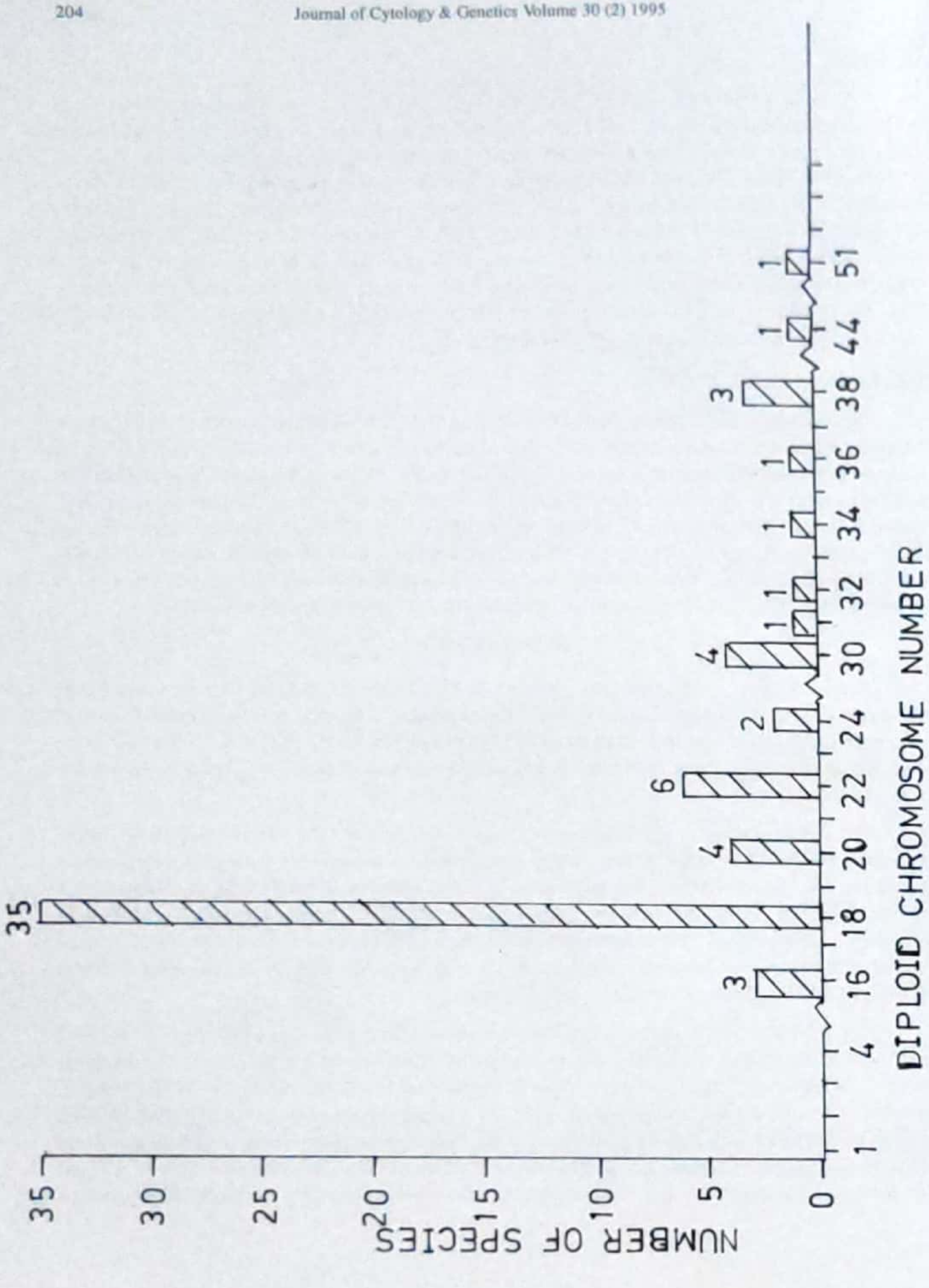


Fig. 25: Histogram showing distribution of diploid chromosome number in Cassidinae.

are obviously derived ones, whereas XO can be assigned to the loss of the minute y. The loss can be withstood by the species as the y is genetically inert and such a change has occurred on several occasions among Coleoptera (Smith & Virkki 1978). *C. viridis* depicts 2 distinct numbers $14+Xy_p$ (Petitpierre, 1977) and $11+Xy_p$ (Petitpierre 1985). The species seems to have been splitted into 2 morphologically indistinguishable races or may be sibling species: the northern race has $11+Xy_p$ and is common in Central Europe and spread to Catalonia (Spain), whereas the southern race with $14+Xy_p$ appears to be distributed in South Spain (Petitpierre 1985). Yadav et al. (1987) reported 2n=18 in *A. dorsata*. The number has been now revised to 2n=16. However, 2n=16 ($7+Xy_p$) in *Glyphocassis trilineata* (Yadav et al. 1987) is a new report. Earlier, Yadav & Pillai (1975) reported 2n=18 ($8+Xy_p$) in this species. As such, this species needs to be reinvestigated. Dimorphism of y chromosome is a common situation in polyphagan Coleoptera, the additional y being a product of simple fission (Smith & Virkki 1978).

Taxonomically, Cassidinae are considered closely related to Hispinae. According to Crowson (1967), a complete series of intermediate forms connect these subfamilies. Petitpierre (1988) discussed the cytotaxonomy of chrysomelidae. Since then there is not much addition to the chromosomal data of beetles belonging to subfamilies Hispinae and Cassidinae which remain limited. However, even the meagre data give good indication of phylogenetic relationships. $8+Xy_p$ male is the most widespread meioformula obtaining in these subfamilies. Another shared character is the presence of conspiciously large-sized metacentric chromosomes in species having low chromosome numbers. However, Stolaini Cassidines are highly derived species both with regard to the diploid number and multiple sex chromosome systems. The Hispinae, on the other hand, show a tendency towards decrease in the number of chromosomes. As compared to the other subfamilies of Chrysomelidae, Hispinae and Cassidinae are chromosomally rather conservative. The situation in some species of *Cassida* and the Stolainini (Petitpierre 1988) may, however, be taken as exceptional.

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Short Communication

GENETICS OF POD AND SEED SHAPE IN GROUNDNUT

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SUMMARY

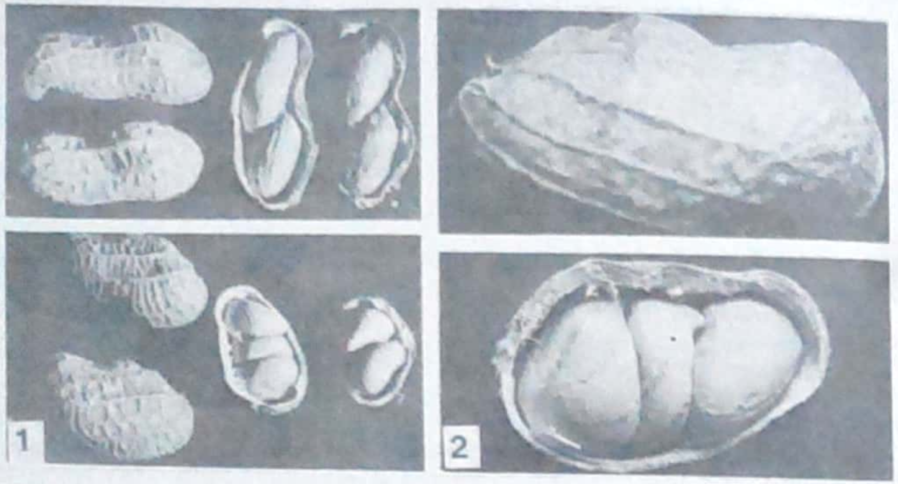
Inheritance of button type seeds was studied for the first time in groundnut, using 4 cross combinations. A stable advanced derivative of cross Co 1 x NCAc 17090 was found to produce pods with flat, button-shaped seeds instead of normal round or elongated ones. Also, the pods of this genotype had hard humps on their ventral side. The segregation pattern in F_2 and F_3 generations of crosses between 4 genotypes producing normal shaped pods and seeds and the one with humped pods and button type seeds indicated that pod and seed shapes in groundnut is controlled by 2 independent loci, *Psd psd* and *I i*. The action of *Psd* is partially inhibited by the dominant *I*, which led to the formation of flat, button-shaped but viable seeds instead of the usual round, oval-shaped or elongated seeds. The genes for humped pods and button type seeds were either closely linked or pleiotropic.

Key Words: Groundnut, seed, pod, inheritance.

The pod of groundnut, *Arachis hypogaea* L., is one-loculed indehiscent lomentiform carpel (Gregory et al. 1951). It consists of 1 to 5 seeds separated by moderate constriction. The unconstricted and deeply constricted pods are commercially unacceptable because of difficulties in mechanical shelling. Absence of pod constriction was reported to be controlled by 2 independent dominant genes (Badami 1928), 3 complementary genes (Hassan 1964) or interaction of nuclear and cytoplasmic factors (Coffelt & Hammons 1974). The seeds of groundnut are round to elongated in shape (Hayes 1933). The inheritance of unconstricted humped pods and button type seeds in groundnut has been reported here.

PBDR 25, a stabilized advanced derivative of the cross Co 1 x NCAc 17090 was observed to produce small (1 - 2.5 cm long), unconstricted. 2-4 seeded pods with hard humps on the ventral side of the pod (Figs. 1, 2). In 2-seeded pods, the seeds at one end were always flat while in 3- or 4-seeded pods the middle seeds were flat at both ends, looking like buttons. The genotype was selfed for 5 generations and found to breed true for the above traits. It was then crossed as male parent with 4 cultivars, namely , M 13, GAUG 10, C 363 and Karad 4-11, all having moderately constricted 2-seeded pods with usual round or elongated seeds. The F_1 generation was space-planted for realising maximum number of F_2 progeny. The F_2 generation was grown in kharif 1989 and observation on pod and seed characters were recorded after harvest. The F_2 plant-to-F_3 progeny rows were grown in summer 1990. The validity of expected genetic ratios was tested by the chi-square test.

All the F_1 hybrids produced unconstricted humped pods with button type seeds, as in PBDR 25 (Figs. 1, 2). In F_2, the segregation of plants with humped pods and button type seeds and those



Figs. 1 & 2: Pod and seed shapes in groundnut. 1. Normal pods and seeds (top) and humped pods containing button type seeds (bottom). 2. A close view of pods of genotype PBDR 25 showing button type seeds (bottom) and humps on ventral side of the pod (top).

TABLE 1: Inheritance pattern of button type seeds in groundnut.

Cross	Segregation in F_2				F_3 progeny grown from button type F_2 plants		
	Button type	Normal type	Total	X^2 (13:3)	Segregating	Non-segregating	X^2 (6:7)
GUAG 10 x PBDR 25	162	25	187	3.55	45	59	1.42
Karad 4-11 x PBDR 25	52	9	61	0.64	11	20	0.34
M 13 x PBDR 25	83	16	99	0.48	-	-	-
C 384 x PBDR 25	43	6	49	1.36	-	-	-

X^2 values were non-significant

producing normal type of pods and seeds was distinct in all the 4 cross combinations, and showed a good fit to the expected ratio of 13 button type : 3 normal type, indicating presence of inhibitory genes (Table 1). There were no plants with intermediate seed shape in the F_2 generation. The number of segregating and non-segregating families was recorded in F_3 generation for 2 cross combinations, GAUG 10 x PBDR 25 and Karad 4-11 x PBDR 25. In both the crosses, the ratio of segregating and non-segregating families derived from F_2 plants with button type seeds showed a good fit to the expected ratio of 6 : 7. 4 of the 20 F_3 families derived from normal seeded F_2 s of the cross GAUG 10 x PBDR 25 also segregated for plants with normal seeds and those with button type seeds.

These results suggest that the button type seeds are controlled by 2 independent loci. It is proposed that the locus *Psd psd* is essential for normal (round or elongated) shape of seeds. Another locus, *I i*, inhibits the action of *Psd-* when in dominant condition. Thus, F_2 plants with the genotypes *I- Psd-*, *I- psd psd* and *ii psd psd* produce button type seeds, whereas those with ii Psd- will have normal seed shape.

The button type seeds were viable and produced healthy plants, suggesting that the inhibitory gene, *I*, affects the action of *Psd-* only during the later stages of seed development. Since the humped pods and button type seeds are always associated, it is proposed that these traits are either closely linked or pleiotropic. This is the first report on the presence of inhibitory genes for seed development in groundnut.

The authors are thankful to the Director, NRCG, Junagadh for providing the necessary facilities.

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BOOK REVIEW

Baeuerle, P.A. (ed) 1995. **Inducible Gene Expression.** Vol.1 : Environmental stresses and nutrients, Vol.2 : Hormonal signals, Birkhauser, Boston-Basel-Berlin. pp. xii + 284 (each volume), Price per set: DM 298.-/ $ 140.00/ £ 98.-, ISBN 0-8176-3800-8.

This two volume collection of "Inducible Gene Expression" elegantly summarizes the rapid progress made in recent years on the molecular basis for the control of gene expression, especially in the higher eukaryotes. Gene expression is controlled most effectively and economically at the level of trascription initiation, although regulatory mechanisms also operate at later stages of transcription or at times post transcriptionally. The present volumes have been brought out with the central idea of helping the readers to compare the different mechanisms used for molecular signalling to achieve differential gene expression.

Differential gene expression is one of the most challenging and intensely investigated topics of modern biology. The pertinent questions relate to how from the midst of thousands of genes present in a multicellular organism does a particular tissue or cell pick up a single set of genes for expression while rendering the rest of them silent. Take for instance, in the human body made up by about 10^{13} cells, each one of its cell carry a chromosomal DNA equivalent of 3 billion base pairs, which is in vast excess of the information that is needed to code for all the functional genes known or estimated. The entire complement of genes is present in each and every cell of an individual tissue but only a single subset qualify to be the 'chosen ones'. The silencing or activation for expression, also depend on the state of development at which an organism is. The genes may be activated or rendered silent for the rest of the life of the organism. So, how does the tissue or cell accomplish this feat?

The intense investigations in molecular biology of the 1960s and 1970s unravelled several mechanisms pertaining to the control of gene expression using the prokaryotic organisms, especially the bacterium *Escherichia coli* and the viruses (phages) infecting it, as model systems. These studies having laid the foundation and together with the galloping advances made in recombinant DNA and genetic engineering methodologies of the 1970s and 1980s, the biologists today have been attempting to unravel the mystiques of the process in higher organisms including man. The networking is extremely complex in higher organisms and transactivators often exert their effects combinatorally. A major difference between prokaryotes and eukaryotes appear to be in that in the former a negative control is more commonly utilised whereas the latter organisms adopt mostly positive control mechanisms to regulate gene expression.

In the first volume of the present series, concise reviews have been provided on various eukaryotic transactivators that allow cells to respond to different extracellular stimuli by the expression of new proteins. There are chapters devoted to features of gene expression in heat shock response and influences by an oncogene transcription factor JUN, as well as such other factor as NFkB which respond to a variety of pathogenic conditions and other stress, and PPAR (Peroxisomal Proliferative Activator Receptors) belonging to the group of steroid hormone superfamily which respond to administration of xenobiotics. This volume also contains an article on the molecular details of how the helixloop-helix region of dioxin (a drug) receptor modulates signal transduction, followed by a chapter on transcriptional regulation by heavy metals, exemplified by the metallothionine genes. The volume rightly starts with a chapter on prokaryotic transcriptional control and how it differs from eukaryotes, providing the reader with a better perspective for things that follow the chapter. While the major target for regulation of gene expression is at the level of transcription initiation, it can also be achieved at later stages (post-transcriptionally) by suitable modulators. One such modulator is iron and this aspect of regulation is discussed in the last chapter.

The second volume deals with eight eukaryotic transactivators that allow cells to respond to hormonal stimuli by the expression of new proteins. These are the physiological signals used by the organism to control and coordinate matabolic changes, cell proliferation, differentiation and development. The responses covered include the effect of cyclic AMP, serum growth factors (and various growth promoting stimulii), the glucocorticoid hormone, thyroid hormone and retinoic acid receptors as well as transactivators which modulate gene expression through phosphorylation of cellular receptors and others involved in cell cycle regulation and signal transduction. The regulation of nuclear transport and the activity of the morphogen Dorsal in *Drosophila* form the topic of the final chapter.

All the chapters in the 2 volumes are contributed by experts in the appropriate fields, mostly from the leading European laboratories. The choice of topics has been excellent because the progress in each of these areas has been phenomenal over the past few years and it has become impossible to keep track without the aid of such reviews. The illustrative presentation of data in most chapters make the reading easy. These volumes will certainly prove to be very useful to the graduate students in advanced molecular biology and more so to research workers interested in the area of eukaryotic transcription.

The set of two volumes is a welcome addition to any good library notwithstanding the fairly high cost of approximately Rs.5000.

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OBITUARY

P. N. MEHRA (1907 - 1994)

The cytogenetists are deeply anguished to know that Professor Pran Nath Mehra died of a cardiac arrest on 19th November 1994. He was the Life and Honorary member of the Society of Cytologists and Genetists, India. He played an important role as Professor of Botany together with Prof. G.P. Sharma of the Department of Zoology of Panjab University, Chandigarh in organising the First Congress on Cytology and Genetics from 5th to 9th October 1971 under the auspices of the Panjab University.

Professor Mehra was born on 27th October 1907 at Amritsar. He obtained his M.Sc. and D.Sc. degrees in Botany from the Panjab University. He was the Professor and Head of the Department of Botany of the Panjab University for a number of years. He had the splendid opportunity to shape and progress the department to its excellence. He was known as the eminent Botanist of the country.

He made a yeoman service to the Panjab University in various capacities as Director of UGC centre of Advanced Research, as Head of the Pharmacy Department, as Dean of the Faculties of Science and Agriculture for many years.

He had the unique honour of being elected as Fellow of many academic bodies such as National Academy of Sciences, Allahabad and the Indian National Science Academy, New Delhi. He was also the President of the Section of Botany of Indian Science Congress Session at Roorkee, President of the Indian Botanical Society in 1971, of the Asian Zone of the World Palynological Organisation in 1971, of the P.R. White Committee for promotion of tissue culture (1971), the General President of the Indian Palynological Congress in 1974 at Chandigarh.

He has delivered a number of special and invited lectures and to list them here is really a difficult task. To mention a few are Sir Charles Seward Memorial Lecture (1967), Mendel Memorial Lecture (1968), Maheswari Memorial Lecture (1968), P.R. White Memorial Lecture (1971), Chavan Memorial Lecture (1973), Joshi Memorial Lecture (1974), Street Memorial Lecture (1978), Puri Commemoration Lecture (1985) and a host of others.

Professor Mehra has been the recipient of many awards like Education Minister's Gold Medal (1951), G. Erdtman International Gold Medal (1975), Sunder Lal Hora Medal (1984), Seth Memorial Gold Medal (1984). Most important one is the Padma Shri Award of the Government of India in 1972 in recognition of his exceptional services to the cause of science particularly of Botany in India.

The areas of Botanical research done by him include Cytology in particular, Morphology of Bryophytes and Vascular plants, Taxonomy, Evolution, Tissue culture and Morphogenesis. He must have authored more than 300 research papers and a number of research monographs. His achievements are remarkable and his students are spread all over the world. His demise is a tremendous loss to all the botanists in general and to cytogeneticists in particular. May his soul rest in peace.

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SUGGESTIONS TO CONTRIBUTORS

Research articles encompassing cytology and genetics are accepted for publication in The Journal of Cytology and Genetics. All authors shall be the members of the Society of Cytologists and Geneticists, India before the paper is submitted for publication. The maximum length of the research paper is usually restricted to 5 printed pages (including one page of illustrations) unless the author is willing to pay the excess charges for additional pagination. **Short communications** presenting interesting findings of current interest and **Review articles** on significant topics will be published out of turn at the discretion of the editor in the first available issue. All manuscripts should be addressed to **Prof. B.H.M. Nijalingappa, Editor, The Journal of Cytology and Genetics, Department of Botany, Bangalore University, Jnanabharathi, Bangalore 560 056.**

All papers will be first assessed by a reviewing editor. Papers found unsuitable in terms of overall requirements of the journal will be returned to the authors. Returned papers cannot be resubmitted. The others will be sent for a detailed review. The authors of these papers will be informed of acceptance, rejection, or need for revision on the basis of referee's opinion. It is a basic condition that the papers submitted have not been, and will not be published elsewhere, either simultaneously or at a later date. The papers should be written in a concise form and in clear, grammatically correct language. The style of the manuscript should conform to the format followed in the latest issue of The Journal of Cytology and Genetics.

Preparation of manuscript

Manuscripts should be typed double-spaced with ample margins allround on one side of white bond paper and submitted in duplicate. The pages should be numbered consecutively, starting with the title page and through the text, reference list, tables and figure legends. The title should be brief, specific and contain words useful for indexing. Each paper should be preceded by a short **SUMMARY** which should be a lucid digest of the whole paper, complete in one paragraph with no numbered parts and not exceeding 100 words. This is followed by 3-5 **KEY WORDS** which should be chosen carefully in a form that can be fed into a data bank.

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

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

Citations in the text of works listed in the References should be set out as follows: Nagl & Ehrendorger (1947), more than 2 authors: Evans et al. (1972), indication of page or figure Teppner (1974; 63, Fig.1). Units of measure should be metric and conform to SI notation and should be abbreviated in standard form (e.g., µm, mg, 1, M) and not followed by full points.

Tables and figures should be used sparingly. The appropriate place of their insertion in the text should be indicated in the margin of the manuscript. Tables must be typed double-spaced on separate sheets, numbered and cited in arabic numerals with caption as concise as possible. While choosing illustrations, note that the print area of the journal is 13 x 17 cm. Line drawings should be neatly executed in black ink and with clear, well-spaced lines and when more than one drawing is included, drawings should be mounted close to each other for only page width reproduction. Photomicrographs and other photographs should be original, high contrast, glossy prints in sharp focus and may be grouped to make up a plate. Photocopies of the original plate are not acceptable. In both line drawings and photomicrographs, enough space should be left for the legend at the bottom of the page. Number all figures (photographs, line drawings or graphs) consecutively from one. Both in the text and legends, the figures should be referred to as Fig. 1,2,3 and so on. Superimposed numbers and explanatory should be dark on light ground, or light on dark ground and should be bold enough to withstand reduction during reproduction and must be done with stencils of appropriate size or with Letraset (instant letters). Legends should be concise and typewritten on a separate sheet. Copies of original illustrations may accompany the duplicate manuscript. Photomicrographs or other photographs must have a scale bar which should be defined clearly in the legend. A similar magnification bar should be indicated in the case of line drawings too.

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(a) YADAV J S & GAHLAWAT S 1993 Karyological investigations on four species of coccinellid beetles (Polyphaga: Coleoptera) *J Cytol Genet* **28** 67-70

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