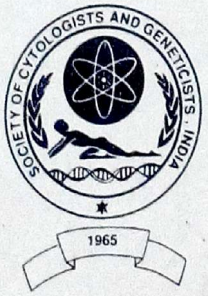
ISSN 0253-7605

# The Journal of Cytology and Genetics

Chief organ of the Society of Cytologists and Geneticists, India

Volume 28, Number 2, July-December 1993

Editor
B.H.M. Nijalingappa

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| Annual Subscription Rates (Effective from 1993) | | |
|---|---|---|
| **Personal** | Rs. 100 (India); | US $30 (Abroad) |
| **Institutional** | Rs. 250 (India); | US $45 (Abroad) |

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Department of Botany, Bangalore University, Bangalore-560 056, India

J. Cytol. Genet. 28: 77-80 (1993)

# GENETIC VARIABILITY IN TASAR SILKWORM (*ANTHERAEA MYLITTA* DRURY)

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(Received 12 October 1992, revised accepted 31 May 1993)

## SUMMARY

Variability for silk yield and 8 contributing characters were studied among 20 genotypes of *Antheraea mylitta*. Sufficient variability was recorded for all characters. High genetic coefficient of variation was found for silk yield, effective rate of rearing and fecundity (28.59, 24.44 and 13.35 respectively). Heritability estimates were high (14.71% - 65.14%) for all characters. Silk yield, fecundity, egg hatching percentage and effective rate of rearing possess high heritability as well as high genetic advance which indicate additive gene effect for these characters. Larval duration, shell weight, cocoon weight and silk ratio showed high heritability with low genetic advance, possibly due to intra- and interallelic interactions.

**Key Words:** *Antheraea mylitta*, variability, heritability, genetic advance.

## INTRODUCTION

Tasar silkworm, *Antheraea mylitta* produces natural silk of commercial importance in many states of India. Since many characters which are of economic value are quantitatively inherited and highly influenced by environmental conditions, it is difficult to judge whether observed variability is heritable or due to environment. It is, therefore, imperative to partition the observed variability into heritable and non-heritable components. The present investigation was, therefore, undertaken to evaluate the variability (genotypic as well as phenotypic) in silk yield and related characters in 20 genotypes for future breeding programme.

## MATERIALS AND METHODS

Twenty genotypes belonging to 11 ecological races of Bihar, Madhya Pradesh, Maharastra and Orissa and 9 varieties evolved at our Institute, constituted the basic material for the study. The experiment was carried out in randomised blocks design with 5 replications each having one disease free laying (DFL). Observations were recorded on 9 quantitative characters, viz., fecundity, egg hatching, larval duration, larval weight, effective rate of rearing, cocoon weight, shell weight, silk ratio and silk yield. The genetic coefficient of variation was computed following the formula of Burton quoted by Johnson et al. (1955). Similarly, heritability, in broad sense, was worked out as suggested by Lush (1949). For estimating the expected genetic advance, the formula of Lush (1949), Johnson et al. (1955) was used. Genetic advance was worked out according to the method given by Allard (1960).

## OBSERVATIONS

Mean values of 9 genetic characters studied in 20 genotypes are presented in Table 1. The analysis of variance indicated significant differences among the genotypes in respect of all characters under study as mean sum of squares of each character are significant at 1% level (Table 2).

TABLE 1: Mean values of 20 genotypes of *Antheraea mylitta*.

| Genotypes | Silk yield (g) | Fecundity (No.) | Hatching (%) | Larval duration (days) | Larval weight (g) | Effective rate of rearing (%) | Cocoon weight (g) | Shell weight (g) | Silk ratio (%) |
|---|---|---|---|---|---|---|---|---|---|
| Ecological races | | | | | | | | | |
| Daba | 260.3 | 289.4 | 63.7 | 35.4 | 39.5 | 51.1 | 14.1 | 2.5 | 18.2 |
| Sukly | 186.9 | 289.8 | 63.8 | 33.8 | 39.0 | 42.6 | 14.3 | 2.3 | 16.5 |
| Laria - P | 117.9 | 311.0 | 72.4 | 32.8 | 36.2 | 25.2 | 12.7 | 2.0 | 15.8 |
| Raily | 124.4 | 256.2 | 72.0 | 34.6 | 35.6 | 27.4 | 13.0 | 2.2 | 17.1 |
| Modal | 98.7 | 277.4 | 80.5 | 31.8 | 34.5 | 19.8 | 12.7 | 2.1 | 17.2 |
| Sukinda T.V. | 224.4 | 250.8 | 64.9 | 32.6 | 38.2 | 57.9 | 14.0 | 2.4 | 17.3 |
| Palma | 133.8 | 284.8 | 61.4 | 36.0 | 36.4 | 39.8 | 13.2 | 2.1 | 16.4 |
| Raily (G) | 195.2 | 254.0 | 63.5 | 32.2 | 38.0 | 52.1 | 14.1 | 2.2 | 15.7 |
| Raily (N) | 160.5 | 282.8 | 59.9 | 38.0 | 40.7 | 42.4 | 13.9 | 2.1 | 15.8 |
| Bhandara | 155.1 | 257.0 | 72.5 | 32.0 | 34.3 | 45.3 | 11.9 | 2.0 | 16.9 |
| Laria (M) | 51.1 | 242.2 | 60.1 | 35.0 | 31.0 | 26.6 | 11.4 | 1.9 | 18.4 |
| Evolved varieties | | | | | | | | | |
| Laria - 8 | 120.6 | 252.0 | 77.6 | 35.4 | 35.7 | 29.4 | 13.0 | 2.1 | 16.4 |
| R - 57 | 110.8 | 268.4 | 72.4 | 35.2 | 36.2 | 30.4 | 12.7 | 2.0 | 15.6 |
| GE - 1 | 80.4 | 289.2 | 74.2 | 35.0 | 35.6 | 21.7 | 12.1 | 1.8 | 14.7 |
| GE - 2 | 114.4 | 256.8 | 75.7 | 34.0 | 35.7 | 27.9 | 12.5 | 2.0 | 15.8 |
| GE - 3 | 181.8 | 275.6 | 78.2 | 35.4 | 35.3 | 48.1 | 13.0 | 2.0 | 15.4 |
| Y.M.G. | 109.0 | 276.2 | 71.2 | 35.8 | 35.4 | 33.2 | 13.0 | 1.8 | 13.9 |
| Nagri - 1 | 94.1 | 260.2 | 74.5 | 34.8 | 34.3 | 25.5 | 13.2 | 1.1 | 16.0 |
| Nagri - 2 | 136.9 | 287.6 | 55.3 | 34.0 | 36.1 | 46.0 | 13.3 | 2.0 | 15.5 |
| Nagri - 3 | 123.7 | 260.2 | 55.1 | 36.0 | 35.8 | 50.2 | 12.2 | 1.9 | 15.6 |

Range, mean with standard error, critical differences at 5% and standard deviation are presented in Table 2. A wide range of phenotypic variation was observed in silk yield, fecundity, hatching percentage and effective rate of rearing whereas cocoon weight (11.45 - 14.38 g), shell weight (1.80 - 2.56 g), silk ratio (13.93 - 18.4%), larval duration 31.8 - 38.0) and larval weight (31.04 - 39.54 g) showed comparatively lower range of phenotypic variation.

The genetic coefficient of variation for different characters (Table 3) ranged from 3.75 for larval duration to 28.59 for absolute silk yield (28.29), effective rate of rearing, (24.44) and fecundity (13.53) while the same was low for others.

Heritability value (Table 3) showed a range of 43.71 for effective rate of rearing to 65.14 for larval weight. Higher heritability was observed for all characters viz., larval weight (65.14), fecundity

TABLE 2: Phenotypic variability in *Antheraea mylitta*.

| Characters | Range | Mean | S.E. | C.D. at % | Treatment mean sum of squares |
|---|---|---|---|---|---|
| Absolute silk yield (g) | 51.1 - 260.3 | 139.0 | 30.2 | 87.4 | 12792.2* |
| Fecundity (No.) | 242.2 - 311.0 | 271.0 | 18.5 | 53.4 | 8344.4* |
| Hatching (%) | 55.1 - 80.5 | 68.2 | 4.8 | 14.1 | 323.5* |
| Larval duration (days) | 31.8 - 38.0 | 34.5 | 0.9 | 2.6 | 12.6* |
| Larval weight (g) | 31.0 - 39.5 | 36.2 | 0.9 | 2.7 | 22.6* |
| Effective rate of rearing (%) | 19.8 - 57.9 | 37.1 | 7.1 | 20.5 | 678.3* |
| Cocoon weight (g) | 11.4 - 14.3 | 13.0 | 0.4 | 1.1 | 3.0* |
| Shell weight (g) | 1.8 - 2.5 | 2.0 | 0.1 | 0.2 | 490.8* |
| Silk ratio (%) | 13.9 - 16.4 | 16.2 | 0.5 | 1.5 | 5.8* |

* Significant at 5%

TABLE 3: Estimates of genotypic coefficient of variation, heritability, expected genetic advance and genetic advance in percentage of mean in *Antheraea mylitta*.

| Characters | Genotypic coefficient of variation | Heritability | Expected genetic advance | Genetic advance percentage of mean |
|---|---|---|---|---|
| Absolute silk yield | 28.5 | 45.0 | 55.1 | 39.5 |
| Fecundity | 13.3 | 64.4 | 0.9 | 22.0 |
| Hatching (%) | 9.2 | 43.9 | 8.6 | 12.5 |
| Larval duration | 3.7 | 49.2 | 1.8 | 5.4 |
| Larval weight | 5.2 | 65.1 | 3.1 | 14.8 |
| Effective rate of rearing | 24.4 | 43.7 | 12.3 | 70.8 |
| Cocoon weight | 5.0 | 57.1 | 1.0 | 7.9 |
| Shell weight | 7.0 | 60.0 | 0.2 | 12.0 |
| Silk ratio (%) | 5.7 | 59.1 | 1.4 | 9.0 |

(64.49), shell weight (60.00), silk ratio (59.18), cocoon weight (54.14), larval duration (49.26), silk yield (45.01), hatching percentage (43.49) and effective rate of rearing (43.71).

The expected genetic advance and genetic advance in percentage of mean showed a wide range from 5.41 for larval duration to 70.82 for effective rate of rearing.

## DISCUSSION

The data indicate that absolute silk yield, effective rate of rearing and fecundity contribute comparatively high genetic coefficient of variation than other characters.

In the present study, all characters were found to have a high heritability. Sen et al. (1976) and Siddiqui et al. (1988b) had reported high heritability for most of the quantitative characters which were found to be useful in tasar silkworm breeding as this could enable the breeder to base his selection on the phenotypic performance. Johnson et al. (1955) while studying $F_4$ and $F_5$ generations of a cross in soybean, have suggested that heritability value estimates with genetic gain is more useful than heritability value alone in predicting the resultant effects for selecting the best individuals. In the present case, high heritability was accompanied by high genetic advance for absolute silk yield, fecundity, effective rate of rearing, egg hatching percentage and larval weight which indicated that these characters are probably controlled by additive gene effects (Panse 1957). On the contrary, shell weight, cocoon weight, silk ratio and larval duration have shown a high heritability and low genetic advance which is probably due to inter- and intraallelic interactions. Further, low heritability indicated the presence of dominant gene action (Kabi & Bhaduri 1981). In the present study, a high heritability was found for shell weight, cocoon weight and effective rate of rearing and low heritability as reported by Siddiqui et al. (1988a). This is indicative of the fact that dominant gene effects are determining these characters. The present study indicated that reciprocal recurrent selection may be helpful to improve these characters.

## ACKNOWLEDGEMENT

We are grateful to Dr. K. Thangavelu, Director, Central Tasar Research and Training Institute, Ranchi for constant guidance and encouragement.

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J. Cytol. Genet. 28: 81-83 (1993)

# MITOTIC EFFECTS OF THE STEM DECOCTION OF *EPHEDRA FOLIATA* ON *ALLIUM CEPA* ROOT TIP CELLS

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(Received 7 November 1992, revised accepted 17 May 1993)

## SUMMARY

Mitotic effects of shoot decoction of the medicinal plant *Ephedra foliata* Varn. 'asmania' on *Allium cepa* root tip cells were studied. The shoot decoction showed a dose dependent mitodepressive effect. It caused disturbance in mitosis and there was accumulation of telophases in the treated roots. The treated roots showed significant increase in cytological aberrations over the control, however, there was no dose dependence.

**Key Words:** *Ephedra foliata*, mitodepression, cytological aberrations, stem decoction.

## INTRODUCTION

In our traditional system of medicine, crude extracts and decoctions of medicinal plants are often used. Many plants are reported to possess toxic substances that may cause heritable changes in the consumers. Studies carried out by various workers like Keck & Hoffman-Ostenhof (1952), Mota (1952), Shehab et al. (1978), Shehab (1979, 1980), Adam & Farah (1989), established the chromosome breaking capabilities of water extracts of many plants. Evaluation of crude extract of Indian medicinal plants for their cytological effects on plant cells is in progress in this laboratory. This paper deals with *Ephedra foliata* Boiss & Kot., a valuable cardiac stimulant. A decoction of the stem and root is reported to be used for rheumatism and syphilis (Kirtikar & Basu 1935).

## MATERIAL AND METHODS

Green shoots of *Ephedra* were washed in tap water and cut into small pieces. Ten grams of these shoot chips were boiled in 100 ml of distilled water for 10 min. The filtrate was made 100 ml by adding more water and stored as stock solution. Five different aqueous dilutions of this extract (decoction) i.e. 0.25%, 0.5%, 1.0%, 1.5% and 2% were used for present investigation. The methodology was followed as described by Saggoo et al. (1991).

## RESULTS AND DISCUSSION

The treatment of *Allium* roots with *Ephedra* stem decoction (ESD) resulted in fluctuations in the frequency of various mitotic phases. There was great disturbance in the prophase/metaphase/anaphase and telephase ratio (Table1). Interestingly, the frequency of the telophases was higher in all the cases except the roots treated with 1% ESD solution. The accumulation of telophase in the roots treated with ESD may be due to prolongation of the duration of this phase. However, the possibility, as suggested by Adam & Farah (1989), of decrease in number of cells entering prophase cannot be ruled out.

The *Ephedra* shoot extract showed a mitodepressive effect on onion roots. The reduction in the mitotic index is clearly dose dependent (Table 1). Water extracts of other plants have also proved to be mitodepressive in *A. cepa* roots such as *Achillea fragrantissima* (Shehab et al. 1978) *Pulicaria crispa*

TABLE 1: Mitotic analysis of root tip cells of *Allium cepa* following treatment with different concentrations of stem decoction of *Ephedra foliata*.

| Conc. of Decoction | Duration of recovery | No. of cells observed | P:M:AT ratio | MI Mean ±SD | Cytological aberrations (%) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Physiological aberration | Clastogenic aberration | Total |
| Control | | 2158 | 1.57:1:2.77 | 19.96 ±1.67 | 2.47 | 0.67 | 3.15 |
| 0.25% | 0 HR | 981 | 0.16:1:2.24 | 19.46±0.64 | 3.66 | 4.71 | 8.38 |
| | 24 HR | 185 | 0 :1:9.0 | 15.14±1.94* | - | 10.71 | 10.71 |
| 0.5% | 0 HR | 1275 | 0.27:1:1.71 | 17.49±1.00** | 10.31 | 12.11 | 22.42 |
| | 24 HR | 1292 | 0.44:1:1.21 | 15.40±1.68* | 13.04 | 10.55 | 23.59 |
| 1.0% | 0 HR | 1321 | 0.25:1:1.08 | 13.25±1.76* | 20.57 | 10.28 | 30.85 |
| | 24 HR | 767 | 0.46:1:2.17 | 11.34±1.47* | 19.53 | 11.50 | 31.03 |
| 1.5% | 0 HR | 980 | 0.52:1:1.59 | 10.41±2.05* | 7.38 | 12.25 | 19.63 |
| | 24 HR | 485 | 0.33:1:7.50 | 10.93±1.32* | 13.21 | 3.77 | 16.98 |
| 2.0% | 0 HR | 582 | 1:0:12.33 | 6.68±2.35* | 13.75 | 13.75 | 27.50 |
| | 24 HR | 707 | 0.05:1:0.59 | 5.09±1.41* | 38.89 | 30.55 | 69.44 |

HR: Hours of recovery; P:M:AT ratio: Prophase: metaphase: anaphase + telophase ratio; MI: Mitotic index.

* Significant to control at 0.01 level of probability (t-test); ** Significant to control at 0.05 level of probability (t-test).

(Shehab 1979), *Teucrium pilosum* (Shehab 1980) and *Ipomoea carnea* (Alam et al. 1987). Such reduction in the mitotic activity may be ascribed to partial blockage of DNA synthesis thus minimizing the number of cells entering mitosis (Schneiderman et al. 1971).

The treatment of onion roots with different concentrations of ESD induced stickiness of chromosomes, failure in the normal functioning of spindle, multipolarity, hyperploidy, lagging of chromosomes, etc. which fall under physiological abnormalities. Such abnormalities ranged between 3.66 to 20.57% in the treated roots. Of these, stickiness of chromosomes is of most common occurrence. These abnormalities can be attributed to abnormal activity of spindle. Numerous physical and chemical agents are reported to induce such type of abnormalities (Kihlman 1955, Sharma & Sharma 1960).

As far as the clastogenic aberrations are concerned, these showed dose dependent increase and their frequency ranged between 4.71 to 13.7%. Among these, chromosome breakage was most common. Formation of rings, bridges at anaphases and telophases and presence of fragments as laggards at telophase are the other clastogenic aberrations induced by ESD treatment in the roots of *Allium cepa*. The production of chromosome breaks by treatment of a plant extract have earlier been reported by Keck & Hoffman-Ostenhof (1952), Mota (1952), Shehab (1980). The production of bridges at anaphases and telophases can be attributed to stickiness of chromosomes or to breakage and reunion of chromatids or subchromatids (Dempong & Maxwell 1973). Similar observations regarding

induction of similar abnormalities by various plant extracts in root tip cells have earlier been reported by Shehab et al. (1978), Alam et al. (1987), Adam & Farah (1989), Saggoo et al. (1991).

The data scored in Table 1 showed that the period of 24 h of recovery of treated roots over water was not sufficient for normalisation of cell division. Rather adverse effects of recovery of roots on quantum of abnormalities are quite apparent.

*Ephedra foliata* contains alkaloids such as ephedrine and pseudoephedrine but the concentration of such alkaloids is low. The cytotoxic nature of ESD observed above may be due to combined effect of these alkaloids, proteins etc. From the above facts it can be recommended that overutilization of crude extracts of *E. foliata* is not safe.

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J. Cytol. Genet. 28: 85-91 (1993)

# SENSITIVITY OF MALE GERM CELLS TO MUTAGENS FOR INDUCTION OF DOMINANT LETHALS IN *BOMBYX MORI* L.

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(Received 3 November 1992, accepted 29 June 1993)

## SUMMARY

Sensitivity of different male germ cells to 3 mutagens (MC, EMS and X-ray) for induction of dominant lethals was found to be different. The scale of sensitivity to MC was in order : Spermatozoa > Spermatids > Spermatocytes, to EMS in the order: Spermatids > Spermatozoa > Spermatocytes and to X-rays it was: Spermatocytes > Spermatids > Spermatozoa. This differential sensitivity of germ cells to mutagens was perhaps due to different mode of action of each of the mutagens.

**Key Words:** *Bombyx mori*, mutagens, male germ cells.

## INTRODUCTION

As in all higher organisms, in *Bombyx mori* also, complex stages of growth, development and differentiation during embryogenesis are under active control of the genetic endowment of the organism. Each and every stage of these above mentioned phenomena require precise regulation of specific gene or genes. Alterations, if any, in the structural, organisational and functional plan of these genes (mutation), are quite likely to affect the phenocritic periods of development in such a way that organisms die early during development. These losses in fertility are collectively deemed as affect of dominant lethal mutations. Origin of such mutations can be quantified by a simple method - subject the male to mutagenic treatment, allow a female to pair with it, and then count the number of larvae hatched/pupae formed from amongst the eggs laid by such females.

Though slightly poorer in objectivity, such assay of dominant lethal mutation is very simple and is widely used to test the mutagenicity of unknown chemicals or to prepare comparative chart of the mutagenic efficiency of mutagens. Relative sensitivity of various types/stages of germ cells to a certain mutagen can also be studied with this method. The method can be further extended to know the extent of interpopulational or interracial differences in sensitivity to a certain mutagen. This paper incorporates the data precisely on these 2 aspects relative sensitivity of various stages of male germ cells to 3 mutagens and estimation of interpopulational differences.

## MATERIALS AND METHODS

Three multivoltine races of mulberry silkworm, *Bombyx mori* viz. Nistari, G and Pure Mysore were used for the present study. One-day-old fifth instar male larvae of nearly equal weight, as well as 2- and 7-day-old male pupae, all maintained at

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24-28°C and 70-80% relative humidity, were subjected to mutagen treatment for the present study. The germ cells of 1-day old fifth instar larvae correspond to primary and secondary spermatocytes, while late pupal stage (seventh day) does so mostly to spermatozoa. Chronologically, these larvae and pupae contain within them the cells belonging to all the stages of spermatogenic cycle right from the cells to spermatozoa.

Mitomycin C (MC) of SIGMA Chemical Company, St. Louis, USA and ethyl methanesulfonate (EMS) from Kochlight Laboratory Limited, England were used for mutagen tests. Three concentrations of MC and EMS viz., 0.05, 0.1 and 0.15% were prepared in 0.85% saline solution (Murakami 1972).

The different concentrations of MC and EMS were injected in the male silkworm larvae (V-1) through last abdominal leg using microsyringe (Hamilton make). The male pupae were injected through their wing bud (Datta et al. 1975). A dose of 0.04 ml of solution was injected in each individual under all treatments. For control batches, 0.85% saline solution in equal volume (0.04 ml) was injected. For X-ray irradiation, Muller MG-50 X-ray apparatus of Saha Institute of Nuclear Physics, Calcutta (India) was utilised to irradiate the larvae and pupae with 500, 1,000, 2,000, 3,000 and 5,000 R. The factors maintained to get the required doses were; 8Kv, 9mA, distance 42.5 cm from target without filter, at 500 R/min.

The silkworm larvae of treated and control variants were reared separately on mulberry leaves. The pupae were maintained at 25 ± 1°C. The male moths that emerged out of the treated lots, were crossed with untreated females. The fertilized females were allowed to lay the eggs for 24 h on laying sheets. After incubation and brushing of hatched worms the laying sheets were removed for scoring fecundity, sterile egg, embryonic death and hatching percentage. The relative dominant lethal mutation rate was determined from the following relationship (Datta et al. 1978 a, b): Relative dominant lethality (%) is expressed as:

$$\left(1 - \frac{\%\text{ of larvae hatched amongst the eggs laid by females mated to treated males}}{\%\text{ of larve hatched amongst the eggs laid by females mated to untreated males (control variant)}}\right) \times 100$$

## OBSERVATIONS

The results, as a whole, indicated that dominant lethal mutations (DLM) were induced in cells of 3 stages of germ cells namely, spermatocytes, spermatids and spermatozoa. However, the sensitivity of different stages of germ cell to different mutagens were different (Table 1). The induction of dominant lethals was dependent on the concentration/doses of mutagens as the RDL% increased with the increase in the concentration/doses.

The late pupal stage (seventh day), which corresponds to spermatozoa stage, was found to be most sensitive to MC. Stagewise degree of sensitivity for MC can be represented as : Spermatozoa > Spermatids > Spermatocytes.

Besides this, a sharp increase in the number of unhatched blue eggs with the treatment by MC in all 3 stages as compared to sterile eggs were recorded which indicates that MC produces more embryonic lethals than zygotic one.

The second day of pupal stage which corresponds mostly to the spermatids and spermatozoa, was found to be most sensitive to EMS. Stagewise degree of sensitivity for EMS can be represented as: spermatids > spermatozoa > spermatocytes. Similar to that of MC, the EMS was also found to induce more embryonic lethals than that of zygotic one.

TABLE 1: Induction of dominant lethals by different mutagens mitomycin C (MC), ethyl methanesulfonate (EMS) and X-rays in the male cells of Bombyx mori races (Mysore, CI and Pure Mysore)

| Mutagens | Conc. (%) / Dose (Gy) | Relative dominant lethals (%) in | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mysore | | | CI | | | Pure Mysore | | |
| | | V-1 | PE-2 | PL-7 | V-1 | PE-2 | PL-7 | V-1 | PE-2 | PL-7 |
| MC | 0.05 | 3.88 | 8.63 | 89.45 | 2.46 | 8.61 | 85.66 | 1.17 | 2.28 | 39.66 |
| | 0.10 | 5.60 | 15.31 | 76.55 | 6.44 | 13.82 | 82.14 | 4.58 | 10.59 | 68.72 |
| | 0.15 | 16.02 | 20.22 | 68.58 | 12.50 | 18.69 | 86.30 | 9.24 | 15.77 | 83.50 |
| EMS | 0.05 | 3.53 | 42.60 | 20.63 | 3.75 | 34.16 | 18.39 | 1.83 | 32.80 | 16.40 |
| | 0.10 | 9.89 | 69.67 | 28.13 | 8.72 | 62.48 | 24.32 | 6.94 | 59.90 | 21.71 |
| | 0.15 | 18.69 | 95.86 | 40.03 | 15.49 | 88.42 | 32.42 | 13.28 | 82.97 | 30.46 |
| X-ray | 500 | 68.13 | 50.83 | 16.73 | 63.58 | 47.34 | 9.75 | 60.42 | 46.20 | 9.12 |
| | 1000 | 88.82 | 71.93 | 30.41 | 87.27 | 60.43 | 17.44 | 82.53 | 58.21 | 12.78 |
| | 2000 | 96.58 | 82.58 | 43.41 | 95.90 | 73.66 | 35.12 | 93.49 | 70.44 | 21.47 |
| | 3000 | 99.24 | 89.22 | 47.72 | 98.34 | 80.12 | 52.44 | 97.26 | 79.70 | 38.83 |
| | 5000 | 100.00 | 94.18 | 72.47 | 100.00 | 91.05 | 67.73 | 100.00 | 88.09 | 65.28 |

V-1: 1-day-old fifth instar larvae (corresponds to primary and secondary spermatocytes stage of germ cells in the testis)
PE-2: 2-day-old pupa (corresponds to mostly spermatid stage of germ cells in the testis)
PL-7: 7-day-old pupa (corresponds to mostly spermatozoa stage in the testis)

Among spermatocytes, X-ray showed highest degree of lethality while late pupal stage which corresponds to spermatozoa, was observed to be least sensitive. Most interesting observation recorded in X-ray treated batches was a sharp increase in the number of sterile eggs with the increase in the doses of X-ray in all 3 stages as compared to blue eggs thereby indicating that X-ray produced more zygotic lethals than the embryonic ones. However, a parallel increase in both the lethal types was recorded with an increase in the doses of X-ray.

In all 3 stages, difference in respect of percentage of eggs hatched under 3 different doses in case of MC and EMS and 5 doses in case of X-ray is significant ($P < 0.05$). The relationship of dose rate with hatching percentage in 3 stages for all the 3 mutagens is significant and negative in all the races. The higher correlation coefficient values ($r$) confirm the validity of the equations (Table 2).

## DISCUSSION

It is evident from the present investigation that MC acts directly on genetic material at meiotic and post-meiotic stages and induces dominant lethals at spermatocytes, spermatids and spermatozoa stages among which the spermatozoa are highly sensitive and the spermatocytes are least so. This result is in conformity with the findings of Tazima and his coworkers (Tazima 1968).

In silkworm (Tazima 1980, Murakami 1982, 1983) and in mammals (Ehling 1971) where the spermatozoa are followed by spermatids in their sensitivity to MC, Tazima & Oninaru (1966)

TABLE 2: Regression models for predicting hatching percentage (Y) from different dose rate (X) of mitomycin C (MC), ethyl methanesulfonate (EMS) and X-ray.

| Muta-gen | Race | V-1 | | PE-2 | | PL-7 | |
|---|---|---|---|---|---|---|---|
| | | Regression equation | Correla-tion coefficient (r) | Regression equation | Correlation Coefficient (r) | Regresssion equation | Correla-tion coeffi-cient (r) |
| MC | Nistari | Y=93.11-97.38X | -0.989* | Y=94.68-128.74X | -0.995** | Y=89.24-624.38X | -0.979* |
| | G | Y=92.18-76.01X | -0.974* | Y=94.07-122.18X | -0.989* | Y=88.07-523.68X | -0.979* |
| | Pure Mysore | Y=96.14-61.14X | -0.974* | Y=93.63-102.92X | -0.979* | Y=88.64-527.36X | -0.979* |
| EMS | Nistari | Y=92.34-107.50X | -0.989* | Y=90.46-598.24X | -0.989* | Y=91.77-236.88X | -0.975* |
| | G-race | Y=96.01-98.06X | -0.989* | Y=91.69-550.62X | -1.00** | Y=89.54-191.32X | -0.969* |
| | Pure Mysore | Y=92.92-81.38X | -0.974* | Y=93.80-530.92X | -0.994** | Y=94.05-279.38X | -0.974* |
| X-ray | Nistari | Y=48.09-0.0132X | -0.983* | Y=57.566-0.0134X | -0.832* | Y=85.229-0.013X | -0.966** |
| | G-race | Y=50.99-0.014X | -0.989* | Y=63.949-0.0135X | -0.831* | Y=88.041-0.0127X | -0.984* |
| | Pure Mysore | Y=52.55-0.014X | -0.974* | Y=66.896-0.0136X | -0.832* | Y=93.734-0.012X | -0.995** |

* Significant at 5% level; ** Significant at 1% level

suggested that it is perhaps due to the possibility that MC can act on one of the double helices of spermatozoa DNA where DNA synthesis is no more in progress.

It is obvious from the results of the second set of experiment that the EMS acts directly on the genetic material at meiotic and post-meiotic stages and induced dominant lethals at spermatocytes, spermatids and spermatozoa stages. The results lend support to earlier findings of Tazima and his collaborators who showed, from the mutagenic test of EMS with egg mutants *pe* and *re*, that post-meiotic germ cells (spermatids in early pupa) are highly sensitive to EMS (Tazima, 1968, 1974). Of interest is the observation that cells of meiotic stage were less sensitive to EMS than those of post-meoitic stage. The cytogenetical studies performed to test the effect of EMS on meiotic metaphase I confirmed the occurrence of various chromosomal aberrations after treatment by EMS solution (Datta et al. 1976). Tazima & Onimaru (1968) postulated that the chemical mutagen causes breakage in one of the 2 helices of DNA. This may be true in this experiment also, and repairing of 1 strand might have been completed at a later stage (Datta et al. 1978a, b). In silkworm (Tazima 1968, 1974, Tazima & Onimaru 1968, Tazima, 1978), as also in *Drosophila* and mammals, the late spermatids are reported to be most sensitive to the chemical mutagens (Fahmy & Fahmy 1957, Ehling 1971, Generoso et al. 1974).

The X-rays induce dominant lethals at all the 3 stages of germ cells and among them the spermatocytes are most sensitive while the spermatozoa are least so. This observation finds support from the earlier findings of Sado (1961) and Tazima (1961).

Tazima (1961) reported that "...extreme sterility was observed when germ cells were irradiated at late meiotic prophase". Genetic sterility induced by irradiation has been reported in *Luffa acutangula* by Kalyar & Roy (1975). By cytological investigation of microsporogenesis in irradiated *Trillium erectum*, Sparrow (1951) showed that the frequency of chromosome fragmentation reached its peak at late meiotic prophase, and declined gradually thereafter. Tazima (1958, 1959, 1961) observed that extremely sensitive stage to the sterilizing effect of X-rays occur early in the fifth instar of male. Though, secondary spermatogonia in silkworms are also easily destroyed by moderate dose of X-rays, histological examination of irradiated tests of the silkworm was conducted by Sado (1961) and the results recorded by him were in good agreement with those reported from mice (Oakberg 1955), grasshopper (Cocchi & Uggeri 1944) and *Drosophila* (Friesen 1937, Welshons & Russel 1957) showing an extreme sensitivity of late spermatogonia to the killing effect of X-ray. Furthermore, it was shown that in silkworm, spermatocytes are more sensitive to irradiation in late meiotic prophase, than in early meiotic prophase. This finding is consistent with the observation of Sparrow (1951) in *Trillium*.

Most remarkable observation in the silkworm pertain to the relation between induced sterility and the irradiated stages of spermatogenic cells. A transitory sterile period due to the destruction of late spermatogonia has been observed both in mice (Russel 1954) and *Drosophila* (Friesen 1937, Auerbach 1954, Alexander & Stone 1955, Ives 1960) by the successive brood technique. However, this technique is hardly applicable to the silkworm, where sperms from different irradiated cell types get mixed up together during their storage in the pupal testis. In addition, the adult moths are so short-lived that successive brood technique cannot be applied in this insect. On the contrary, in mice and adult *Drosophila*, sperms are injected successively without being stored for long time and therefore, a temporary sterile periods appear at a definite time after irradiation when non-irradiated spermatogonia are expected to mature. After this period, fertility recovers, because by this time sperms are produced from regenerated spermatogonia.

Contrary to the findings in mice and adult *Drosophila*, sterility due to the destruction of spermatogonia is hardly observed in silkworm even though these cells are easily killed by radiation. But the newly regenerated spermatogonia have enough time to develop into functional sperms.

Ionizing radiations induce mutations in germ cells belonging to all the stages of the gemetogenic cycle, thought with markedly different rates. On the contrary, chemical mutagens induce mutations only in certain spermatogenic stages. The stage-specific induction of mutation by chemical agents is very likely due to their different pathways and therefore, to their different effects on the structure and various macromolecular processes during the development of the germ cells (Ehling 1974). Accordingly, chemical mutagens are characterised by their stage-specific induction of mutation (Ehling 1971).

From the foregoing account it may be concluded that for induction of dominant lethal mutations in male silkworm, spermatozoa are the most sensitive to MC and spermatocytes the least; spermatids are most sensitive to EMS and spermatocytes the least; and in case of X-rays, spermatocytes are most sensitive while spermatozoa the least.

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J. Cytol. Genet. 28: 93-98 (1993)

# INDUCED CHLOROPHYLL MUTANTS IN RICE

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(Received 29 March 1993, revised accepted 25 June 1993)

## SUMMARY

Seeds of scented rice cultivars, Khao Dawk Mali 105 and Milagrosa were irradiated with 20, 30, 40 and 50 kR gamma rays. For recurrent treatments $M_1$ seeds of the 4 irradiated doses of each variety were reirradiated at 20, 30, 40 and 50 kR gamma ray doses. In both the cultivars, in single treatment, chlorophyll mutants were observed in all the doses and were more in Khao Dawk Mali 105 than Milagrosa. The spectrum induced include albina, viridis, xantha and other types. The frequency of chlorophyll mutants was less in recurrent treatments and the other types were conspicuously absent. Efficiency as well as effectiveness were more in single treatments when compared to recurrent ones.

**Key Words:** Chlorophyll mutants, mutagenic effectiveness, efficiency.

## INTRODUCTION

In rice, Ramaiah & Parthasarathy (1938) obtained mutants for chlorophyll deficiency, leaf, panicle and spikelet characters. Cream, albino and variegated mutants were reported in 10% of $R_1$ plants by Chang & Hsieh (1957) and Hsieh (1959) suggesting them to be plastid mutations. Shastry & Ramiah (1961) reported a steady increase of chlorophyll mutants with increase in activity of $^{35}S$ when compared to X-rays and thermal neutrons. Nilan (1957) opined that the spectrum of chlorophyll mutants induced is specific to the type of mutagen used in rice and barley. Basu & Basu (1969) reported a predominance of albino type over others by subjecting the rice cultivars with $^{32}P$, $^{35}S$ and X-rays. Bhan & Kaul (1976) treated 3 varieties of rice with gamma rays, EMS and dES alone and in combinations and found that chlorophyll mutation frequency was enhanced with increasing dose but dropped at very high doses. Rao (1977) studying the effect of gamma rays and EMS on seed fertility in relation to chlorophyll mutation frequency proposed that the frequency of mutation was completely independent of the degree of fertility. Nallathambi & Raja (1982) reported a higher frequency of chlorophyll mutants in azide treated population followed by combination and gamma ray treatments. Reddi & Rao (1988) studied the relative effectiveness and efficiency of single and combination treatments using gamma rays and azide in inducing chlorophyll mutations in rice. Gupta & Sharma (1990) studied the effectiveness and efficiency of EMS and gamma rays in rice and found the latter to be more efficient on the basis of average mutagenic efficiency.

Earlier studies on chlorophyll mutants in rice were confined mostly to those obtained either from single or combination treatments involving gamma rays. Therefore, the present study was undertaken to demonstrate the radiosensitivity of scented rice cultivars to single and recurrent treatments of gamma rays in inducing chlorophyll mutations.

## MATERIALS AND METHODS

For single treatments, dry, well- filled seeds of scented rice cultivars *Khao* Dawk Mali 105 and Milagrosa with 14% moisture content (obtained from Dr. T.T. Chang, IRRI, Manila, Philippines) were subjected to 20, 30, 40 and 50 kR gamma rays at a dose rate of 80 rads per second. For recurrent treatments, $M_1$ seeds of the 4 irradiated doses of each variety were reirradiated at 20, 30, 40 and 50 kR gamma ray doses. Comparable controls were maintained.

The frequency and spectrum of chlorophyll mutants were scored 10 days after germination. The mutagenic efficiency and effectiveness were estimated following the method of Konzak et al. (1965).

## RESULTS AND DISCUSSION

In a single treatment, in the cultivars Khao Dawk Mali 105 and Milagrosa mutants segregating for chlorophyll mutants were observed in all the doses of gamma rays and were more in the former both on $M_1$ panicle and $M_2$ seedling basis. The highest frequency of 24.2 and 4.35% chlorophyll mutants was observed in 50 and 40 kR doses in Khao Dawk Mali 105 and Milagrosa respectively (Table 1). The spectrum of chlorophyll mutants induced include albina, viridis, xantha and other categories like striata and viridoalba. In both the varieties viridis type was at its maximum followed by xantha, albino and other categories in Khao Dawk Mali 105 and albino, xantha and other types in Milagrosa. Effectiveness and efficiency was more in Khao Dawk Mali 105 when compared to the other variety Milagrosa.

TABLE 1: Chlorophyll mutants induced in rice by gamma rays in single treatment in $M_2$.

| Variety/ Gamma rays (kR) | Segregated seedlings % | Albina % | Viridis % | Xantha % | Other types % | Effectiveness M/Con. | Efficiency | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | M/L | M/S |
| Khao Dawk Mali 105 | | | | | | | | |
| Con | - | - | - | - | - | - | - | - |
| 20 | 23.88 | 1.44 | 90.72 | 7.91 | - | 1.19 | 6.21 | 4.11 |
| 30 | 23.95 | 1.79 | 75.81 | 4.24 | 0.09 | 6.80 | 1.22 | 2.49 |
| 40 | 9.66 | 1.79 | 91.84 | 4.11 | - | 0.24 | 0.53 | 1.73 |
| 50 | 24.16 | - | 94.42 | 4.53 | 0.17 | 0.48 | 1.34 | 1.86 |
| Milagrosa | | | | | | | | |
| Con | - | - | - | - | - | - | - | - |
| 20 | 3.55 | 2.15 | 77.60 | 3.62 | 0.91 | 0.18 | 0.22 | 0.30 |
| 30 | 1.95 | 33.74 | 51.80 | - | 4.82 | 0.07 | 0.13 | 0.09 |
| 40 | 4.33 | 2.16 | 93.59 | 2.14 | 0.86 | 0.11 | 0.14 | 0.16 |
| 50 | 3.79 | 2.82 | 85.90 | 8.50 | 2.82 | 0.80 | 0.11 | 0.10 |

In recurrent treatment population, in $M_2$, panicles segregating for chlorophyll mutants were observed in all the doses in both the cultivars just as in single treatment. When compared to a single treatment the frequency of chlorophyll mutants in both the varieties on $M_1$ panicle and $M_2$ seedlings

TABLE 2: Chlorophyll mutants induced in var. Khao Dawk Mali 105 induced by gamma rays in recurrent treatment in $M_2$

| Gamma rays (kR) | Segregated seedlings % | Albina % | Viridis % | Xantha % | Effectiveness M/Con. | Efficiency M/I. | Efficiency M/S |
|---|---|---|---|---|---|---|---|
| Con | - | - | - | - | - | - | - |
| 20 kR | | | | | | | |
| Con | 1.36 | - | 57.10 | 42.90 | 0.06 | 0.12 | 0.30 |
| 20/20 | 2.50 | 7.69 | 76.92 | 15.39 | 0.12 | 0.99 | 0.14 |
| 20/30 | 2.14 | 11.11 | 55.56 | 33.33 | 0.07 | 0.08 | 0.05 |
| 20/40 | 2.68 | - | 62.50 | 37.50 | 0.06 | 0.22 | 0.06 |
| 20/50 | 7.09 | 21.74 | 43.48 | 34.79 | 0.14 | 0.59 | 0.21 |
| 30 kR | | | | | | | |
| Con | 2.22 | - | 100.00 | - | 0.07 | 0.11 | 0.50 |
| 30/20 | 6.86 | 15.63 | 50.00 | 34.40 | 0.34 | 1.07 | 0.43 |
| 30/30 | 3.11 | - | 70.60 | 29.41 | 0.10 | 0.23 | 0.21 |
| 30/40 | 11.26 | - | 49.47 | 50.53 | 0.28 | 3.89 | 0.41 |
| 30/50 | 8.45 | - | 70.60 | 29.41 | 0.17 | 0.39 | 0.26 |
| 40 kR | | | | | | | |
| Con | 1.55 | - | 100.00 | - | 0.03 | * | 0.12 |
| 40/20 | 0.73 | 11.11 | 66.67 | 22.21 | 0.03 | * | 0.06 |
| 40/30 | 5.47 | - | 75.00 | 25.00 | 0.18 | * | 0.12 |
| 40/40 | 4.38 | - | 100.00 | - | 0.11 | 0.27 | 0.09 |
| 40/50 | 5.02 | 8.30 | 50.00 | 41.67 | 0.10 | 0.34 | 0.11 |
| 50 kR | | | | | | | |
| Con | 2.10 | - | 73.33 | 26.67 | - | 0.31 | 0.28 |
| 50/20 | 2.31 | 16.67 | 83.33 | - | 0.11 | 0.28 | 0.15 |
| 50/30 | 0.85 | - | 100.00 | - | 0.02 | 0.13 | 0.03 |
| 50/40 | 3.60 | 35.29 | 35.29 | 29.40 | 0.05 | 0.25 | 0.07 |
| 50/50 | 3.07 | 9.09 | 72.73 | 18.18 | 0.06 | 0.23 | 0.08 |

* Improved upon treatment

was less in the recurrent treatments. The spectrum induced include albina, viridis and xantha and other types were conspicuously absent. Just as in the single treatment viridis type was at its maximum in both the varieties followed by xantha and albino in Khao Dawk Mali 105 and albino and xantha in Milagrosa. Mutagenic efficiency as well as effectiveness was decreased in the recurrent treatments in the cultivars Khao Dawk Mali 105 (Table 2) whereas in Milagrosa they increased slightly in the recurrent treatment populations (Table 3).

TABLE 2. b : Chlorophyll mutants induced in var. Mahsuri induced by gamma rays in successive treatment in $M_2$

| Gamma rays (kR) | Segregated seedlings % | Albina % | Viridis % | Xantha % | Effectiveness M/Gm | Efficiency | |
|---|---|---|---|---|---|---|---|
| | | | | | | M/L | M/S |
| Con | - | - | - | - | - | - | - |
| 20 kR | | | | | | | |
| Con | 0.12 | - | - | 100.00 | - | - | - |
| 20/20 | 0.41 | 100.00 | - | - | 0.02 | 0.02 | 0.05 |
| 20/30 | 2.68 | - | 100.00 | - | 0.08 | 0.13 | 0.04 |
| 20/40 | 2.42 | 16.67 | 83.30 | - | 0.06 | 0.14 | 0.05 |
| 20/50 | 2.17 | - | 83.30 | 16.67 | 0.04 | 0.12 | 0.06 |
| 30 kR | | | | | | - | |
| Con | 1.07 | - | 80.00 | 20.00 | 0.03 | 0.05 | 0.03 |
| 30/20 | 1.86 | - | 100.00 | - | 0.09 | 0.08 | 0.04 |
| 30/30 | 2.94 | - | 70.00 | 30.00 | 0.09 | 0.15 | 0.10 |
| 30/40 | 4.81 | - | 95.83 | 4.17 | 0.12 | 0.57 | 0.07 |
| 30/50 | 7.10 | 3.23 | 83.91 | 12.90 | 0.14 | 0.31 | 0.19 |
| 40 kR | | | | | | | |
| Con | 3.92 | 6.67 | 93.33 | - | 0.09 | 0.45 | 0.43 |
| 40/20 | 5.36 | - | 61.54 | 38.46 | 0.26 | 0.34 | 0.23 |
| 40/30 | 1.02 | - | 100.00 | - | 0.03 | 0.04 | 0.19 |
| 40/40 | 2.70 | - | 100.00 | - | 0.06 | 0.11 | 0.03 |
| 40/50 | 2.37 | - | 100.00 | - | 0.04 | 0.35 | 0.03 |
| 50 kR | | | | | | | |
| Con | 3.40 | 4.76 | 66.70 | 28.60 | 0.06 | 0.08 | 0.17 |
| 50/20 | 3.61 | 10.00 | 85.00 | 5.00 | 0.18 | 0.46 | 0.12 |
| 50/30 | 2.30 | - | 100.00 | - | 0.06 | 0.45 | 0.03 |
| 50/40 | 2.90 | - | 100.00 | - | 0.05 | 0.47 | 0.47 |
| 50/50 | 2.70 | 28.57 | 71.43 | - | 0.05 | 1.26 | 0.04 |

In mutagenic studies, the most common spontaneous or induced alterations arising in highe plants are the chlorophyll mutations which are mainly gene mutations influencing the gree colouration of photosynthetically active parts. The chlorophyll mutation frequency is an indicator t predict the frequency of factor mutations and is thus an index for evaluating genetic effects o mutagens (Gustafsson 1951, D'Amato et al. 1962, Walles 1973). There was a dose dependen relationship in the chlorophyll mutation frequency in Khao Dawk Mali 105. Swaminathan (1965) an Goud (1967) indicated that genes effecting chlorophyll mutations occurred near the centric region o the chromosome where recombination occurs very rarely. Thus these genes would have greate selective advantage. This is true in case of the present study also.

In the present study, viridis type occurred at a maximum frequency in both the varieties in both the types of treatments. Similar results were reported earlier by Afsar Awan et al. (1980) in azide treated cultivar M5, Reddi & Reddi (1984) in MMS, dMS, dEMS and dES treated cvs. T(N)1, IR 8 and Soma and Reddi & Rao (1988) in gamma rays and azide treated cv. Jaya.

The estimates of mutagenic effectiveness and efficiency indicate the rate of induced mutations in relation to dose/concentration of the mutagens and the undesirable caused in biological systems respectively (Konzak et al. 1965). Chlorophyll mutations are used to assess the effectiveness and efficiency of a mutagen because of their easy detectability and frequent appearance following mutagenic treatments. Vast differences can be noted amongst the values of mutagenic effectiveness and efficiency in the present data in both single and recurrent treatments both in terms of chlorophyll mutants spectrum and mutation frequencies. This may be attributed to differences in the genetic make-up. Sharma & Chatterjee (1962) and Varughese & Swaminathan (1968) are of the opinion that the difference is due to the amount of DNA and its replication time in the initial stages. It might be due to the physiological stage of the cells, ability to repair the damages or several other physical factors (Gelin 1968).

The usefulness of any mutagen depends not only on its effectiveness but also to a large extent upon its efficiency. Effective mutagenesis is brought about by the production of useful mutations with minimum undesirable changes.

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J. Cytol. Genet. 28: 99-103 (1993)

# KARYOMORPHOLOGY OF SOME SPECIES OF *PENNISETUM*

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(Received 2 March 1993, revised accepted 25 June 1993)

## SUMMARY

Karyotype studies on 4 species of *Pennisetum* viz., *P. typhoides*, *P. violaceum*, *P. mollissimum* and *P. schweinfurthii* were carried out. In all the species, somatic chromosome number was 2n = 14. These species differed appreciably among themselves in the total chromatin length, F% and TF% value of their chromosomes. In all the species, the chromosomes were with median and submedian primary constrictions. The differences in the somatic chromosome attributes help in understanding the evolution of this genus.

**Key Words:** *Pennisetum*, karyomorphology, cytotaxonomy.

## INTRODUCTION

Genus *Pennisetum* is a heterogeneous assemblage of species belonging to family Poaceae. Jauhar (1981) suggested this genus to be a suitable material for cytogenetical studies because of smaller number and larger size of chromosomes with one distinct pair having nucleolar organizers. He also reported this genus with a wide range of chromosome numbers of 2n = 10 - 72 being multiples of 7, 8 and 9 with diverse chromosome morphology and considerable size differences. The earlier cytogenetical work was mainly concerned with taxonomic and phylogenetic relationships in this genus. The karyotypic studies may help in understanding systematic relationships among different species and also in transferring specific chromosome or chromosome segment from related species. The present study deals with the karyomorphology of *Pennisetum typhoides* (Burm.) Stapf et Hubb., *P. violaceum* (Lam), L.Rich., *P. mollissimum* Hoechst. and *P. schweinfurthii* Pilger.

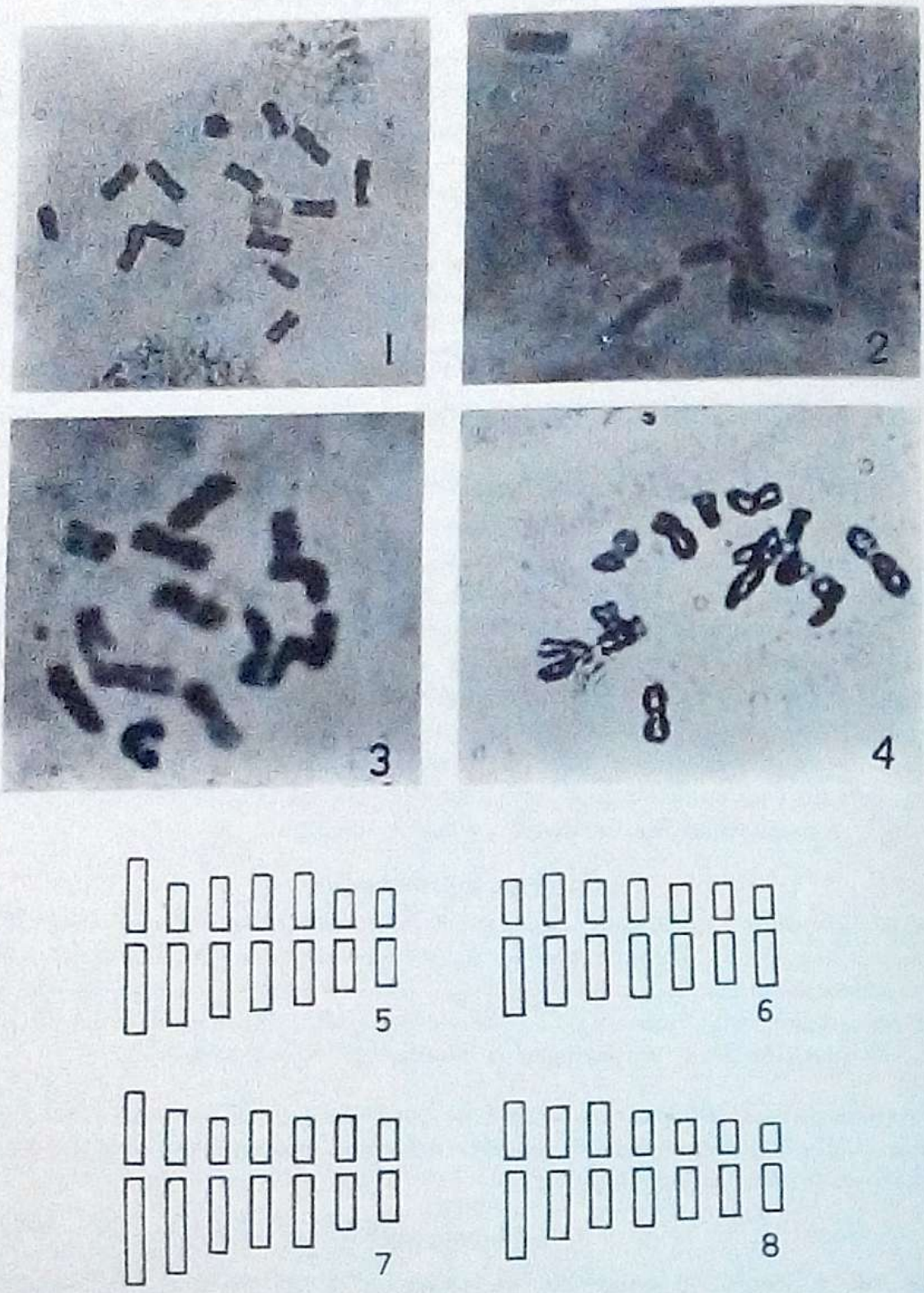
## MATERIAL AND METHODS

The seeds of 4 species (*P. typhoides*, *P. violaceum*, *P. mollissimum* and *P. schweinfurthii*) acquired from ICRISAT were germinated at a constant temperature of 25 ± 1°C. The roots of 0.5 to 1.0 cm in length were pretreated in saturated solution of α - bromonaphthalene for 30 min and fixed in acetic-alcohol (1:3) for 24 h. They were then hydrolysed in IN HCl at 60°C for 10-12 min and root tips were squashed in 1% acetocarmine. Different parameters used for karyotype analysis are arm ratio, centromeric index (F%) and total forma percentage (TF%) as suggested by Huziwara (1962) and Sinha & Roy (1979).

Chromosomes were identified as median and submedian, based upon arm ratio of 0.76-1.0 and 0.51-0.75 respectively. The karyotype formulae (KF) were based upon the length of the chromosomes and categorised as : A (3.5 -4.5 μm); B (4.5-5.5 μm); C (5.5-6.5 μm); D (6.5-7.5 μm) and E (7.5-8.5 μm).

## OBSERVATIONS

All the 4 species of *Pennisetum* (*P. typhoides*, *P. violaceum*, *P. mollissimum* and *P. schweinfurthii*) studied here had the same chromosome number of 2n=14. Although these species closely resemble each other in their gross morphology, they can easily be distinguished on the basis of total chromosome length, chromosome size, relative chromosome length (TCL%), F% and TF%.




**Figs. 1-8:** Somatic chromosomes and idiograms of *Pennisetum* species at mitotic metaphase (2n=14). 1.5, *P. typhoides*. 2.6, *P. violaceum*. 3.7, *P. mollissimum*. 4.8, *P. schweinfurthii*. 5-8 Idiograms of *Pennisetum* species. 5. *P. typhoides*. 6. *P. violaceum*. 7. *P. mollissimum*. 8. *P. schweinfurthii*.

Karyomorphologically, *P. typhoides* consisted of 3 median and 4 submedian chromosomes (Figs. 1 &5). The haploid chromatin length in this species was 41.17 µm with individual chromosome length ranging from 4.26-8.22 µm. Average chromosome length was 5.58 µm. Arm ratio and arm index varied from 0.59-0.85 and 1.17-1.68 respectively. F% and TCL% were 37.29-45.30 and 10.34-19.72. TF% was 42.5. Karyotype formula (KF) : 1A(M) + 1A(SM) + 1B(SM) +1C(M) +1C(SM)+1D(SM)+1E(M).

In *P. violaceum*, total haploid chromosome length was 36.02 µm. The range and mean chromosome length were 4.37-6.06 µm and 5.15 µm respectively. Arm ratio was 0.55-0.75 and arm index varied from 1.32-1.82. F% and TCL% ranged from 36.53-44.27 and 12.04-16.80 respectively. TF% was 39.67. This species had 1 median and 6 submedian chromosomes (Figs. 2,6). KF : 2A(SM)+3B(SM) +1C(M) + 1C(SM).

*P. mollissimum* is characterised with 2 median and 5 submedian chromosomes (Figs. 3,7). Its haploid chromosome length and average chromosome length were 41.76 µm and 5.97 µm respectively. Chromosome length varied from 4.24-8.43 µm with their arm ratio 0.54-0.83 and arm index 1.19-1.84. TCL% ranged from 10.15- 20.23. TF% was 40.04 and F% varied from 35.19-42.37. KF : 1A (M) + 1B(M) +3C(SM) + 1D(SM) + 1E(SM).

*P. schweinfurthii* had the minimum haploid chromosome length of 34.61µm among the 4 species studied. It had 1 median and 6 submedian chromosomes (Figs. 4,8). The chromosome length ranged from 3.65-6.88 µm whereas average chromosome length was 4.95 µm. Arm ratio and arm index had the range of 0.62-0.93 and 1.06-1.61 respectively. F% and TCL% varied from 38.36-48.42 and 10.54-19.82 whereas TF% was 41.92. KF : 3A (SM)+1B(SM)+1B(M)+1C(SM)+1D(SM).

## DISCUSSION

Among the 4 species studied *P. mollissimum* had the longest chromosome with the highest total length of the haploid complement. The smallest chromosome and the least haploid chromosome complement length were observed in *P. schweinfurthii*. Average chromosome length was also highest in *P. mollissimum* and least in *P. schweinfurthii*. The karyotype symmetry (Stebbins 1971) in all the 4 species was similar and of 1A type.

The present study showed the presence of median and submedian primary constrictions in all the species. In *P. typhoides* there were 3 median and 4 submedian pairs of chromosomes whereas Varmani & Gill (1972) and Tyagi (1975) reported one subterminal pair. Varmani & Gill (1972) also classified chromosome one as median with secondary constriction whereas Lobana & Gill (1973) on the basis of karyotype established from pachytene chromosomes found this chromosome to be submetacentric. The minor variation reported by different workers and in the present study with respect to the position of centromere is not unexpected as already suggested by Jauhar (1981). There may be differential condensation of different chromosomes during somatic metaphase.

Morphologically, *P. typhoides* and *P. violaceum* are closely related species. These shared similar habitat and have compactly arranged spikelets in the spike (Fig. 9a, c). These were also found to have close relationship in their isozyme patterns. Isozyme pattern homology was 66.7%, 75% and 100% for

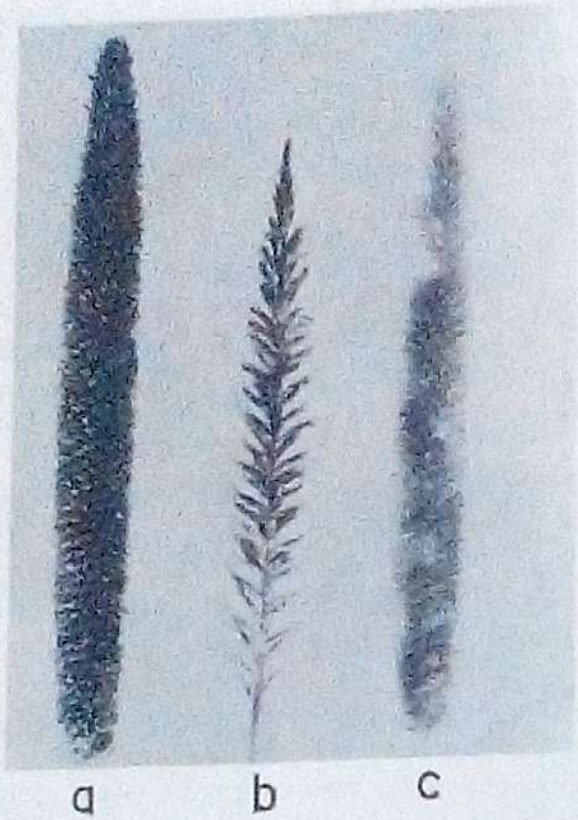



Fig. 9: Spikes of *Pennisetum*. (a) *P. typhoides*, (b) *P. schweinfurthii*, (c) *P. violaceum*.

peroxidase, esterase and amylase respectively (Mohindra 1989). *P. violaceum* was also considered as the probable progenitor of *P. typhoides* based on hybridization experiment. Hanna (1986) has put *P. violaceum* in the primary gene pool. The karyotype similarity with respect to centromeric index also substantiate the above view.

*P. schweinfurthii* is characterized by loosely arranged florets as compared to compactly arranged florets of *P. typhoides* and *P. violaceum* (Fig. 9b). Karyotypically, it had the smallest chromosome size varying from 3.65-6.88 μm and minimum haploid complement length (34.61 μm). Mohindra (1989) observed low affinity index in its isozyme pattern with *P. typhoides* for peroxidase (40%), esterase (14.3%) whereas no similarity in isozyme pattern for amylase.

According to Stapf & Hubbard (1934) *Pennisetum* species were grouped under 3 sections on the basis of their morphological differences. *P. typhoides* and *P. violaceum* with similar chromosome numbers were grouped under section *Penicillaria*. Jauhar (1981) because of similar morphology and chromosome number placed *P. mollissimum* under the same section. In the present study, despite similarity in karyotype and chromosome number, *P. schweinfurthii* was placed under section *Heterostachya* because of its different morphological characteristics with loosely arranged florets and linear-laceolate leaves. Therefore, these species may be grouped under 2 different sections with *P. typhoides, P. violaceum* and *P. mollissimum* in Penicillaria and *P. schweinfurthii* in Heterostachya.

This, it could be revealed that the identification of specific chromosome both from karyotype studies and isozyme pattern could be used for the characterization of interspecific hybrids. Due to high similarity of *P. typhoides* and *P. violaceum* in their morphology, karyotype and isozyme pattern, these could easily be crossable in nature through embryo rescue technique and can be exploited for resistance to downy mildew. *P. mollissimum* with similar karyotype could be exploited in wide hybridization programmes. Incompatibility of crosses between *P. schweinfurthii* and *P. typhoides* might be due to stylar incompatibility and high mortality rate of seedlings. However, *P. schweinfurthii* could also be used for transferring resistance to ergot and downy mildew through interspecific hybridization followed by embryo rescue technique.

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J. Cytol. Genet. 28: 105-106 (1993)

# GENETIC STUDIES OF A LARVAL COLOUR MUTANT IN *ANOPHELES STEPHENSI* - A MALARIA VECTOR

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(Received 20 April 1993, revised accepted 24 June 1993)

## SUMMARY

This paper describes the inheritance of a larval colour mutant designated as "Green" (*g*) isolated from laboratory strains of *An. stephensi*. The gene "*g*" is recessive and autosomal with full penetrance, uniform expression and high viability in both the sexes.

Key Words: *Anopheles stephensi*, larvae, colour mutants, genetics,

## INTRODUCTION

*Anopheles stephensi* Liston is one of the important malaria vectors in the Indian subcontinent. It belongs to subgenus Cellia and series Neocellia. During recent years, considerable research in the genetics of mosquitoes especially *Anopheles* has been directed towards the isolation and characterization of mutants. A few larval and adult mutants have been described (Naran & Seawright 1982, Mahmood & Sakai 1982, Rathore et al. 1983 a, b, Adak et al. 1984). These mutant markers have been identified on 3 linkage groups. The present paper describes the mode of inheritance of a mutant, green (*g*) for *An. stephensi*.

## MATERIAL AND METHODS

The mutant green larvae appeared spontaneously in stocks originally collected in Bangalore. After the initial discovery, the green larval mutants were crossed with their wild type sibs; subsequently, by crossing mutants *inter se* a large cage population of pure green larvae mosquitoes were established. The green colour is very conspicuous during later larval instars and pupal stages. The entire body of the larva, except the eyes, is green. Some of the larva were dark green and others were light green. By repeated inbreeding of *gg* homozygotes, a pure culture of bright green larvae has been established and is being maintained. The mutant has been independently isolated from Bangalore, Poona and Delhi strains. This suggests that this mutant is fairly common in India.

A characteristic feature of the mutant mentioned above is that it does not differ from the wild type larvae in early stages of development. It is only when they reach the third instar that the differences in pigmentation become conspicuous.

In all crosses, 25 females and 25 males were placed in 20 x 20 x 20 cm cage made of iron frame covered with nylon mosquito net. The males and females used in the experiment were originally isolated as single pupae in vials, then used before being introduced into the experimental cage.

## RESULTS AND DISCUSSION

The results of crosses green (*g*) larvae with wild type mosquitoes, scored in freshly emerged adults are given in Table 1. The $F_1$ adults were backcrossed in both the directions with respective

TABLE 1: Mode of inheritance of green larva in *Anopheles stephensi*.

| No. | Cross | Number of larvae | | | $X^2$ |
|---|---|---|---|---|---|
| | | Green | Wild | Total | |
| 1 | Green male X Wild female | - | 390 | 390 | |
| 2 | Green female X Wild male | - | 699 | 699 | |
| 3 | $F_1$ male (Green male X Wild female) X Green female | 258 | 326 | 584 | 7.92** |
| 4 | $F_1$ male (Green female X Wild male) X Green female | 141 | 128 | 269 | 0.64* |
| 5 | $F_1$ female (Green female X Wild male) X Green male | 110 | 108 | 218 | 0.02* |
| 6 | $F_1$ female (Green male X Wild female) X Green male | 134 | 185 | 319 | 8.16** |
| 7 | $F_1$ male (Green male X Wild female) X $F_1$ female (Green male X Wild female) | 177 | 455 | 632 | 3.06** |
| 8 | $F_1$ male (Green female X Wild male) X $F_1$ female (Green female X Wild male) | 69 | 271 | 340 | 4.03* |

* not significant ** significant

mutants of both sexes. Crosses 4 and 5 fit the expected 1:1 ratio of normal to mutant, however, crosses 3 and 6 did not show the ratio of 1:1 at the 5% level of deviation. $F_1$ mosquitoes in each case were crossed to get the $F_2$ generations. The results of crosses 7 and 8 in each case fit the expected 3:1 ratio of normal to mutants.

The $F_2$ ratios of 3:1 in both types of crosses led to 2 satisfactory conclusions. Firstly, the gene *g* responsible for giving rise to green larvae is recessive, secondly, it is autosomal and not sex-linked.

The gene *g* is an excellent larval marker for *An. stephensi* since it expresses itself in larval and pupal stages with full penetrance and high viability in both the sexes. The mutant colony has been vigorous, requiring no more care than the wild type. The gene *g* is thus an excellent marker for *An. stephensi*.

## ACKNOWLEDGMENT

This work was supported by the grants from the UGC-DSA programme, New Delhi.

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J. Cytol. Genet. 28: 107-112 (1993)

# CYTOMORPHOLOGY OF FOUR SPECIES OF *SISYMBRIUM*

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(Received 17 May 1993, revised accepted 25 June 1993)

## SUMMARY

Cytomorphological investigations in 4 species of genus *Sisymbrium* L. viz., *S. irio* L., *S. loeselii* L., *S. officinale* (L.) Scop. and *S. brassiciforme* C.A. May have been made. All the 4 species are characterised by normal chromosomal behaviour. 2n=42 has been observed for the first time in *S. brassiciforme*.

**Key Words:** *Sisymbrium*, morphology, karyotype, meiosis.

## INTRODUCTION

Species of *Sisymbrium* (Brassicaceae), especially *S. loeselii* and *S. irio* grow widespread on roadsides and shady moist places. *S. officinale* is an introduced species in Kashmir (Jafri 1973) and is very rare. *S. brassiciforme* occurs only at the altitudes of Ladakh. Plants of *S. loeselii* and *S. irio* have medicinal properties. While leaves and flowers of *S. loeselii* are given in scurvy and scrofula, the seeds of *S. irio* are expectorant, stimulant, and restorative used in asthma. Infusion of leaves of *S. irio* is given in affections of throat and chest in Spain (Chopra et al. 1956). The present study was taken up to study their cytomorphological details and to understand their interrelationships.

## MATERIALS AND METHODS

The species included in this study are *Sisymbrium irio* L., *S. loeselii* L., *S. officinale* (L.) Scop. and *S. brassiciforme* C.A. May. Morphological details were studied from both fresh and pressed specimens. For meiotic details, young floral buds were fixed in a mixture of absolute alcohol, acetic acid and chloroform (1:1:1) for 24 h. Anthers were squashed in 1% propionocarmine. All the observations were made from temporary slides. For mitotic studies, freshly germinated seeds were pretreated with saturated solution of aesculin to which traces of 8-hydroxyquinoline had been added for a period of 6 h at 6-8°C. After washing, the root tips were fixed in propionic-alcohol (1:2) and then stored in 60% alcohol. For staining, the usual Feulgen technique was followed.

## OBSERVATIONS

### Morphology

The plants of genus *Sisymbrium* are characterized by lyrate to pinnatipartite leaves and yellow flowers. Morphological features of the 4 species *S. loeselii, S. irio, S. officinale* and *S. brassiciforme* are summed up in Table 1.

TABLE 1: Morphological features of species of *Sisymbrium*.

| Characters | Species | | | |
|---|---|---|---|---|
| | *S. irio* | *S. loeselii* | *S. officinale* | *S. brassiciforme* |
| Plant height (cm) | 14-18 | 25-100 | 33-40 | 45-60 |
| Leaf shape | Pinnate | Lyratepinnate partite | Pinnate-sect | Lyrate |
| No. of flowers per raceme | 16-23 | 62-75 | 33-35 | 35-50 |
| Raceme length in fruit (cm) | 10-13 | 32-38 | 10 | 35-40 |
| Flower size (across in mm) | 3.0 | 3.0 | 6.0-7.5 | 4.0-4.5 |
| Sepal length (mm) | 2.5-3.0 | 3.5-4.0 | 2.0 | 3.0 |
| Petal length (mm) | 4.0 | 7.0-8.0 | 4.0 | 6.5-7.0 |
| Stamen length (mm) (small : long) | 2-2.5:3-3.5 | 3-3.5:4-5 | 2.0-3.0 | 3-3.5:5-5.5 |
| Anther length (mm) | 0.5-1.0 | 1.0-1.5 | 0.5 | 1.5 |
| Pedicel length (mm) | 6.0 | 7.0-9.0 | 2.0-3.0 | 7.0-9.0 |
| Style length (mm) | 0.5 | 1.0-1.5 | 0.5 | 1.5 |
| Fruit length (mm) | 45-50 | 25-52 | 15-18 | 80-85 |

TABLE 2: Salient features of the somatic chromosomes of species of *Sisymbrium*.

| Species | TCL (μm) | MCL (μm) | Chromosomes | | L/S |
|---|---|---|---|---|---|
| | | | Longest (μm) | Shortest (μm) | |
| *S. irio* | 19.78 | 1.41 | 1.77 | 1.1 | 1.6 |
| *S. loeselii* | 16.49 | 1.19 | 1.51 | 0.92 | 1.64 |
| *S. officinale* | 23.75 | 1.69 | 2.4 | 1.34 | 1.79 |

TCL, Total chromatin length; MCL, Mean chromatin length.

## Cytology

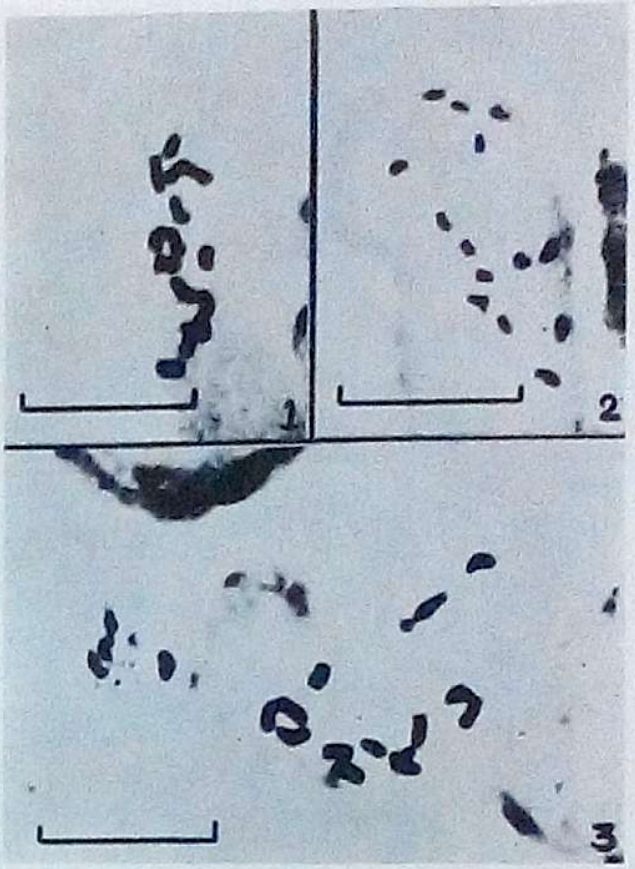
Karyotypic details of 3 species viz. *S. loeselii, S. irio* and *S. officinale* were studied. These three species are diploid with 2n=14 (Figs. 1-3). The salient features of their somatic chromosomes are tabulated in Table 2.

Meiosis in all the species studied was normal with the formation of bivalents followed by regular anaphasic segregation (figs. 4-14). In *S. brassiciforme* the chromosome count of n=21 has been made for the first time. However, in other 3 species studied, 7 bivalents have been observed in each pollen mother cell at first meiotic division. In none of the pollen mother cells of *S. brassiciforme* studied were any multivalent association observed. The only apparent abnormality observed was the stickiness of bivalents at metaphase I. This also did not impair the future course of meiosis in this species. The chiasma frequency and other details of all the 4 species have been summed up in Table 3.

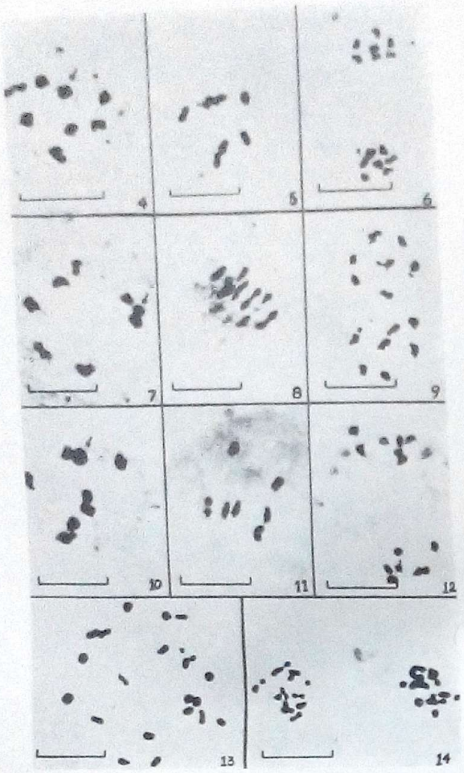
TABLE 3: Meiotic details of species of *Sisymbrium*.

| Species | n | Meiotic stage | No. of cells scored | Chiasma frequency | | | | | | No. of nucleoli per cell | No. of nucleolar II | Pollen stainability (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total per | | Interstitial per | | Terminal per | | | | |
| | | | | cell | bivalent | cell | bivalent | cell | bivalent | | | |
| S. irio | 7 | Dip. | 40 | 14.41 | 2.06 | 1.02 | 0.15 | 13.39 | 1.91 | 0-1 | 0-1 | 97.34 |
| S. loeselii | 7 | Dip. | 40 | 12.49 | 1.78 | 0.94 | 0.13 | 11.55 | 1.65 | 1 | 1 | 97.70 |
| S. officinale | 7 | Dip. | 40 | 15.13 | 2.16 | 3.23 | 0.46 | 11.90 | 1.70 | 1 | 1 | |
| | 7 | M I | 40 | 12.38 | 1.77 | 0.00 | 0.00 | 12.38 | 1.77 | | | 96.60 |
| S. brassiciforme | 21 | Dia. | 35 | 44.18 | 2.10 | 8.09 | 0.39 | 36.09 | 1.72 | 1-2 | 1-2 | 95.90 |

Due to small size of bivalents at metaphase I chiasma frequency at this stage could be analysed only in *S. officinale*. In the other 3 species, the bivalents appear as highly condensed dots only at this stage.




**Figs. 1-3:** Somatic chromosomes of species of *Sisymbrium*. 1, *S. irio* (2n=14). 2, *S. loeselii* (2n=14). 3, *S. officinale* (2n=14). (Scale=10 μm)




Figs. 4-14: Meiosis in species of *Sisymbrium*. 4-6, *S. irio* (n=7). 4, Diplotene. 5, Metaphase I. 6, Anaphase I. 7-9, *S. loeselii* (n=7). 7, Diplotene. 8, Metaphase I. 9, Anaphase I. 10-12, *S. officinale* (n=7). 10, Diplotene. 11, Metaphase I. 12, Anaphase I. 13,14, *S. brassiciforme* (n=21). 13, Diakinesis. 14, Anaphase I. (Arrow=Nucleolus; Scale = 10 μm)

## DISCUSSION

All the 4 species investigated are annual herbs reproducing by means of seeds alone. *S. loeselii*, widely distributed in Kashmir valley and adjoining areas ranging in altitudes from 5000 ft to 12000 ft, is often found in diverse habitats. However, this adaptability often gets reflected in the phenotype of its plants especially in plant size; plants as small as 25 cm and as tall as 1 m are met with. Moreover, the plants of *S. loeselii* seem to be great colonisers. This variation in the morphological characters can only be possible if the genotype of this species is highly flexible. Khoshoo (1966) has also commented that the genotypic dynamism confers an important advantage on the taxa enabling them to inhabit diverse habitats.



*S. irio*, although not as widespread as *S. loeselii* is a highly polymorphic species with respect to its leaf, flower and pedicel size. Some forms of this species are at times confused with the plants of *S. loeselii*. However, rocket-shaped leaves and siliquae overtopping the young buds in *S. irio* distinguishes it from *S. loeselii*.

The chromosome size ranges from 0.92 μm (*S. loeselii*) to 2.4 μm (*S. officinale*). Although all the 3 species, whose somatic chromosomes could be studied are diploid with 2n=14, total chromatin length is maximum in *S. officinale* (23.74 μm). Keeping in view the ratio between the size of the longest and the smallest chromosome, the karyotypes of *S. officinale* is more asymmetrical than the other two. However, *S. irio*, with minimum total chromatin length (19.75 μm) appears to be more advanced as compared to *S. officinale* (23.75 μm). On the basis of L/S and TCL, it seems that within this genus the evolution has progressed on 2 different lines, one towards greater asymmetry and another towards reduction in chromatin length. In the absence of any earlier record of somatic chromosome study in this genus, it was not possible to compare the results.

The present observation of 2n=14 in *S. irio*, *S. loeselii* and *S. officinale* conforms to the earlier reports (Jaretzky 1932, Rohweder 1937, Podlech & Dieterle 1969, Aryavand 1978). The present observation of n=21 in *S. brassiciforme* is at variance with the earlier record of 2n=14 (Fedorov 1969) indicating its hexaploid nature. Meiosis in all the 4 species was normal characterised by formation of bivalents only at prophase I and regular anaphasic segregations. All these species are based on x=7. However, some species of this genus with x=8 are also on record (Fedorov 1969). At the same time, the presence of 2n=26 in *S. litorale* (Fedorov 1969) can be either due to the presence of a third base number (x=13) or its being hypotetraploid with 7 as its base number. A perusal of available literature reveals that out of 70 species, which represent this genus, so far only 28 species have been cytologically studied. Eighty per cent of these species are based on x=7 which suggests that 7 is the ancestral base number in the genus *Sisymbrium*.

## ACKNOWLEDGEMENT

The senior author wishes to thank CSIR, New Delhi for providing financial assistance in the form of Senior Research Fellowship.

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J. Cytol. Genet. 28: 113-116 (1993)

# CHROMOSOMES OF AN ISOLATED HIGH ALTITUDE POPULATION OF *ARMIGERES(ARMIGERES) SUBALBATUS* (CULICIDAE: DIPTERA)

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(Received 21 May 1993, revised accepted 25 June 1993)

## SUMMARY

Investigations on the chromosomes of mosquitoes is an integral part of cytotaxonomy and phylogenetic relationship studies in the family Culicidae. The present piece of information about the chromosomes of 2 high altitude populations of *Ar. subalbatus* is a contribution to this aspect of researches on mosquitoes. Gonial metaphase chromosomes from pupal testes of this species present a normal karyotype of 6 chromosomes similar to majority of the non-anopheline mosquitoes except that all are extra large in size. The aberrant population had chromosomes with structural deformities like breaks, despiralization, heterochromatization, late disjunction at anaphase and vacuolization.

**Key Words:** Chromosomes, cytotaxonomy, *Armigeres subalbatus*.

## INTRODUCTION

Family Culicidae is a large group with over 2680 species belonging to 33 genera and 120 subgenera (Stone 1967). The fauna of the Oriental region prevalent in India is represented by nearly 245 species but the chromosome information is available for about 30 of them. Many workers have made valuable contributions in elaborating the accounts of mosquito karyotypes from three main genera, namely *Anopheles, Culex* and *Aedes,* highlighting the significance of chromosome cytology in the cytotaxonomy and evolution of the family Culicidae (Rai 1963, 1980, Mukherjee et al. 1966, Kitzmiller 1967, 1976, Kanda 1968, Aslamkhan & Baker 1969, Avirachan et al. 1969, Baker & Aslamkhan 1969, Sharma et al. 1970, 1977, Rai & Hartberg 1975, Mittal & Dev 1977 a, b, Rooney 1980, Zhu et al. 1981, Rai et al. 1982). Besides these 3 genera, there are many species of which cytological investigations have not been carried out to any appreciable extent because of the fact that majority of these species have limited seasonal prevalence and specific ecological preferences. The members of the genus *Armigeres* to which the present material belongs, are found chiefly in forested localities where there is sufficient rain water to provide them with suitable breeding places. Genus *Armigeres* is Oriental in distribution of which a few species have the capacity to harbour *Wuchereria,* a source of elephantiasis. In the present study, the results of normal chromosome behaviour as well as that of aberrant population of *Ar. subalbatus* are included.

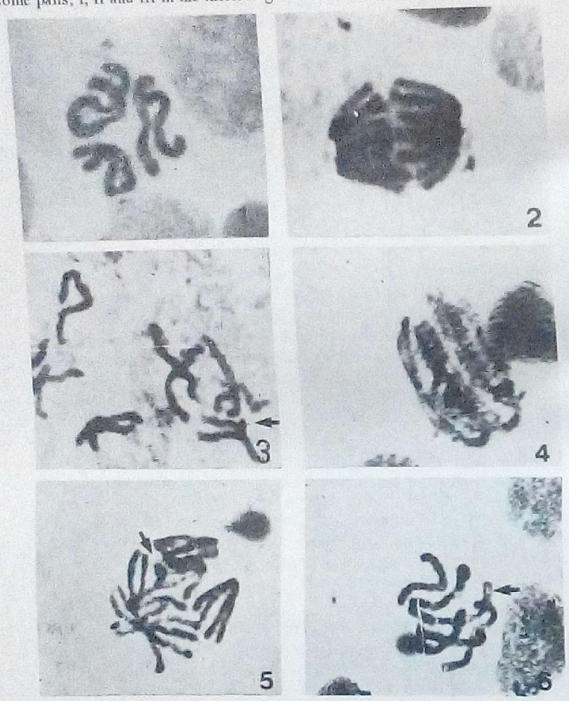
## MATERIAL AND METHODS

*Armigeres subalbatus* is a large-sized mosquito with equally large larvae and pupae. The collections were conducted from a village Gagal, at an altitude of about 1000 M (3000 ft), near Dharamsala in the Kangra Hills of Himachal Pradesh. The adults, larvae and pupae were abundant during the monsoon months of July and August, when ample breeding places were available for the species. Hundreds of fully grown larvae and pupae, exclusively belonging to this species, were drained out of containers like drums, canisters, earthen pots and other such articles under dense vegetation. The adults were collected from

nearby bushes during day time. The chromosome preparations from pupal testes were made by making the temporary squash preparations in a diluted 2% lacto-aceto-orcein by following the standard technique of French et al. (1962). The entire chromosome analysis was made from these temporary squashes.

## OBSERVATIONS

*Ar. subalbatus* has a diploid number of 6 with large-sized chromosomes which can be numbered as chromosome pairs, I, II and III in the increasing order of their size. Chromosome pair I measures




Figs. 1-6: *Armigeres* (*Armigeres*) *subalbatus*. 1, Spermatogonial metaphase (2n=6). 2, Normal anaphase. 3, Chromosome breaks. 4, Heterochromatization of chromosomes. 5, Late disjunction of some chromatids at anaphase. 6, Vacuolization of chromosomes.



about 4.8 µm, II-6.0 µm and III-7.5 µm revealing a significant size difference between one another. The mitotic and meiotic cycles proceed much in the same way like most of the species of *Aedes* and *Culex* in which the dividing cells pass through all the stages of cell division. Though there is not much of a difference in various phases of cell division, yet *Ar. subalbatus* chromosomes present clearly defined phenomenon of somatic pairing leading to the formation of the so-called "mitotic bivalents" at gonial metaphase (Fig. 1). The anaphase chromosomes are also well organised as all the chromatids maintain their synchronized movement towards the poles of the spindle (Fig. 2).

In one of the aberrant populations collected from extremely foul water in the abandoned drums, there was abnormally high rate of damage suffered by the chromosomes. The structural abnormalities like chromosome breaks, despiralization, hetero-chromatization, late disjunction and vacuolization were seen in many cells of the testicular tissue (Figs. 3-6). The mosquitoes of the effected stock did not have any visible morphological deformities. The effect was non-lethal as the adults were strong enough to escape from puparium and were active fliers. However, their mating competitiveness could not be ascertained due to inadequate rearing procedures available for the species.

## DISCUSSION

The 6 chromosomes of *Ar. subalbatus* confirm the uniformity of this diploid number in all the mosquitoes irrespective of the genus to which a species belongs. In 1943, Sinoto & Suzuki found the same number in this species and all the chromosomes were metacentric. Kanda (1968) published details of its metaphase karyotype. He also found 6 metacentric chromosomes forming 3 unequal homologous pairs. The gonial metaphase chromosomes of the present population are considerably larger than the chromosomes in majority of the species in the genera *Anopheles*, *Culex* and *Aedes*. The exception is presented by *C. tarsalis* (Asman 1974) which has equally large chromosomes.

*Armigeres* is a comparatively lesser known genus with very few species of common occurrence in the Indian region. The present mosquito species seems to withstand sufficient genetic damage as is evident form the present results of an aberrant stock. Thus, it is pertinent to acquire chromosome information of the remaining species of the complex to reach at some conclusion.

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J. Cytol. Genet. 28: 117-119 (1993)

# CHROMOSOME ANALYSIS IN A SPIDER, *MISUMENA MENOKA* TIKADER

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## SUMMARY

The present study deals with the chromosomes of spider, *Misumena menoka* Tikader (2n=22+X). All the chromosomes irrespective of the autosomes and sex-chromosome are acrocentric and medium-sized. The sex-chromosome exhibits positive heteropycnosis at stages before metaphase.

**Key Words:** *Misumena menoka*, chromosomes, heteropycnosis.

## INTRODUCTION

Family Thomisidae, which is one of the large families of spiders, includes spiders with crab-like movements. Inspite of their worldwide distribution and frequent availability, only 35 species belonging to its 3 subfamilies, namely, Philodrominae, Dietinae and Misumeninae, have so far been recorded cytologically (Tugmon et al. 1990, Mittal 1991) which is a meagre information as compared to the taxonomically recorded species (Roewer 1954, Tikader 1980) thus leaving a large void in the karyological knowledge of this family. Keeping these gaps in view, the present study on thomisid spider, *Misumena menoka*, has been undertaken for its chromosomes.

## MATERIALS AND METHODS

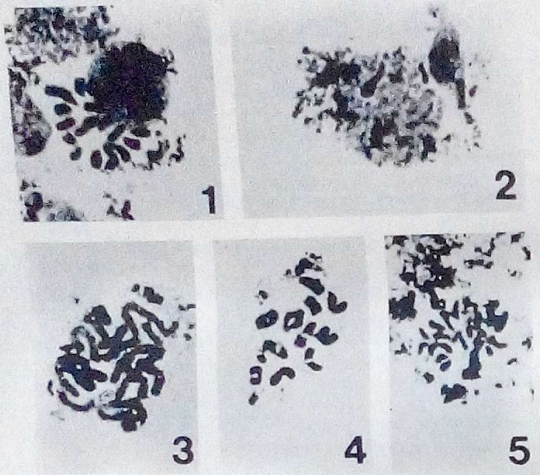
The spiders were collected from the botanical garden, Sector-14, Panjab University, Chandigarh from January to March, 1993 from different host plants i.e., *Pyrus japonica*, *Prunus persica*, *Buddleja madagascarensis* and *Adhatoda vasica*. Six male specimens of *M. menoka* were dissected for their testes following the procedure as laid down by Mittal (1963). After hypotonic treatment (Ford & Hamerton 1956), the testes were squashed and stained in 2% lacto-aceto-orcein (French et al. 1962). After a careful examination of the slides, photography was done using Olympus microscope.

## OBSERVATIONS

The spermatogonial metaphase of this spider exhibits 23 (22+X) acrocentric, distinct chromosomes forming a rosette having 7 chromosomes in its centre (Fig. 1). The X-chromosome is recognizable from the autosomes as the largest element in the complement.

During meiosis I the sex-chromosome exhibits positive heteropycnosis right from premeiotic interphase to diakinesis (Figs. 2,3).

Each of the diplotene, diakinesis and metaphase I stages houses 11 autosomal bivalents and a univalent X-chromosome. At diakinesis/metaphase I, the bivalents display tetrad structure while the X as dyad (Fig. 4).




Figs. 1-6: Male mitotic and meiotic stages of *Misumena menoka*. 1, Spermatogonial metaphase; 2. Premeiotic interphase nucleus; 3, Diplotene; 4, Diakinesis/ Metaphase I; 5. Metaphase II showing 2 groups of chromosomes with 12 and 11 chromosomes.

Meiosis I being reductional both for the autosomes and sex-chromosome, results in 2 types of metaphase II plates (Fig. 5), 50% on an average with 12 chromosomes including the X-chromosome and the remaining g 50% with 11 autosomes only.

## DISCUSSION

The tally of the chromosomally known species of Thomisidae is raised to 36 from 35 species by the presently worked out species, covering its 3 subfamilies, namely, Philodrominae, Dietinae and Misumeninae (Tugmon et al. 1990, Mittal 1991). The family appears quite important from the evolutionary point of view as well exhibiting a great diversity of chromosomes from 2n=23 to 2n=29 with 3 types of sex-determining mechanisms, i.e., XO, $X_1X_2O$ and $X_1X_2X_3O$ in the male of different species.

In the subfamily Philodrominae, 2n=28 ($26+X_1X_2$) is predominantly met with as it is found in 9 out of 13 species. However, each of its remaining 4 species, namely, *Philodromus aureolus* 2n=27 (26+X), *Philodromus* sp. 2n=25 ($22+X_1X_2X_3$), *Tibellus duttoni* 2n=29 (28+X) and *Tibellus oblongus* 2n=26 ($24+X_1X_2$) exhibit different diploid numbers. It is interesting to observe that both the diploid numbers of chromosomes and sex-mechanism exhibit diversity to certain extent.



Both the species of the subfamily Dietinae, differ in their chromisome makeup i.e., 2n=24+$X_1X_2X_3O$ in *Tarrocanus viridis* and 2n=22+$X_1X_2$ in *Oxytate setosa*.

Subfamily Misumeninae to which the present species belongs now becomes known cytologically by 21 species. Misumeninae monotonously possess 2n=23 in all of its species with XO type of sex-mechanism in the male, with the sole exception of *Xysticus triguttatus* in which 2n=24 with $X_1X_2O$ sex-mechanisms has been recorded (Painter 1914). Chromosome garniture of the presently worked out species, *M. menoka*, 2n=22+X is in conformity with those of the 19 already worked out species of this subfamily (Mittal 1991). Thus, its inclusion in Misumeninae is further strengthed cytologically. Further, 2n=23 can be regarded as a 'modal number' for this subfamily as has already been suggested by Mittal (1991).

## ACKNOWLEDGEMENT

The financial help rendered to the second author (MK) in the form of Research Associateship by CSIR is gratefully acknowledged.

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J. Cytol. Genet. 28: 121-124 (1993)

# EFFECT OF AIR POLLUTION ON MEIOTIC DIVISIONS IN *TRIDAX PROCUMBENS* L.

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## SUMMARY

Recent studies have shown that airborne pollutants from factories can disturb the ecosystem and exert hazardous effects on plants. The present paper deals with abnormalities in cell divisions observed in the meiotic divisions of *Tridax procumbens* exposed to air pollution from the titanium dioxide pigment industry. Significant increase over controls has been observed in the occurrence of abnormalities in cell division in plants growing adjacent to the factory.

**Key Words:** Air pollution, meiotic divisions, *Tridax procumbens*.

## INTRODUCTION

It is now widely recognised that the technological explosion which brought unprecedented benefits to mankind has its impact on environment by polluting the air, water and soil. As there is growing concern about conservation of the environment, studies are being conducted by scientists to assess the impact of industrial effluents and airborne pollutants on vegetation. Various reports have shown that air and water pollutants can affect growth and development in plants adversely (Kozlowski 1980, Takemoto et al. 1988, Olszyk et al. 1992). The influence of pollutants from the titanium dioxide pigment industry on cell division in plants has not been evaluated yet. The present paper deals with meiotic abnormalities observed in *Tridax procumbens* exposed to air pollution from the factory. This was accomplished by studying meiotic divisions in *T. procumbens* growing at different sites potentially in danger of chemical pollution.

## MATERIAL AND METHODS

This titanium dioxide industry is situated at Kollam in Kerala State. During processing the titanium ore and chlorine are reacted in the presence of petroleum coke. The gases leaving the chlorinator contain chlorides of all the metals which occur in the raw materials. The exact nature of air pollutants present in the atmosphere cannot be measured accurately as the pollutants are carried away by wind currents.

For the study of influence of airborne pollutants from the factory on meiotic divisions in *T. procumbens*, 4 sites were chosen (25 m, 50 m, 100 m, 150 m) in the region within the reach of the industrial exhaust. The fifth site chosen 40 km away from the factory, in the region not in danger of air pollution, served as the control. For meiotic studies, flower buds were fixed in Carnoy's fluid and smeared in acetocarmine. Photographs were taken before making the slides permanent.

## OBSERVATIONS

Meiosis in the control plants of *T. procumbens* was normal and 18 bivalents were observed at diakinesis (Fig.1). Only about 5% of the pollen mother cells showed abnormalities in divisions. On the

pollutants. Cytomixis showing extrusion of chromatin from one nucleus into the cytoplasm of the adjoining cell has been found to occur frequently (Fig. 2). Breakage of chromosomes followed by fusion at random resulted in exchange of segments between chromosomes. As a result, rings and chains, characteristic of translocations were observed (Fig. 3) Clumping of chromosomes was the most predominant abnormality observed in the flower buds collected from plants growing at different sites. As may be observed from the Table 1, 31.14% of the pollen mother cells in the plants growing adjacent to the factory showed chromosome clumping in contrast to only 1.99% incidence in the control. In the extreme cases all the chromosomes were clumped together (Fig.4). Clumping was also found to impede normal separation of the chromosomes and as a result sticky bridges were observed at anaphase I and II of meiosis (Figs. 5,6). Lagging chromosomes were also seen in varying frequencies (Fig. 7). Multipolar anaphases were also observed occasionally. Pollen sterility was high in plants growing near the factory. Over 42% of the pollen grains in the plants collected from site No. 1 showed sterility while the control plants showed only 8% pollen.

TABLE 1: Percentage of pollen mother cells showing abnormalities in *Tridax procumbens* collected from different sites adjacent to the titanium pigment industry.

| Sampling area | Total PMC's analysed | Percentage of pollen mother cells showing | | | |
|---|---|---|---|---|---|
| | | Translocation configuration | Chromosome clumping | Chromosome breaks | Other abnormalities |
| Site No.1 | 167 | 11.98** | 31.14** | 5.39** | 9.59** |
| Site No.2 | 155 | 10.32** | 24.52** | 5.16** | 9.68** |
| Site No.3 | 172 | 8.14** | 13.37** | 4.10** | 8.14** |
| Site No.4 | 193 | 6.74** | 8.81** | 2.59* | 5.18* |
| Site No.5 | 201 | 1.00 | 1.99 | - | 1.99 |

* Significant at P<0.05 ** Significant at P<0.01

## DISCUSSION

The effects of industrial effluents and other pollutants on plants have been the subject of many investigations (Mossman 1989, Smith 1989). Observations made during the present study showed higher incidence of chromosomal aberrations and pollen sterility in *T. procumbens* growing near the factory. Recent studies have shown that effluents and airborne pollutants from factories can produce a wide spectrum of abnormalities in plants. Studies on algal flora of tannery effluent polluted waters of Kanpur showed poor growth in polluted water (Pandey & Chandah 1990). Agarwal & Agarwal (1990) observed that Kota saree printing effluents exerted toxic effects on seed germination and seedling growth of *Cyamopsis tetragonoloba*. Sengupta & Ghosh (1992) observed that heavy metals like cadmium and mercury induced diverse chromosomal aberrations in mitotic cells of *Lathyrus sativus*. Increased $SO_2$ concentration in the atmosphere caused decrease in total plant weight, shoot length and number of leaves, branches and flowers (Murray & Wilson 1991).

Earlier studies on the impact of factory effluents on vegetation in Kerala State have shown that effluents from the titanium factory produced floral abnormalities and somatic mutations in *Tradescantia* clone $O_2$ (Abraham 1989). Clay factory wastes induced spindle abnormalities and chromosomal aberrations in the mitotic cells of *Chlorophytum heynei* (Abraham & Abraham 1992). The predominance of chromosome clumping can be noticed in all the plants collected from the first 4 sites. Obviously, their presence in much higher frequencies in the plants growing adjacent to the factory is possibly due to greater dispersion of aerial toxicants in this area. It is known that the titanium dioxide pigment industry has employed an advanced technology and is pollution proof. However, the results of the present study shows that airborne pollutants that escape to the atmosphere are likely to affect cell divisions and can produce sterility in plants.

## ACKNOWLEDGEMENT

The award of a Junior Research Fellowship by the University of Kerala to one of us (CJ) is gratefully acknowledged.

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J. Cytol. Genet. **28**: 125-129 (1993)

# EXTRANUCLEAR NUCLEOLUS-LIKE BODY IN *BALANOPHORA*

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(Received 10 June 1993, revised accepted 5 July 1993)

## SUMMARY

A conspicuous nucleolus-like body appears frequently in the cytoplasm of megaspore mother cell, megaspore, dyad cell, female gametophyte and zygote of two species of *Balanophora*. It is extruded from the nucleus and is RNA-rich with a feeble reaction for proteins. It appears to interfere with the function and destiny of the concerned part in which it occurs.

**Key Words:** *Balanophora*, extranuclear nucleolus.

## INTRODUCTION

Balanophoraceae are highly specialized angiosperm root parasites known for their achlorphyllous fungoid habit. Species of the genus *Balanophora* have been repeatedly investigated over the years (Ekambaram & Panje 1935, Zweifel 1939, Fagerlind 1945, 1948, Arekal & Shivamurthy 1978, Shivamurthy 1975, Shivamurthy et al. 1981a, 1981b, Gedalovich-Shedletzky & Kuijt 1990).

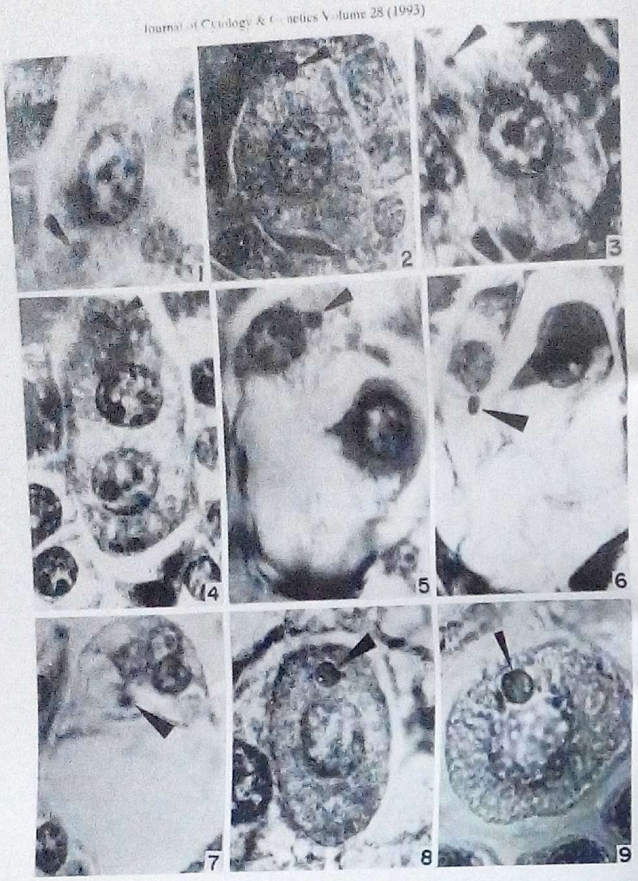
While investigating the reproductive biology of 2 species of *Balanophora* we frequently came across with an extranuclear nucleolus-like body in the cytoplasm of megaspore mother cell, dyad cell, functional megaspore, developing female gametophyte and in the zygote. The observations presented herein are based on *B. abbreviata* Blume collected from Ranganathittu near Mysore (Karnataka) and *B. fungosa* Forst. ssp. *indica* (Arn.) Hansen var. *indica* Sorensen et al. from Mercara (Karnataka) and Kodaikanal (Tamil Nadu).

## MATERIAL AND METHODS

The inflorescences at various stages of development were fixed separately in formalin-acetic-alcohol, Carnoy's fluid, Clarke's fixative and Navashin's fluid (Krishnamurthy 1988). Customary methods of dehydration and imbedding were followed for microtomy. Sections 8-14 µm thick were taken and stained with Heidenhain's haematoxylin with erythrosin in clove oil as counterstain. Some sections were stained according to the periodic acid-Schiff's (PAS) reaction for polysaccharides, ninhydrin-Schiff's method (Jensen 1962) and mercuric chloride-bromophenol blue (Mazia et al. 1953) for proteins and with methyl green-pyronin (Branchet 1953) and cresyl violet (Cecich et al. 1972) for RNA.

## OBSERVATIONS

A small circular deeply stained body simulating a nucleolus, in size and shape, appears frequently in the cytoplasm of megaspore mother cells during prophase I. This body was observed in the cytoplasm at the lower end of megaspore mother cell (Fig.1). It becomes more prominent during meiotic divisions and is often located at the peripheral region of cytoplasm. When the dyad cells are formed, it gets incorporated into the lower dyad cell which without passing through meiosis II, degenerates promptly, while the upper dyad without this body, completes meiosis II and a triad results



Figs. 1-9: Extranuclear nucleolus-like body (arrow) in *Balanophora*. 1-8, *B. abbreviata*, 9 *B. fungosa* ssp. *indica* var. *indica*. 1. Part of l.s. of gynoecium containing megaspore mother cell at prophase I; note the nucleolus-like body in the cytoplasm. 2, Degenerated and functional dyad cells showing nucleolus-like body in the functional dyad cell. 3, Functional megaspore at prophase with nucleolus-like body at the upper end. 4, An young 2-nucleate embryo sac with this body at the upper end. 5, An older 2-nucleate embryo sac containing this body at the upper end. 6, 4-nucleate embryo sac after assuming U-shape with the deeply stained body below the 2 nuclei of the morphologically upper end. 7, Antipodal end of the 8-nucleate embryo sac with the deeply stained nucleolus-like body. 8, Part of l. s. of a mature fruit indicating nucleolus-like body above the nucleus within the zygote. 9, Extrusion of nucleolus-like body from the nucleus of transversely cut megaspore mother cell showing positive reaction for RNA stained with methyl green-pyronin. (Figs. 1 - 6 & 9 X1660; Fig. 7 X950; Fig. 8 X1500)



finally. In some instances, it appears in the upper end of a functional dyad cell (Fig. 2). After the organization of megaspore tetrad in the usual course, it is found to appear in the functional megaspore at its morphological upper end (Fig. 3). This body appears very prominent at the 2-nucleate embryo sac stage (Fig. 4). It continues to stay at that end as the embryo sac undergoes curvature and becomes 4- and 8-nucleate. The lobe of the embryo sac in which it is located is rendered the antipodal end containing sparse cytoplasm although that morphological upper end should have become the egg apparatus end (Figs. 4-7). Rarely, this body was seen in the zygote cytoplasm (Fig. 8) in which case the zygote had remained undivided despite the surrounding endosperm had 16 cells at which stage, in the normal course of development, an embryo of 12 cells is consistently observed. But for the presence of nucleolus-like body such a zygote is similar in all respects to a normal zygote.

In several instances it was noticed that the nucleolus-like body extruding itself from the nucleus of concerned cell in which it was observed (Fig. 9). A regular nucleolus, however, was present in the nucleus of such cells. The nucleolus-like body responded negatively to PAS test but stained red with methyl green-pyronin and violet with cresyl violet indicating the presence of RNA (Fig. 9). This body showed feeble positive reaction to proteins when it was stained with ninhydrin-Schiff's reagent and mercuric chloride bromophenol blue.

## DISCUSSION

The frequent appearance of small, circular, deeply staining nucleolus-like body in the cytoplasm at different stages of megasporogenesis and female gametophyte development in the present study has been enigmatic since there is paucity of information on it in the literature. It is found both during meiotic and mitotic cycles of megasporogenesis and megagametogenesis respectively. Ekambaram & Panje (1935) who noted such a structure during megasporogenesis in *B. dioica* could neither trace its origin nor attribute any functional role to it. Bernard (1900) observed the appearance of such bodies in the embryo sac of *Helosis guyanensis* (Balanophoraceae) and called them 'spheres directries'. Flint & Johansen (1958) who studied the nucleo-cytoplasmic relationships in the Fritillaria type of megagametogenesis, demonstrated the entry of nuclear substance into the cytoplasm from the nucleus in the megaspore mother cells. According to them, these substances control or participate in many nuclear activities such as, the formation of tractile elements or spindles, holding nuclei in position or causing them to move, presumably causing the 3 chalazal spindles to fuse and the control of respective functions of the nuclei in the developing gametophytes. Occurrence of nucleolus-like bodies in the cytoplasm during part of the division cycle have been illustrated and described in *Lilium*. That these 'nucleoloids' contain both RNA and proteins and are structurally similar to nucleoli has been elucidated (Dickinson & Heslop-Harrison 1970). In the present study, it is observed that in megaspore mother cell this body is extruded into the cytoplasm just before or soon after diakinesis. In the case of megaspores it arises during meiosis II. During the female gametophyte development it is extruded into the cytoplasm from the nucleus.

The investigation has established that this body is nuclear in origin, RNA-rich and contains small quantity of proteins and, therefore, nucleolus-like. The point of interest is its presence in the megaspore mother cell either at the upper part or at the lower part, away from the nucleus. When it gets incorporated into the dyad cell after meiosis I that dyad cell degenerates without completing meiosis II

and producing two megapores as per the normal course of development. Moreover, when it appears in the cytoplasm of 2-nucleate embryo sac at the upper pole which should have been the egg apparatus pole as in all angiosperms, it is rendered the antipodal pole. And later the antipodal cells are never organized but the three antipodal nuclei and a polar nucleus unite to form a polyploid fusion nucleus. Could we logically, therefore, assign a functional role to this cytoplasmic nucleolus-like body? The function being its interference with the normal chain of biochemical reactions leading to the deviation of destiny of cells or parts of cells namely, the dyad cell degenerating as such, the egg apparatus pole functioning as the antipodal pole of the embryo sac and the antipodal nuclei not organizing as cells but fusing with the polar nucleus. Further, when it occurs in the cytoplasm of the zygote no further cell divisions ensure even though the other part of the 'seed' developed according to the general pattern of morphogenesis.

Electron microscopic studies of the nucleolus of the nucleus have revealed the presence of types of particulate bodies which are believed by Hyde et al. (1965) and Rho & Bonner (1961) as ribosomes. It is also established that in the cytoplasm there are a number of soluble 'gene inducers' which regulate the gene activity. Ultrastructural and histochemical studies of extranuclear nucleolus-like body, therefore, would certainly result in the identification of particulate bodies, if any, which should be characterised and their functional regulatory role determined to explain the interference of these bodies in the normal chain of biosynthetic reactions within the cell or a part in which the nucleolus-like body is located, leading to the inhibition of function and the distortion of morphological destiny of the part in which it occurs.

## ACKNOWLEDGEMENT

One of us (GRS) is thankful to the Chairperson, Department of Studies in Botany, Manasagangotri, Mysore for facilities.

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J. Cytol. Genet. 28: 131-135 (1993)

# OCCURRENCE OF CYTOMIXIS AND MINIPOLLEN GRAINS IN EMS INDUCED MUTANT OF *DESMODIUM TORTUOSUM*

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(Received 22 May 1993, revised accepted 8 July 1993)

## SUMMARY

The cytomictic sterile mutant with minipollen grains was isolated in $M_2$ generation of 0.2% EMS/16 h treated progeny in forage legume *Desmodium tortuosum*. The mutant was characterised by phenotypic variations, highly abnormal meiotic behaviour (24.03%), occurrence of cytomictic cells (33.46%) and formation of minipollen grains (46.92%). The meiotic anomalies were, stickiness (1.53%), multivalents (3.87%), laggards (6.52%) bridges (4.32%) and cells with micronuclei (7.79%). The minipollen grains were half of the normal pollen grains in size. The plant was sterile with no seed setting. The present study deals with the occurrence of cytomixis, its possible role in pollen formation and sterile nature of mutant plant.

**Key Words:** Forage legume, *Desmodium tortuosum*, EMS, cytomixis.

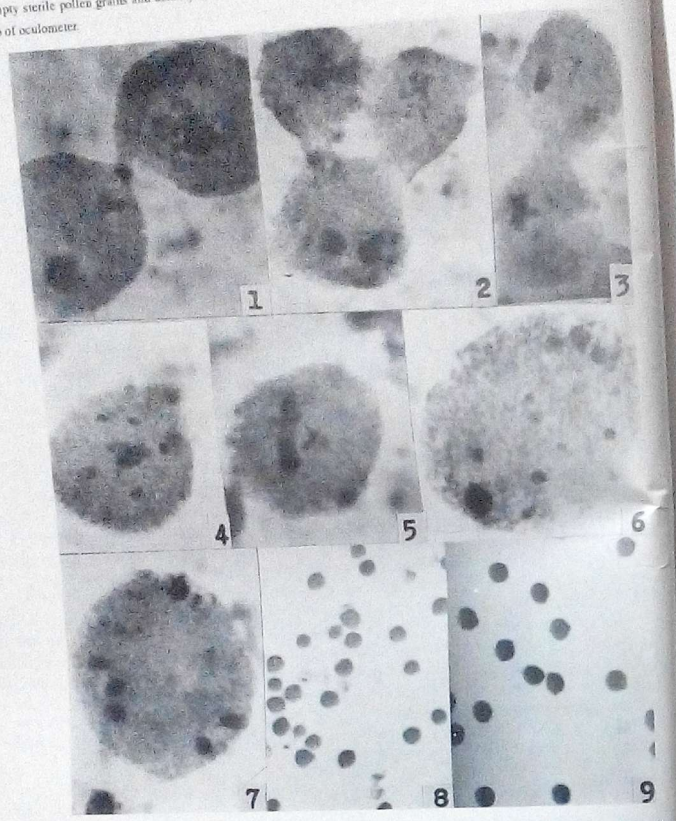
## INTRODUCTION

The cytoplasmic connections between 2 or more cells and its role in chromatin movement through cytoplasmic channel have been well described both in treated with mutagenic agents and nontreated material by many workers (Levan 1941, Sarvella 1958, Kamra 1960, Ghatnekar 1964, Bobak & Herich 1978, Patil 1983). The occurrence of cytomixis and its impact on meiotic system have been assessed by these workers with different viewpoints. Some have described the process as an artifact caused by improper fixation or due to mechanical action or abnormal environmental conditions. According to Bobak & Herich (1978) it may be the result of total breakdown of cytokinetic process or it may be a manifestation of pathological events. Gottschalk (1970) differs with these views and considers that it cannot be a fixation artifact or degenerative process in treated material. The cytomixis occurs only in the plants showing irregular physioligical and cytological behaviour or it is restricted to the genetically imbalanced type of plants (Levan 1941, Gottschalk 1970). However, Sarvella (1958) and others described the process as of natural occurrence. The transfer of chromatin material in cytomixis is now well established (Kamra 1960, Gottscalk 1970) and it can be doubtlessly presumed that the cytomixis has a significant impact on meiotic system and pollen formation. In the present investigation, an attempt has been made to discuss the possible role of cytomixis on pollen formation and sterility in mutant of *Desmodium tortuosum* (Sw.) DC.

## MATERIAL AND METHODS

The mutant plant of forage legume *Desmodium tortuosum* was isolated in $M_2$ generation raised from the seeds treated with 0.2% EMS for 16 h. For meiotic analysis, the flower buds of suitable sizes were fixed in 1:3 acetic-alcohol and smear preparations were made in 1.5% acetocarmine. Cells with cytomixis and other chromosomal abnormalities were recorded and per cent occurrence per meiotic stage has been estimated. Pollen sterility was recorded on the basis of stainability in 1% acetocarmine and 50% glycerine mixture. Total of 7497 pollen counts were made to estimate the occurrence of minipollen grains,

nstained empty sterile pollen grains and densely stained normal pollen grains in the mutant plant. Pollen size was determined vith the help of oculometer.




Figs. 1-9: *Desmodium tortuosum*. 1. Cytomixis between 2 cells at telophase I showing migration of chromatin material. 2. Cytomixis involving 2 cells of metaphase I and 1 cell of telophase I. 3. Cytomixis with laggard in anaphase I. 4. Metaphase I showing 5II + 1 multivalent. 5. Metaphase I showing stickiness. 6. Telophase I showing 4 micronuclei. 7. Telophase I with multinucleate condition. 8. Minipollen grains in a mutant plant. 9. Pollen grains in control plant. (Figs. 1-3, 8, 9 X460, Figs. 4-7 X920)



## OBSERVATIONS

Phenotypically, the mutant plant was different from control in exhibiting bushy appearance with repetitive branching of inflorescence axis. The plant was marked with poor foliation, weak stem and no seed setting.

Meiosis in control plant was normal with formation of 11 bivalents at diakinesis and normal pollen production. The interesting observation in meiotic analysis of mutant plant was the frequent occurrence of cytomixis and chromosomal aberrations. In control, the PMCs are completely separated from one another without cytoplasmic connections between them and are surrounded by nutrient material of tapetal secretion. In contrast to this normal behaviour, the mutant plant reveals the clear cytoplasmic connections between 2 or more cells (Figs. 1,2). Cytomixis involving 2 or 3 cells were quite common. However, telophase I reveals 4-celled event of cytomixis with a rare occurence. Some cytomictic cells were observed with the evidence showing the migration of chromatin material through the cytoplasmic channel (Fig. 1). Cytomixis was observed between the different stages as well as similar stages of meiotic division (Figs. 2,3). However, cytomictic events between 2 different stages of meiosis were of a rare occurrece. The mutant plant exhibits a toatl of 33.47% cytomictic cells. The meiotic abnormalities encountered were formation of multivalents (3.97%) and stickiness (1.53%) in

TABLE 1: Meiotic abnormalities, cytomictic cells and pollen fertility in mutant plant *D. tortuosum*.

| | | Meiotic abnormalities | Cytomixis | Pollen grains |
|---|---|---|---|---|
| No. of cells/pollen grains scored | | 2426 | 1691 | 7497 |
| No. of cells with aberrations/ cytomixis/ No. of mini- and sterile pollen grains | | 583 | 566 | 6289 |
| Diakinesis | Stickiness | 0.78% | 2.07% | |
| | Multivalents | 0.87% | | |
| Metaphase I | Stickiness | 0.75% | 1.95% | |
| | Multivalents | 3.00% | | |
| Anaphase I | Laggards | 1.53% | 7.87% | |
| | Bridges | 1.07% | | |
| Telophase I | Laggards | 2.97% | 8.87% | |
| | Bridges | 2.10% | | |
| | Micronuclei | 2.77% | | |
| Metaphase II | -- | -- | 1.95% | |
| Anaphase II | Laggards | 0.91% | 2.42% | |
| | Bridges | 0.33% | | |
| Telophase II | Laggards | 1.11% | 8.34% | |
| | Bridges | 0.82% | | |
| | Micronuclei | 5.02% | | |

diakinesis and metaphase I (Table 1, Figs. 4,5), laggards (6.52%) and bridges (4.32%) in anaphase/telophase I and II and micronucleate cells (7.79%) in telophase I and II (Fig. 6). Pollen analysis in mutant plant reveals the presence of smaller, densly stained, oval-shaped minipollen grains of average size (26.6 µm) with high frequency of occurrence (46.92%, Fig.8). The size of the minipollen was nearly half of the size of normal pollen grains. The plant also found to exhibit shrunken, empty and unstained sterile pollen grains (36.96%). Normal pollen grains occurred in the studied plant were densly stained, oval in shape and of average size (44.7 µm) with remarkably less frequency (16.12%, Fig.9). The mutant plant showed regular flowering but yielded no seeds.

## DISCUSSION

The occurrence of cytomixis and meiotic chromosomal abnormalities were the interesting events in relation to the formation of minipollen grains and sterile nature of mutant plant.

Cytomixis has a similar effect like that of chromosomal deletion or addition. Only difference being that such chromosomal aberrations belong to intercellular transfer of chromatin material as an effect of physiological disturbance (Ghatnekar 1964). Cytomixis may be caused by the destruction of individual part of the sporogenous tissue or even it may be concurrent interaction among the cells during microspergenesis based on deficiency of nutrients (Bobak & Herich 1978).

There are reports explaining the correlative account of cytomixis with formation of micronuclei and minipollen grains. During cytomixis, the active migration of chromosome or entire nucleus occurs towards the area of cytoplasmic bridge. The migration can take place in one direction (Gottschalk 1970) or in 2 or more directions (Kamra 1960). In such cases, unusual genetic situation arises by getting or losing chromatin material in pollen mother cells undergoing final stage of microsporogenesis. As a consequence of this behaviour the respective microsporocytes may not produce functional germ cells. In addition, the supernumerary nuclei resulted by nuclear migration during cytomixis affect the nucleo-cytoplasmic relation. Consequently, certain disorders being formed like imbalance of nucleo-cytoplasmic ratio which affects the development of respective genome and pollen grain (Gottschalk 1970). Similar events must have occurred in studied mutant plant, leading to the formation of pollen grains of reduced size. The minipollen formed in mutant will be genetically defective because of loss of genetic material thus affecting the fertility of plant. Segregation of unusual number of chromosomes towards the poles in anaphase I and presence of unequal volume of chromatin material in 4 daughter nuclei after anaphases II are also the possible factors leading to the formation of genetically defective, unequal-sized pollen as observed in *Lathyrus sativus* (Shaik & Godward 1972).

Occurrence of good number of micronuclei/multinuleate cells in mutant plant appears to be related to cytomixis. Chromosomes/fragments which have migrated in the cells become persistent and thus giving rise to a micronuclei/multinucleate cells at later stages (Figs. 6,7). Similar results are also recorded by Ghatnekar (1964) in $M_2$ mutant of *Vicia faba*.

Sterile nature of mutant plant is ascribed to the cytomixis and meiotic chromosomal abnormalities. If the chromatin material is lost or transfered by cytomixis, one can expect the pollen mother cells with abnormal chromosomal status which will lead to formation of abnormal pollen.



Later, this leads to the decrease in fertility. Kamra (1960) reported 40% cytomixis in sterile mutant of barley. The chromosomal abnormalities encountered in mutant plant can also lead to pollen abortion and decreases in fertility.

On the basis of reports on cytomixis and observations made in present study, it can be conceived that the formation of minipollen grains, cells with micronuclei and multinucleate cells might be the consequences of cytomixis leading to pollen sterility. In addition, the presence of other meiotic irregularities also might be one of the factors responsible to certain extent for the sterile nature of mutant plant.

## ACKNOWLEDGEMENTS

Financial assistance by University Grants Commission, New Delhi to VDM and research facilities offered by Karnatak University, Dharwad are gratefully acknowledged.

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J. Cytol. Genet. 28: 137-140 (1993)

# EFFECT OF GIBBERELLIC AND ABSCISIC ACIDS ON IN VITRO FLOWERING IN SAFFLOWER (*CARTHAMUS TINCTORIUS* L.)

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(Received 19 April 1993, revised accepted 30 July 1993)

## SUMMARY

In vitro capitula induction was observed in 5 varieties of safflower (*Carthamus tinctorius*) from the inner surface of the cotyledon obtained from 2- or 3-day-old seedlings. Effect of $GA_3$ and ABA on flowers induced in vitro was studied. In general, it was observed that ABA and $GA_3$ reduced the frequency of flowering in all the 5 varieties used when compared to the control. However, with the increase in concentraiton of $GA_3$ (0.002 to 0.008; 0.05 to 0.2 mg/l) and ABA (0.008 - 0.1 mg/l) the percentage of capitula induction decreased. The data recorded also indicate that $GA_3$ has more inhibitory effect on floral induction than ABA.

**Key Words:** *Carthamus tinctorius*, in vitro flowering.

## INTRODUCTION

Since Skoog (1955) observed for the first time occasional de novo floral bud formation in cultured stem segments of *Nicotiana tabaccum*, many investigators started using this technique in order to elucidate the mechanism of floral inducation. Induction of in vitro flowers in safflower from the cotyledonary explant was also reported (Tejovathi & Anwar 1984). In an earlier study, isoperoxidase pattern of induced in vitro capitula in safflower was observed (Tejovathi & Anwar 1986). Most of the published work in different plant systems dealt with the effects of environmental, physiological, nutritional and hormonal factors regulating in vitro flowering. In the present study, a modest attempt is made to understand the effect of a growth regulator, gibberellic acid ($GA_3$) and a growth inhibitor, abscisic acid (ABA) on the frequency of induction of in vitro flowers in safflower.

## MATERIAL AND METHODS

Seeds of five varieties of safflower, A-1, Mangira, APRR-3, Bhima and HUS-305 were sterilised with 0.1% mercuric chloride in a sterile flask in aseptic conditions for 8-10 min and then thoroughly washed with sterile distilled water for 2 or 3 times and were germinated on wet filter paper in sterile test tubes.

Cotyledons (devoid of apical meristem) were excised from 2- or 3- day- old seedlings and inoculated on Murashige & Skoog's (MS) medium supplemented with 0.1 mg/1 NAA, 0.5 mg/1 BAP, 0.008-0.1 mg/1 ABA, 0.002-0.2 mg/1 $GA_3$ and 2% sucrose. ABA and $GA_3$ were obtained from Sigma chemicals USA. The cotyledons were inoculated in such a way that the cut ends of explants were in contact with the medium. All cultures were maintained under continuous flourescent light (800-1000 lux) at 26 $\pm$1°C and were subcultured every 2 wk on the medium of the same composition but supplemented with 9% sucrose.

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## RESULTS AND DISCUSSION

Initiation of shoot buds from the cut ends of the cotyledons in all the 5 varieties was noticed within 10-15 d after inoculation. Floral induction was observed from the cut end of the inner surface of the cotyledon (Fig.1) within 55-60 d in control where flowering frequency ranged from 40% (HUS-305) to 73.3% (Mangira and A-1).

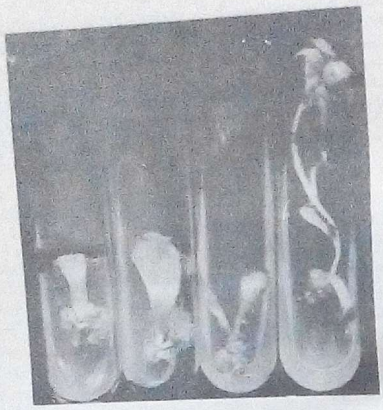

Fig. 1: In vitro induction of capitulum from the cotyledonary explant in safflower.

The data on percentage capitula induction frequency on medium supplemented with different levels of $GA_3$ is given in Table 1. At lower levels of $GA_3$ (0.002-0.01 mg/1) floral induction was noticed within 65-70 d after inoculation while at higher levels (0.05, 0.1, 0.2 mg/1) the formation of capitula was not observed. In general, with increase in the concentration of $GA_3$, the frequency of capitula induction decreased in all the varieties used in this study. However, 0.01 mg/1 of $GA_3$ supplemented medium resulted in relatively increased frequency of capitula induction. Nevertheless, the percentage frequency of capitula induction was more in the control.

Gibberellic acid ($GA_3$) with lower concentrations induced floral buds, while at higher concentrations it showed inhibitory effects both in *Chrysanthemum morifolium* and *Torenia fournieri* (Tanimoto & Harada 1981)

Table 1, shows the data on percentage capitula induction ABA-supplemented media. Observations recorded from different concentrations of ABA-supplemented media shows that with increase in the level of ABA there was a reduction in percentage of capitula induction. Among the different concentrations used (0.009 - 0.1 mg/1) relatively increased frequency of capitula induction



TABLE 1: Effect of $GA_3$ and ABA on in vitro capitula induction in safflower.

| Conc. (mg/l) | Percentage of cultures (genotypes) showing capitula induction | | | | |
|---|---|---|---|---|---|
| | A-1 | Mangira | APRR-3 | HUS-305 | Bhima |
| $GA_3$ | | | | | |
| 0.002 | 33.3 | 26.6 | 13.3 | 6.6 | 10.0 |
| 0.004 | 33.3 | 20.0 | 13.3 | 6.6 | 10.0 |
| 0.006 | 20.0 | 16.6 | 10.0 | 6.6 | 10.0 |
| 0.008 | 13.3 | 13.3 | 6.6 | 6.6 | 6.6 |
| 0.010 | 70.0 | 60.0 | 33.3 | 20.0 | 33.3 |
| 0.05 | - | - | - | - | - |
| 0.1 | - | - | - | - | - |
| 0.2 | - | - | - | - | - |
| ABA | | | | | |
| 0.008 | 66.6 | 53.3 | 46.6 | 33.3 | 36.0 |
| 0.01 | 50.0 | 50.0 | 40.0 | 33.3 | 34.5 |
| 0.05 | 50.0 | 40.0 | 30.0 | 16.6 | 20.0 |
| 0.1 | 16.6 | 13.3 | 10.0 | 6.6 | 10.0 |
| Control (MS + 0.5 BAP + 0.1 NAA) | 73.3 | 73.3 | 46.6 | 40.0 | 50.0 |

was seen when concentration of ABA was 0.008 mg/1. Nevertheless, the percentage of capitula induction was more in control.

Application of ABA on *Pharbitis* and *Chenopodium* could not induce under "strictly non-induction" phase but it enhanced the flowering response of "slightly induced" plants (Zeevart 1976). However, the present study shows that ABA could also stimulate floral induction under a non-inductive phase in safflower. Nitsch (1971) reported that $GA_3$ inhibited flowering while ABA stimulated flowering in short-day plants of *Cichorium intybus*.

The present study shows that $GA_3$ and ABA exert an inhibitory effect on caputula induction when compared to the control. However, $GA_3$ has more inhibitory effect on floral induction than ABA. Among the 5 varieties used in the present study, both $GA_3$ and ABA exhibited the least inhibitory effect on var. A-1 follwed by var. Mangira, while var. HUS-305 was more sensitive.

Generally, it is proposed that $GA_3$ exerts its physiological effect by altering the auxin status of the tissue. Kuraishi & Muir (1964) while working on *Hyosyamus niger* plants noted 40 times increase in the auxin level due to $GA_3$ treatment. In the present study, the MS medium is already supplemented with both the auxin (NAA 0.1 mg/1) and cytokinin (BAP 0.5 mg/1). Addition of $GA_3$ may be responsible for increasing the concentrations of auxins to a level which probably becomes inhibitory/toxic.

Another possible mode of action of $GA_3$ is that it acts at the gene level and cause de-repression of specific genes that are involved in the normal growth and development of the vegetative tissues. The observed inhibitory effect of $GA_3$ on the frequency of induced in vitro flowers may probably be explained by attributing repressive role of $GA_3$ on certain gene (s) that may be involved in flower initiation.

## ACKNOWLEDGEMENTS

We thank Prof. G.M. Reddy and Prof. T.P. Reddy for their interest, encouragement and for necessary facilities to carryout this work. We also acknowledge the financial assistance provided by UGC under a Research Project (No. 3-234/90 RBB-1).

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J. Cytol. Genet. 28: 141-144 (1993)

# SPONTANEOUS CYTOMIXIS IN *JASMINUM*

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(Received 24 June 1993, revised accepted 26 July 1993)

## SUMMARY

In *Jasminum*, cytomixis occurs from early to late stages of meiosis. Cytoplasmic communicating channels between pollen mother cells with or without migrating chromatin materials and nucleoli were noticed. This paper gives an account of spontaneous cytomixis in 22 accessions of *Jasminum sambac, J.pubescens, J.rottlerianum, J.malabaricum, J.arborescens, J roxburghianum, J.cordifolium, J.sessiliflorum, J. rigidum* and *J. ritchei*.

**Key Words:** *Jasminum*, cytomixis.

## INTRODUCTION

The jasmines (Oleaceae) being pollen and seed sterile are propagated almost entirely by vegetative means. Almost all species of *Jasminum* under investigation exhibit the phenomenon of cytomixis. As originally defined by Gates (1911) cytomixis refers to the passage of chromatin materials from one pollen mother cell to the adjacent one through cytoplasmic communicating channels. The occurrence of cytomixis and various meiotic abnormalities in *Jasminum* is spontaneous and not an artefact of fixation and staining as has already been verified (George & Geethamma 1993, Geethamma 1993). Here, the results of an attempt made to illustrate the phenomenon of cytomixis in 22 accessions under 10 species of *Jasminum* are given (Table 1).

## MATERIAL AND METHODS

The flower buds from different species of *Jasminum* were fixed in chlor-acetic-ethanol (1:1:3). A few drops of ferric acetate were added to the fixed materials to increase the stainability of chromosomes. Anthers were smeared in 2% propionocarmine. Photomicrographs of the required stages were taken from fresh preparations. Data on the names of the species analysed, locality of collection, percentage of cytomixis and the stages of its occurrence are recorded in Table 1.

## OBSERVATIONS

The process of meiosis in almost all the pollen mother cells was highly irregular exhibiting an array of meiotic abnormalities of which cytomixis predominates. Cytoplasmic communicating channels between adjacent PMCs with or without chromatin materials and sometimes migrating nucleoli were found (Figs. 1-3, Table 1). In certain cases more than 2 adjacent cells got involved in the process (Fig. 4).

## DISCUSSION

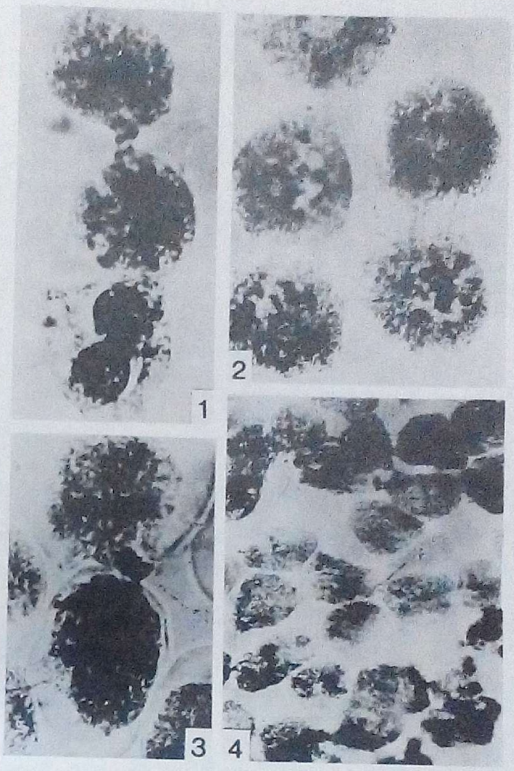
A careful analysis of data in Table 1 reveals that the phenomenon of cytomixis occurs either in early or later stages of meiosis in all the varieties and species of *Jasminum*. In *Oenothera gigas*,

TABLE 1: Data on the species, locality, percentage of cytomixis, stages of its occurrence and pollen sterility in jasmines.

| Species | Locality | Cytomixis (%) | Meiotic stage | Pollen sterility (%) |
|---|---|---|---|---|
| *Jasminum sambac* Ait. | | | | |
| Accn No. 1 | Trivandrum | 55.2 | Prophase I to M I | 81.5 |
| Accn No. 2 | Kariavattom | 53.1 | " | 84.2 |
| Accn No.3 | Coimbatore | 51.9 | " | 58.9 |
| Accn No.4 | Trivandrum | 53.3 | " | 85.2 |
| Accn No.5 | Trivandrum | 62.0 | " | 82.2 |
| Accn No.6 | Coimbatore | 48.2 | " | 78.2 |
| Accn No.7 | Kariavattom | 55.5 | " | 72.3 |
| *J. pubescens* Willd. | | | | |
| Accn No.1 | Trivandrum | 68.45 | Prophase I to A II | 93.5 |
| Accn No.2 | Coimbatore | 81.2 | " | 93.1 |
| Accn No.3 | Bangalore | 80.1 | " | 91.2 |
| Accn No.4 | Kariavattom | 65.0 | " | 89.8 |
| Accn No.5 | Tamil Nadu | 68.52 | Prophase I to M II | 83.5 |
| Accn NO.6 | Munnar | 63.5 | " | 88.2 |
| *J. rottlerianum* Wall. | Kallar | 31.2 | " | 10.2 |
| *J. malabaricum* Wt. | Calicut | 63.5 | " | 78.1 |
| *J. arborescens* Roxb. | Coimbatore | 23.5 | Prophase I to M I | 28.3 |
| *J. roxburghianum* Wall. | Bangalore | 75.3 | " | 86.2 |
| *J. cordifolium* Wall. | Ponmudi | 62.3 | " | 65.5 |
| *J. sessiliflorum* Vahl. | Tamil Nadu | 65.8 | " | 67.8 |
| *J. rigidum* Zink. | Bangalore | 63.5 | Prophase I to A II | 60.3 |
| *J. ritchiei* C. B. Clarke | | | | |
| Accn No.1 | Kallar | 48.7 | " | 92.1 |
| Accn No.2 | Munnar | 55.3 | " | 91.1 |

cytomixis occurs in all the PMCs at all stages of meiosis (Gates 1911) and in a random fashion in certain dicots (Stebbins 1932). However, in *Jasminum* its frequent occurrence from metaphase I to anaphase II even when great care was taken not to cause any traumatic injury or extra pressure to anther sac while fixing and staining suggests cytomixis a spontaneous phenomenon and not an artefact of fixation and staining (George & Geethamma 1983, Geethamma 1993). Cytoplasmic connections with lagging chromatin in them were more frequent after anaphase I. This indicates that apparent migration of nuclear chromatin was either starting or getting completed by these stages and gets manifested and increased in later meiotic stages. Appearance of whole chromatin in one cell leaving the adjacent cell empty was more frequent after anaphase II probably owing to increased inability to divide and separate into daughter cells.






Figs. 1-4: Cytomixis in *Jasmanum* (all x 1250). 1, *J. sambac* (Accn No.1). Cytomixis between 2 PMCs with migrating chromatin materials; a tapetal cell also involved. 2, *J. pubescens* (Accn No.1). Cytomixis between PMCs without chromatin materials. 3, *J. sambac* (Accn No.2). Cytomixis between 2 PMCs with migrating nucleoli. 4, *J. pubescens* (Accn No.2). Several PMCs showing cytomixis.

When the nucleoli are transferred, it may produce an immediate stimulatory metabolic effect in the recipient cell in view of the additional contribution of its RNA content. Cytomixis results in the

production of aneuploid, diploid and polyploid gametes (Sarvella 1958). Though the exact cause of the phenomenon is still not very clear it is well established that migration of genetic materials from one cell to the other leads to discrepancies in chromosome number in individual cells involved (Sakya & Joshi 1990). However, *Jasminum* being pollen and seed sterile, the significance of cytomixis in the origin of aneuploid gametes can be ruled out.

In short, it is inferred that in *Jasminum* both meiotic abnormalities and cytomixis are regularly spontaneous and widespread and appear to be originating from a peculiar gene action or defective gene function bringing about a pathological condition in the cells. Owing to such defective gene function the chromosomes are unable to carry on the dreary process of duplication and movement and lag in condensation and separation in their clumping into unequal masses. Consequently, incomplete cytokinesis and wall formation between cells would be forming bridges which appear like extrusions of cytoplasm with or without chromatin as what is called cytomixis. In *Jasminum*, such an unstable and plastic genetic system with defectively functioning genes causing abnormal meiosis, failure of cytokinesis appearing like cytoplasmic extrusions, sterility of pollen grains and absence of seed set may be a character accumulated through generations of vegetative propagation and domestication.

## ACKNOWLEDGEMENTS

Thanks are due to Prof. Susan Abraham, Department of Botany for facilities. The award of Research Associateship by the University Grants Commission, New Delhi is gratefully acknowledged.

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J. Cytol. Genet. 28: 145-148 (1993)

# CYTOLOGICAL STUDIES OF FOUR SPECIES OF LABIATAE FROM SOUTH INDIA

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## SUMMARY

The chromosome numbers n=26 in *Ocimum canum*, 2n = 50 in *Orthosiphon aristatus*, n = 9 in *Geniosporum elongatum* and n = 17 in *Coleus zeylanicus* are reported here for the first time. It is shown that *O. canum* having n = 13 has evolved as an aneuploid and the taxon with n = 26 is a tetraploid. *G. elongatum* is based on x = 9 and *C. zeylanicus* on x = 17. *O. aristatus* with n = 25 may be an amphidiploid species evolved from 2 dysploid genomes, n = 12 and n = 13, both of which are reported in the genus.

Key Words: Labiatae, cytology, chromosome numbers.

## INTRODUCTION

Tropical South India hosts about 134 species of the Labiatae, distributed in about 29 genera. (Rani & Mathew 1983). Chromosome numbers of about 85 species of these are already known (Gajapathy 1961, Ramachandran 1967, Vembu & Sampathkumar 1978, 1980, Sanjappa 1979, Vembu 1979a, Cherian & Kuriachan 1981, 1984, 1990, 1991, Krishnappa & Basavaraj 1982, Saggoo & Bir 1982, Bir & Saggoo 1985). The results of cytological studies on 4 species of the family are reported here.

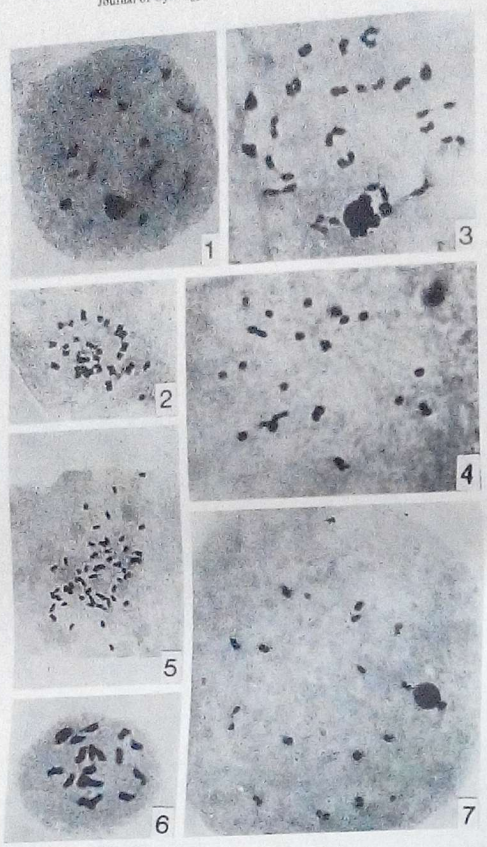
## MATERIALS AND METHODS

Plants for this study were collected from various places in southern India. Root tips were treated with 8-hydroxyquinoline at approximately 4°C prior to fixation. Young flower buds and root tips were fixed in ethanol-acetic acid-chloroform (3:1:1) mixture. The chromosomes were stained in 1% acetocarmine and photomicrographs were taken from temporarily sealed slides. The voucher specimens are preserved in KUBOT.

## RESULTS AND DISCUSSION

*Ocimum canum* Sims.

It is popularly known as hoary basil. The plants are herbaceous, erect, much-branched with white flowers and distributed in the paleotropic regions. The plants collected from Thiruvananthapuram and Comibatore were investigated. Both the above collections were similar in morphological characters but for the size of leaves, inflorescence and flowers, which are larger in the Coimbatore specimens than in the other. The plants from Thiruvanthapuram showed n = 13 (Fig. 1) and 2n = 26 (Fig. 2) chromosomes. The somatic chromosomes were very small ranging from 0.9-3.1 µm in length. The Coimbatore collections showed n=26 (Fig.3). The chromosome number n = 26 is a new count for the species in which several other chromosome numbers such as 2n = 64, 128 (Golubinski 1937), 2n = 24 (Morton 1962), n = 11, 12, 13, 14, 17, 42 (Sharma 1970), n = 32 + 0-4B (Vij & Kashyap 1976), n = 32 (Sanjappa 1979), n = 40 (Saggoo & Bir 1981), 2n = 22 (Krishnappa &




Figs. 1-7: Chromosome numbers in *Ocimum*, *Orthosiphon*, *Geniosporum* and *Coleus* (all x 1000). 1. *O. canum* (Thiruvananthapuram), Diakinesis (n =13), 2. *O. canum* (Thiruvananthapuram), Somatic metaphase (2n = 26), 3. *O canum* (Coimbatore), Diakinesis (n = 26), 4. *O. aristatus*, Metaphase I (n = 25), 5. *O. aristatus*, Somatic metaphase (2n = 50), 6. *G. elongatum*, Diakinesis (n = 9), 7. *C.zeylanicus*, Diakinesis (n = 17).

Basavaraj 1982), 2n = 24 (Singh & Sharma 1981, Pushpangadan & Sobti 1982) and n = 13 (Saggoo & Bir 1986) are already reported.



Morton (1962) reported that *O. canum* with 2n = 24 is based on x = 12. This suggestion may be accepted as 2n = 24 is reported in this species by a number of workers and as x = 12 is, as shown by Sobti & Pushpangadan (1977), the most frequent number of the *Basilicum* group of this genus. The gametic number n = 13 might have evolved from x = 12. Plants with n = 26 observed during this study are obviously tetraploids of the aneuploid number n = 13.

*Orthosiphon aristatus* (Bl.Miq.) Mukherjee (=*O. stamineus* Benth.)

It is an erect herb with white flowers, conspicuous for very long filiform stamens. It is distributed throughout south-eastern Asia to tropical Australia. Plants of this species collected from Thiruvananthapuram showed n = 25 (Fig.4) and 2n = 50 (Fig.5). The chromosomes were very small ranging from 0.9-2.4 µm in length. This is the first chromosome number report for the species. The basic numbers already suggested for the genus are x = 7 (Morton 1962), x = 11, 12, 13, and 14 (Saggoo & Bir 1985) and x = 14 (Cherian 1990). Aneuploid numbers such as n = 12 and n = 13 are known in more than one species of the genus. (Bolkhovskikb et al. 1969, Mehra & Gill 1972, Vembu & Sampathkumar 1980, Saggoo & Bir 1981, Krishnappa & Basavaraj 1982, Cherian 1990, Cherian & Kuriachan 1984). This may suggest that *O. aristatus* is perhaps an amhidiploid species evolved from 2 dysploid genomes, one with n = 12 and the other with n = 13.

*Geniosporum elongatum* Benth. (= G. *indicum sensu* Gamble)

It is a prostrate, very slender plant with orange flowers, distributed in South India and Ceylon. This species was collected from Kanyakumari. It showed n = 9 bivalents (Fig.6). Chromosome numbers of only 2 other species of the genus are so far known. Morton (1962) has reported 2n = 28 for West African specimens of G. *rotundifolium* and suggested a basic number of x = 7. But *G. tenuiflorum* (=*G.prostratum* Benth.) showed n = 9 (Vembu 1979a, Cherian 1990). This together with the present observation of n = 9 in *G. elongatum* would suggest that probably x = 9 represents the basic haploid level of this genus.

*Coleus zeylanicus* Cramer

It is fleshly, herbaceous medicinal species distributed mainly in Ceylon. The plants collected from Thiruvananathapuram showed n = 17 (Fig. 7) and this is the first report in this species. As polyploid species having chromosome numbers in multiples of 17 are reported in the genus (Ramachandran 1967) *C. zeylanicus* may be considered as a diploid with n = x = 17.

ACKNOWLEDGEMENT

One of us (MC) is thankful to the UGC for the award of a research fellowship.

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J. Cytol. Genet. 28: 149-152 (1993)

# KARYOMORPHOLOGICAL STUDIES IN *ASPLENIUM* AND *CYCLOSORUS*

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## SUMMARY

Karyomorphological studies on *Aplenium grevillei, A. indicum, Cyclosorus arbusculus* and *C. gongylodes* from South India have been made. Both species of *Asplenium* belong to 2A karyotype category while *C. arbusculus* and *C. gongylodes* come under 3B and 2B categories respectively. Until more study on DNA quantity, quality and disposition are made, no definite conclusion can be drawn regarding the karyotype evolution of species investigated here.

Key Words: *Asplenium, Cyclosorus*, karyotype.

## INTRODUCTION

Karyomorphological analysis on a few fern species has been reported earlier by Kuriachan (1979), Kuriachan & Ninan (1974), Bhavanandan (1985) and Sankari Ammal & Bhavanandan (1992) from South India. The detailed karyomorphological findings in 4 species, viz., *Asplenium grevillei, A. indicum, Cyclosorus arbusculus* and *C. gongylodes* are presented here.

## MATERIALS AND METHODS

The materials for the present study were collected from Bonacaud, Ponmudi, Munnar, Erumeli, Nilamboor, Palode, Upper Kothayar and Udhagamandalam. Young root tips were used for somatic chromosome studies. The root tips were fixed in acetic-alcohol (1:3) after a pretreatment with 0.002M 8-hydroxyquinoline at 2-8°C for 2 h. Acetocarmine (2%) was used for chromosome staining. The systems proposed by Stebbins (1958), Levan et al. (1964) and Walker (1985) were followed for karyomorphological analysis.

## OBSERVATIONS

***Asplenium grevillei* Wall. ex Hook. and Grev.**

This species was collected from Bonacaud and Upper Kothayar. The root tip cells revealed 72 chromosomes at metaphase (Fig.1). The chromosome length ranged from 3.5 to 5.9 µm. The complement consisted of 1 pair of M-type, 11 pairs of m-type, 13 pairs of sm-type and 11 pairs of st-type chromosomes (Fig. 1a). The TCL of the haploid complement was 154.7 µm. The F% ranged from 17 to 50. The TF% is 31. The karyotype belonged to 2A category. This species is a diploid on the basic number x = 36.

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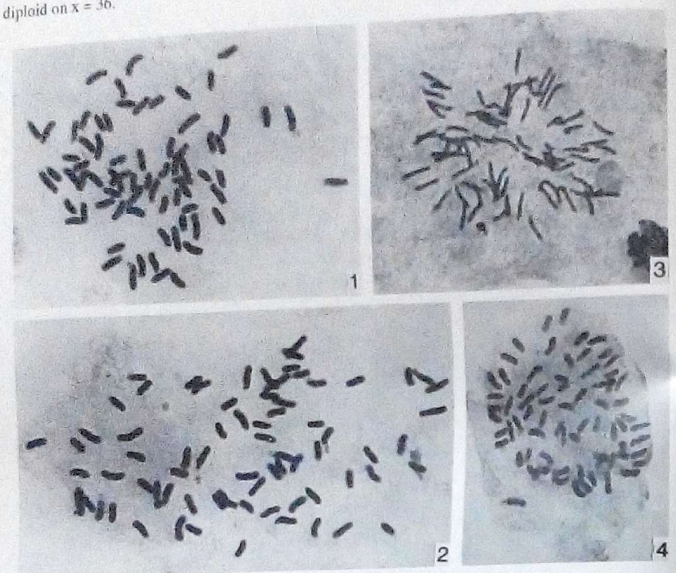
*A. indicum* **Sledge**

The root tip cells of this species were obtained from plants collected from Udhagamandalam. The mitotic cells showed 72 chromosomes at metaphase (Fig. 2). The chromosomes varied in length from 3.2 to 6.3 µm. The somatic complement consists of 14 pairs of m-type, 13 pairs of sm-type and 9 pairs of st-type chromosomes (Fig. 2a). The TCL was estimated as 295 µm. The F% ranged from 19.5-43.8. The TF% was found to be 35.6. The karyotype belonged to 2A category. This species is also a diploid on x = 36.

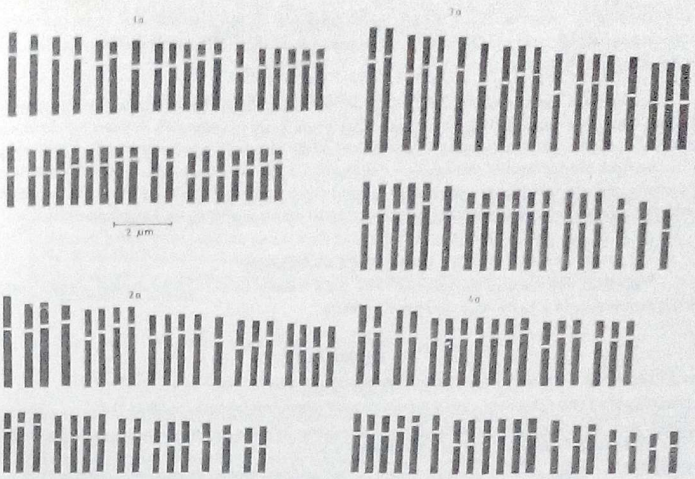



**Figs. 1-4.:** Somatic chromosomes of *Asplenium* and *Cyclosorus*. (all x 840) 1, *Asplenium grevillei*. A root tip cell showing 72 chromosomes at metaphase. 2, *A. indicum*. A root tip cell showing 72 chromosomes at metaphase. 3, *Cyclosorus arbusculus*. A root tip cell showing 72 chromosomes at metaphase. 4, *C. gongylodes*. A root tip cell showing 72 chromosomes at metaphase.

***Cyclosorus arbusculus* Willd.**

This species was collected from Ponmudi, Munnar, Erumeli and Nilamboor. The root tip cells revealed 72 chromosomes at metaphase (Fig.3). The chromosome length varied from 4.0-8.4 µm. The complement consisted of 8 pairs of m-type, 24 pairs of sm-type and 4 pairs of st-type chromosomes






**Figs. 1a-4a:** Idiograms of somatic chromosomes of *Asplenium* and *Cyclosorus*. 1a. *Asplenium grevillei*. 2a. *A. indicum*, 3a. *Cyclosorus arbusculus*. 4a. *C. gongylodes*.

(Fig. 3a). The TCL of the gametic set was 219.3 µm. The range of F% was 17.0-44.8. The TF% was 30.5. The karyotype belonged to 3B category. The species is a diploid on the basic number, x = 36.

*C. gongylodes* **(Schkuhr) Link**

The root tip cells of the materials collected from Palode revealed the presence of 72 chromosomes at metaphase (Fig.4). The chromosome length varied from 2.6 to 5.4 µm. There were 6 pairs of m-type, 23 pairs of sm-type and 7 pairs of st-type chromosomes in the complement (Fig.4a). The TCL of the haploid complement was 147.4 µm. The F% ranged from 15 to 45.8. The TF% was 29.5. The karyotype belonged to 2A category. This species is a diploid on the basic number, x = 36.

## DISCUSSION

Detailed karyomorphological analysis in 4 species, *Asplenium grevillei, A. indicum, Cyclosorus arbusculus* and *C. gongylodes* have been made. All these species are diploids on x = 36. The data reveal that *Asplenium grevillei* and *A. indicum* are almost of the same status in respect of karyotype category. Further, they do not differ much in their F% and TF%. However, they differ much in their TCL value, being 295 µm for *A. indicum* and 154. 7 µm for *A. grevillei* which is almost half of the total TCL value of *A. indicum*. It is not certain whether *A. grevillei* with lower TCL is primitive or advanced

as compared to *A. indicum*. Even though much similarity in the chromosomal types are seen in *Cyclosorus arbusculus* and *C. gongylodes*, they also differ in their TCL, the former having 219. 3 µm and the latter with 147.4 µm.

An increase or decrease in TCL cannot be taken as an indication of primitiveness or advancement since Jones (1984) stated that DNA amount per genome can increase or decrease in evolution, it can be spread amongst all the members of the chromosome complement in equal amounts or in quantities proportional to chromosome length or it may be confined to one or more chromosomes. Therefore, until the species are subjected to detailed DNA study with special reference to the quantity, quality and disposition, no definitive conclusions can be drawn regarding the karyotype evolution.

## ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Prof. Susan Abraham for providing the necessary facilities. One of us (SA) is thankful to the UGC for the award of a research fellowship.

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J. Cytol. Genet. 28: 153-155 (1993)

# THE EFFECT OF FUNGICIDES (BAVISTIN AND DIATHANE M-45) ON CHIASMA FREQUENCY IN PEARL MILLET

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## SUMMARY

An attempt has been made to study the effect of fungicides (Bavistin and Diathane M-45) on distribution of chiasmata between nuclei and within nuclei in the pollen mother cells of *Pennisetum americanum* Schum. It was noted that the fungicides have affected the variance ratio between and within the nuclei. It was found that in the treated populations the variance ratio was higher than in the control. The number of chiasmata at higher doses was lower than that of control. Mostly, positive correlation was recorded among the treated populations.

**Key Words:** *Pennisetum americanum*, fungicides, chiasma frequency.

## INTRODUCTION

Chromosome pairing and chiasma formation during meiosis is under genetic control. These are affected by environmental factors such as variation in temperature, chemical treatments and different types of radiations. A good number of workers have proved the efficacy of some fungicides to induce chromosomal abnormalities in higher plants (Mohan 1975, Soliman & A1-Najjar 1980).

Bavistin and Diathane M-45 are very commonly used by our farmers mainly for seed treatments to help eliminate seed borne diseases and to protect the field crops from fungal diseases. In the present study, an attempt has been made to test if the chiasma frequency, a parameter of wide implications, is altered by these common fungicides in pearl millet (*Pennisetum americanum* Schum.)

## MATERIAL AND METHODS

Dry dormant pure line seeds of the variety Giant Bajra (*Pennisetum americanum*) were soaked in distilled water for 6 h. The seeds were then immersed in Bavistin and Diathane M-45 solutions of 0.05, 0.1, 0.25, 0.5 and 1.0% concentrations. The duration of seed treatment in all the cases was 24 h with intermittent shaking. At the end of the treatment, the seeds were washed thoroughly in running tap water. The treated seeds were sown in nursery beds along with the control. In the field, the plants were kept in the natural conditions and were treated agronomically as usual in regard to irrigation, thinning and weed control. Panicles from treated plants were fixed in acetic-alcohol (1:3) supplemented with a drop of ferric chloride. Anthers were squashed in 2% acetocarmine. The chiasma frequency was calculated in well spread dividing pollen mother cells in flowers of $M_1$ plants at all doses of treatment along with the control. Twenty cells were observed for analysis of variance for chiasma frequency in each case.

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## RESULTS AND DISCUSSION

From Table 1 it is apparent that the variance ratio in the pollen mother cells of the plants grown from the treated seeds were relatively higher in most of the cases except in treatments using 1% Bavistin and 0.5% Diathane M-45. The increased variance ratios are due to the rise in the intranuclear variation in chiasma frequency as compared to internuclear variation. Such a variation might be attributed to the fact that in the treated materials, more than one karyotype might have taken part in the formation of anthers of the flower buds (Sinha & Roy 1976). This might be expected since the seeds, after being subjected to different chemical treatments tend to be chimaeric in nature because of induced mutation at cytological and genetic levels. This was found true for both the chemicals used.

TABLE 1: Analysis of variance of chiasma frequency following fungicide treatments.

| Variation | Mean Chiasmata/ Cell | Mean Sqr. between nuclei | Mean Sqr. within nuclei | Variance ratio | Correlation co-efficient |
|---|---|---|---|---|---|
| Bavistin | | | | | |
| Control | 13.90 | 0.43 | 0.28 | 1.52 | 0.05 |
| 0.05% | 12.95 | 0.32 | 0.19 | 1.63 | 0.07 |
| 0.1% | 13.65 | 0.18 | 0.15 | 0.72 | 0.01 |
| 0.25% | 12.50 | 0.38 | 0.18 | 2.06 | 0.12 |
| 0.50% | 12.45 | 0.35 | 0.18 | 1.88 | 0.10 |
| 1.0% | 11.90 | 0.49 | 0.26 | 1.94 | 0.08 |
| Diathane M-45 | | | | | |
| Control | 13.90 | 0.43 | 0.28 | 1.52 | 0.05 |
| 0.05% | 12.65 | 0.19 | 0.30 | 0.66 | 0.05 |
| 0.10% | 12.85 | 0.36 | 0.20 | 1.80 | 0.09 |
| 0.25% | 12.45 | 0.83 | 0.21 | 3.80 | 0.07 |
| 0.50% | 12.42 | 0.74 | 0.22 | 3.40 | 0.26 |
| 1.00% | 12.28 | 0.70 | 0.21 | 3.30 | 0.23 |

The mean chiasma frequency as recorded in Table 1 was lower in all the doses in both the chemicals. The results show a gradual decrease with increase in concentration in both the cases. This might be explained on the basis that the number of chromosomal interchanges might have increased with the increase of dose. Such a high incidence of chromosomal interchanges at higher dose levels might be responsible for the low chiasma frequency recorded in this case. The low chiasma frequency might also be attributed to the failure of complete pairing. The role of mutated genes in creating such a situation could not be ruled out completely at this stage. The cases of desynaptic mutants have been reported by Riley (1966) in wheat, Sinha & Godward (1969) in *Lens culinaris* and Sinha & Roy (1976) in *Phaseolus*. Such mutants might have arisen due to change in nature of genes or set of genes controlling chiasma formation.



The present study indicates that the chisama frequency is affected by these chemicals a treatments using the fungicide Bavistin produced more disturbances in chromosome pairing tha Diathane M-45. Such a difference between the behaviour of different chemicals with the sam concentrations could be observed in other plant attributes also (Choudhary 1992).

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J. Cytol. Genet. 28: 157-159 (1993)

# STUDIES ON CYTOMIXIS IN *CATHARANTHUS ROSEUS* RAISED FROM GAMMA RAY TREATMENTS

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## SUMMARY

Cytomixis has been found to occur in the $M_1$ generation of *Catharanthus roseus* (L.) G. Don. raised from gamma ray treatment. As a result, in plants exhibiting this phenomenon extra chromosomes were present in many pollen mother cells. Likewise, empty cells devoid of any chromatin materials were also observed. It is suggested that the phenomenon is possibly due to physiological disturbances occurring as a result of the treatment.

**Key Words:** *Catharanthus*, gamma rays, induced cytomixis.

## INTRODUCTION

Cytomixis has been reported as a natural phenomenon in many taxa (Sinha 1985, Banerjee & Sharma 1988) and is known to be induced by various treatments, (Datta & Biswas 1984, Bariar 1985). This phenomenon is also found in hybrids, triploids, haploids and other genetically disturbed populations (Verma et al. 1986). During the course of mutation induction studies in *Catharanthus roseus* using gamma rays, cytomixis was found to occur occasionally in all the treatments. The present paper describes cytomixis occurring in the progenies raised from gamma ray irradiated seeds of *C. roseus*.

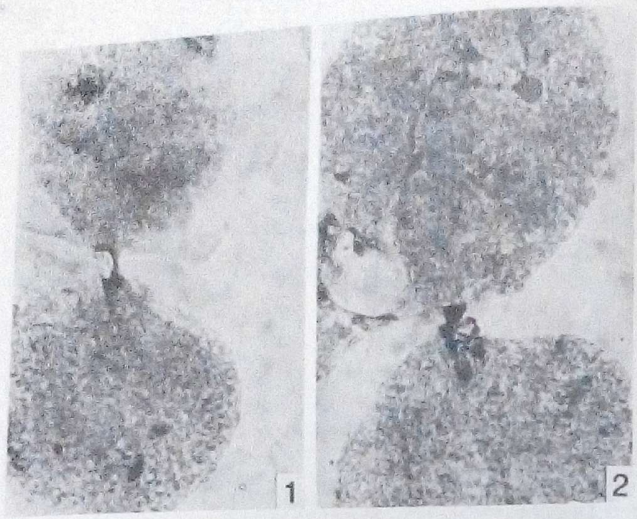
## MATERIAL AND METHODS

*C. roseus* is a medicinally important species. The medicinal principles vincaleukoblastine and vincristine present in *C. roseus* are useful in the treatment of leukemia. During the present study, genetically pure seeds of a locally adapted variety of *C. roseus* were irradiated using different doses of gamma rays (10 kR, 15 kR and 20 kR) to induce variability. Three hundred seeds were used for each treatment. Similar set of untreated seeds grown under identical conditions served as control. $M_1$ generation was raised in the experimental field.

For meiotic studies, flower buds were collected at random from all the treatments and fixed in Carnoy's fluid (3 alcohol : 1 acetic acid). Smear preparations were made using the acetocarmine technique. Pollen mother cells (PMC) from control plants were also examined for comparison with the treatments.

## RESULTS AND DISCUSSION

Control plants of *C. roseus* showed normal divisions and 8 bivalents were clearly observed in each PMC at metaphase I. However, occasionally, in plants raised from different treatments, extrusion of chromatin from one PMC and its entry into the cytoplasm of an adjacent cell has been found to occur. As may be observed from Fig.1 clear chromatin connections and cytoplasmic bridges were observed between cells. The extruded chromatin materials were found in between cells in the




Figs. 1&2: *Catharanthus roseus* (x 1500) 1. Pollen mother cells showing chromatin bridge and cytoplasmic connections. 2. Pollen mother cells showing condensed chromatin between 2 cells.

condensed state as in Fig.2. Extrusion of the entire chromatin of one cell or part of the chromatin materials to another cell resulted in the formation of excess chromatin in many cells. Cells devoid of chromatin were also observed occasionally. The percentage of PMCs showing cytomixis in different treatments are recorded in Table 1. A dose dependent increase in the percentage of cells showing cytomixis is quite evident from the data obtained under different treatments.

TABLE 1: Percentage of cytomictic pollen mother cells showing abnormal chromatin movement in the different treatments and control.

| Treatments | No. of PMCs analysed | Cytomictic PMCs (%) |
|---|---|---|
| 10 kR | 300 | 5.34* |
| 15 kR | 300 | 6.67* |
| 20 kR | 300 | 7.67* |
| Control | 300 | - |

* Significant at 1% level.

Previous reports show the occurrence of cytomixis as a result of different treatments. The phenomenon of cytomixis has been reported as occurring in the PMCs of *Rauwolfia tetraphylla* raised



from gamma ray treatments (Bariar 1985). Datta & Biswas (1984) observed cytomixis in an ethyl methanesulphonate induced mutant plant of *Nigella sativa*. Cytomixis occurring in triploids of mulberry evolved by crossing artificially induced tetraploids with diploids was reported by Verma et al. (1986). Bobak & Henrich (1978) observed cytomixis in the root tip cells of *Vicia faba* treated with the herbicide, trifluraline. Cytomixis in exotic varieties and natural hybrids were also reported by many workers. (Lakshmi & Veeraraghavaiah 1981, Sinha 1985, Banerjee & Sharma 1988). Banerjee & Sharma (1988) observed cytomixis in all the individuals of a particular population of *Rauwolfia serpentina* and suggested a genetic control of this phenomenon. During the present study, cytomixis has been found to occur in progenies raised from different treatments. Possibly, physiological disturbances occurring as a result of the treatment has resulted in the manifestation of this phenomenon.

## ACKNOWLEDGEMENT

The award of SRF by the UGC to one of us (SS) is gratefully acknowledged.

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J. Cytol. Genet. 28: 161-165 (1993)

# MEIOTIC STUDIES ON SOME MEMBERS OF *CASSIA*

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## SUMMARY

Meiotic studies in 9 species of *Cassia*, *C. timorensis*, *C. obovata*, *C. roxburghii*, *C. siamea*, *C. sophera*, *C. spectabilis*, *C. tomentosa*, *C. occidentalis* and *C. surattensis* from South India have been carried out and all the species are tetraploids showing n=14. The chromosome number of *C. timorensis* is reported for the first time.

**Key Words:** *Cassia*, meiosis, tetraploids.

## INTRODUCTION

*Cassia* Linn., belonging to the family Caesalpiniaceae comprises about 600 species (Willis 1973). The cytology of some species of this genus has been reported previously from India (Pantulu 1942, 1960, Sampath & Ramananthan 1949, Ramanathan 1950, 1955, Bir & Kumari 1979, 1981, 1982, Chaudhary & Chaudhary 1988, Saji Mariam George & Bhavanandan 1993) and abroad (Fedorov 1969). A perusal of cytological data shows that the South Indian region received little attention by the cytologists. This fact prompted us to take up the cytological study of *Cassia* from this region.

## MATERIALS AND METHODS

Materials for the present study include 9 species collected from different localities of Kerala and Tamil Nadu. Flower buds were fixed in a modified Carnoy's fluid (6 absolute alcohol : 3 chloroform : 1 glacial acetic acid) and anthers were squashed in 2% acetocarmine. Photomicrographs were taken from temporary slides.

## RESULTS AND DISCUSSION

All the species presently investigated showed n=14 (Table 1, Figs. 1-9). The course of division during meiosis was normal. Previous chromosome reports show that *C. roxburgii* (=*C.marginata*), 2n=26, *C. sophera*, 2n=12 (Chaudhary & Chaudhary 1988), *C. tomentosa*, 2n=24 (Hus 1904, Saxton 1907, Irwin & Turner 1960, Diers 1961), *C. occidentalis*, 2n =13, and 2n = 14 (Bir & Kumari 1979, Gill & Hussaini 1982, Muto 1929, Turner 1956, Irwin & Turner 1960, Miege 1962), *C. surattensis*, 2n = 28 (Pantulu 1960, Irwin & Turner 1960) exist as different cytotypes, either as aneuploids or polyploids.

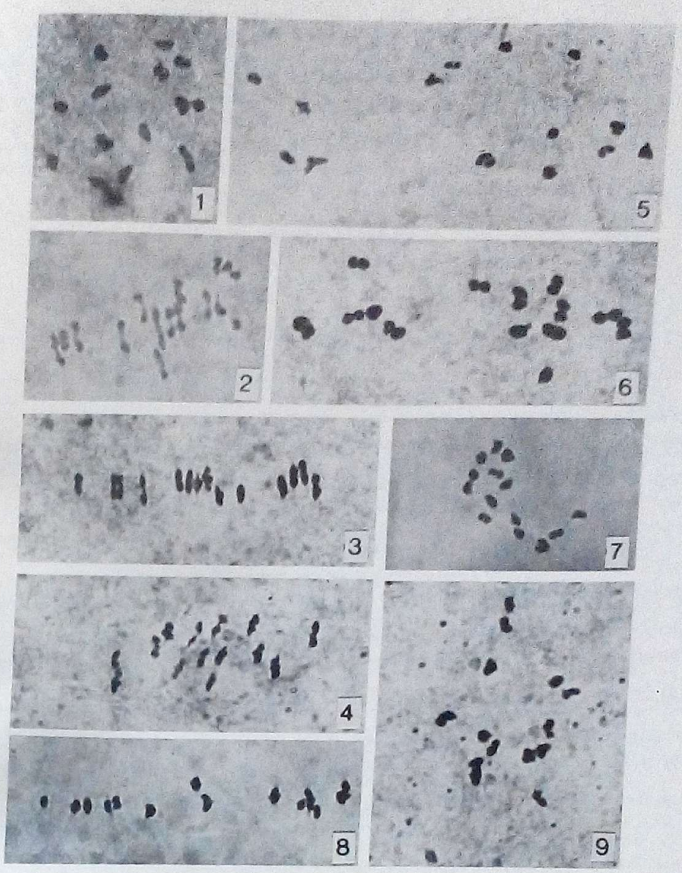
Though *Cassia* is a polybasic genus having a series of basic chromosome numbers such as x = 6, 7, 8, 10, 11, 13, 14 and 15, the members of the Indian subcontinent are based on x = 6, 7 and 8 (Kumari & Bir 1987). Goldblatt (1981) suggests a basic number of x = 7 to be the primary basic number which has undergone an early duplication to with some aneuploidy to x=14 which is common in Caesalpiniaceae with some aneuploidy to x=13 and 12. Fernandes & Queiros (1978) regarded x=14 as secondarily evolved number. A host of cytologists have marshalled considerable chromosome data

TABLE 1: List of materials studied and other supplementary data

| Species | Chrom. No. n | Chrom. No. 2n | Locality | Author/s & Year/s |
|---|---|---|---|---|
| *C. timorensis* DC. | 14 | - | Munnar, Upper Kothayar | Present study |
| *C. obvata* Collad. | 14 | - | Kanyakumari | Present study |
| (*C. obtusa* Roxb.) | - | 28 | | Ramanathan 1950 |
| *C. roxburghii* DC. | 14 | - | Thiruvananthapuram | Present study |
| (*C. marginata* Roxb.) | - | 26 | | Choudhary & Choudhary 1988 |
| *C. siamea* Lamk. | 14 | - | Thiruvananthapuram | Present study |
| | 14 | 28 | | Bir & Kumari 1981, 1982, Choudhary & Choudhary 1988, Pantulu 1942, Irwin & Turner 1960 |
| *C. sophera* L. | 14 | - | Kottarakara | Present study |
| (*C. tenuifolia* DC. *C. sophora* Auth.) | 14 | 28 | | Bir & Kumari 1981, 1982, Irwin & Turner 1960, Pantulu 1960 |
| | 12 | - | | Choudhary & Choudhary 1988 |
| | - | 24 | | Ramanathan 1955 |
| *C. spectabilis* DC. | 14 | - | Thiruvananthapuram, Coimbatore | Present study, Gill & Husaini 1982 |
| *C. tomentosa* L. | 14 | - | Udhagamandalam | Present study |
| | - | 24 | | Hus 1904, Saxton 1907, Irwin & Turner 1960, Diers 1961 |
| *C. occidentalis* L. | 14 | - | Thiruvananthapuram | Present study |
| | 14 | 28 | | Bir & Kumari 1979 |
| | 13 | - | | Gill & Hussaini 1982 |
| | | 26 | | Muto 1929 |
| | | 28 | | Pantulu 1960, Turner 1956 |
| | - | 26,28 | | Irwin & Turner 1960 |
| *C. surattensis* Burm. f. | 14 | - | Kollam | Present study |
| (*C. glauca* Lamk.) | | 28 | | Pantulu 1942, Sampath & Ramanathan 1949 |
| | 28 | 56 | | Irwin & Turner 1960, Pantulu 1960 |

that x=7 would be the original basic chromosome number of angiosperms (Walker 1972). Since n = 14 is the most common haploid number in *Cassia*, it is suggested that the basic chromosome number 7 is well established in the genus.






**Figs. 1-9:** Chromosome numbers of *Cassia* (all x 2000) 1. *C. timorensis*, M I (n = 14), 2. *C. obovata*, M I (n - 14). 3. *C. roxburghii*, M I (n = 14), 4. *C. siamea*, M I (n = 14), 6. *C. spectabilis* M I (n = 14), 7. *C. tomentosa*, M I (n = 14), 8. *C. occidentalis*, M I (n = 14), 9. *C. surattensis* M I (n = 14).

Based on the chromosome data on the North and Central Indian members of *Cassia*, Bir & Kumari (1981) suggested that the genus has a high accelerated pace of evolution through polyploidy (tetraploids, hexaploids, octoploids) and aneuploidy. In contrast to this, the South Indian species of *Cassia* (present study) do not show such an amount of dynamism as shown by their counterparts in the North and Central India. All the species reported in this study from South India are tetraploids on the basic chromosome number x=7 except a solitary report of n = 8 in *C. nigricans* (Saji & Bhavanandan 1993). Thus the cumulative chromosome data from the North, Central and South India show that polyploidy, aneuploidy and hybridization are the chief mechanisms of evolution in *Cassia*.

## ACKNOWLEDGEMENT

One of us (SMG) is grateful to the CSIR, New Delhi for the award of a Senior Research Fellowship.

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J. Cytol. Genet. 28: 167-172 (1993)

# CYTOTOXIC EFFECT OF AQUEOUS LEAF EXTRACT OF *LEUCAS ASPERA* IN *ALLIUM CEPA*

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## SUMMARY

Considering the medicinal use of *Leucas aspera* Spreng. in Bihar, cytotoxic effects of the aqueous extract of its leaves were investigated in the root meristem of *Allium cepa* L. The leaf extract was found to depress the mitotic division to a considerable extent. The absence of any clastogenic abnormalities was an important finding and, hence, the leaf extract of the species was suggested to be safe remedial measure to various human and cattle ailments. The extract, however, caused several non-clastogenic abnormalities all of which were due to spindle disturbances. The total cytotoxic effects were concentration- and period-based.

**Key Words:** *Leucas aspera*, leaf extract, cytotoxic effects.

## INTRODUCTION

The leaves of *Leucas aspera* Spreng. (Labiatae), the species locally known as *guma, dulphi, dulpha* in Bihar (India) are used as a remedy to cough and cold (Kirtikar & Basu 1935), fever (Shiraji 1947), headache, scorpion- and snake-bites (interviews conducted by the authors). Juice of the leaves is also applied externally in psoriasis, scabies, skin-eruption and painful swellings (Anonymous 1986). The leaves are given to cattle for inducing their appetite by the rural people in Bihar. The herb has been reported to contain alkaloid (Shiraji 1947, Chatterjee & Majumdar 1969) and glucoside (Shiraji 1947) in addition to various terpenoids. Considering its medicinal use in human beings and cattles, an investigation was undertaken to see the cytotoxic effects, if any, of the leaf extract of *L. aspera* in *Allium cepa* L., as the aqueous extracts of certain plants are known to induce cellular and chromosomal abnormalities (D'Amato 1956, Kato 1957, 1960 Ono 1960, Ono & Tanikuzi 1960a, b, Abraham & Cherian 1976).

## MATERIALS AND METHODS

Fresh young leaves of *Leucas aspera* were collected from the Experimental Garden, Department of Botany, R.K.College, Madhubani, Bihar. The leaves were thoroughly washed in distilled water and 10 g of washed leaves were ground with 10 ml of single glass-distilled water in a glass mortar and pestle. The preparation was filtered in a new but thoroughly washed white cloth and different dilutions, namely 6.25%, 3.12% and 1.56%, were made from this. The concentrations above 6.25% were proved to be lethal. Onion bulbs with clean roots of 1-2 cm were placed at the mouths of tubes containing the above concentrations for 6, 12, 18, 24, 30, 36, 42 and 48 h. After a particular period of treatment, a few root tips were taken out to analyse cytotoxic effects and the bulbs with rest of the roots were transferred to Knop's solution for recovery for 18 h. The treated and recovered root tips were thoroughly washed in glass-distilled water and fixed in acetic-alcohol (1:2) for 45 min, stained in 2% aceto-orcein: N HCL (9:1) for a 1 h and squashed in 45% acetic acid.

## OBSERVATIONS

The root meristem of *Allium cepa* was treated with 6.25%, 3.12% and 1.56% of the leaf extract of *Leucas aspera* for 6,12,18,24,30,36 and 42 h. As far as mitotic index was concerned, the leaf extract was observed to depress it. The results were concentration and period dependents. The maximum mitotic depression of 88.00% was recorded for 24 h when the meristem was treated with 6.25% of the extract. The mitotic depression gradually decreased with the increase in the dilution of the extract. The minimum depression was, therefore, obtained for the concentration 1.56% when treated for 6 h. The mitotic indices noted in the treated cells were far more lower than the indices observed for their respective controls. Recovery was seen to take place in each and every case. Though the degree of recovery was variable, it was seen to be minimum in 42 h treatments for each concentration (Table 1, Figs. 1-3).

TABLE 1: Mitotic index and mitotic depression recorded in *Allium cepa* root treated with *L. aspera* leaf extract.

| Conc. (%) | Period (h) | TCS | Mitotic index M ± SD | | | Mit. Dep. (%) |
|---|---|---|---|---|---|---|
| | | | Control | Treatment | Recovery | |
| 6.25 | 6 | 2500 | 9.62 ± 0.57 | 4.08 ± 0.19 | 5.90 ± 0.44 | 52.39 |
| | 12 | 2500 | 6.94 ± 0.71 | 3.93 ± 0.15 | 4.44 ± 0.44 | 43.37 |
| | 18 | 2500 | 8.27 ± 0.71 | 2.94 ± 0.10 | 3.23 ± 0.26 | 64.44 |
| | 24 | 2500 | 8.62 ± 0.70 | 1.95 ± 0.30 | 2.80 ± 0.48 | 77.37 |
| | 30 | 2500 | 9.02 ± 1.17 | 1.17 ± 0.80 | 3.31 ± 0.50 | 87.02 |
| | 36 | 2500 | 8.00 ± 0.37 | 1.04 ± 0.02 | 3.04 ± 0.12 | 87.00 |
| | 42 | 2500 | 7.00 ± 0.42 | 0.84 ± 0.07 | 1.31 ± 0.07 | 88.00 |
| 3.12 | 6 | 2500 | 8.07 ± 0.23 | 7.00 ± 0.19 | 7.34 ± 0.42 | 13.25 |
| | 12 | 2500 | 8.60 ± 0.44 | 6.26 ± 0.44 | 6.95 ± 0.13 | 27.20 |
| | 18 | 2500 | 9.07 ± 0.51 | 3.57 ± 0.24 | 4.71 ± 0.64 | 60.63 |
| | 24 | 2500 | 8.00 ± 0.27 | 2.47 ± 0.48 | 3.59 ± 0.49 | 69.12 |
| | 30 | 2500 | 9.60 ± 0.33 | 2.13 ± 0.13 | 2.72 ± 0.33 | 67.39 |
| | 36 | 2500 | 9.00 ± 0.42 | 2.02 ± 0.16 | 2.12 ± 0.18 | 77.55 |
| | 42 | 2500 | 8.00 ± 0.35 | 1.80 ±0.23 | 1.90 ± 0.13 | 77.50 |
| 1.56 | 6 | 2500 | 6.47 ± 0.35 | 5.47 ± 0.36 | 5.71 ± 0.34 | 15.45 |
| | 12 | 2500 | 6.81 ± 0.39 | 5.16 ± 0.09 | 5.52 ± 0.76 | 24.22 |
| | 18 | 2500 | 6.18 ± 0.33 | 4.78 ± 0.26 | 5.01 ± 0.40 | 22.65 |
| | 24 | 2500 | 5.84 ± 0.75 | 4.59 ± 0.42 | 4.74 ± 0.28 | 21.40 |
| | 30 | 2500 | 6.01 ± 0.66 | 3.80 ± 0.30 | 4.55 ± 0.32 | 36.77 |
| | 36 | 2500 | 6.47± 0.79 | 3.11 ± 0.19 | 4.33 ± 0.34 | 21.02 |
| | 42 | 2500 | 6.44 ± 0.26 | 2.62 ± 0.25 | 2.87 ± 0.87 | 59.31 |

Conc, Concentration, TCS, Total cells scanned, Mit. Dep., Mitotic depression.

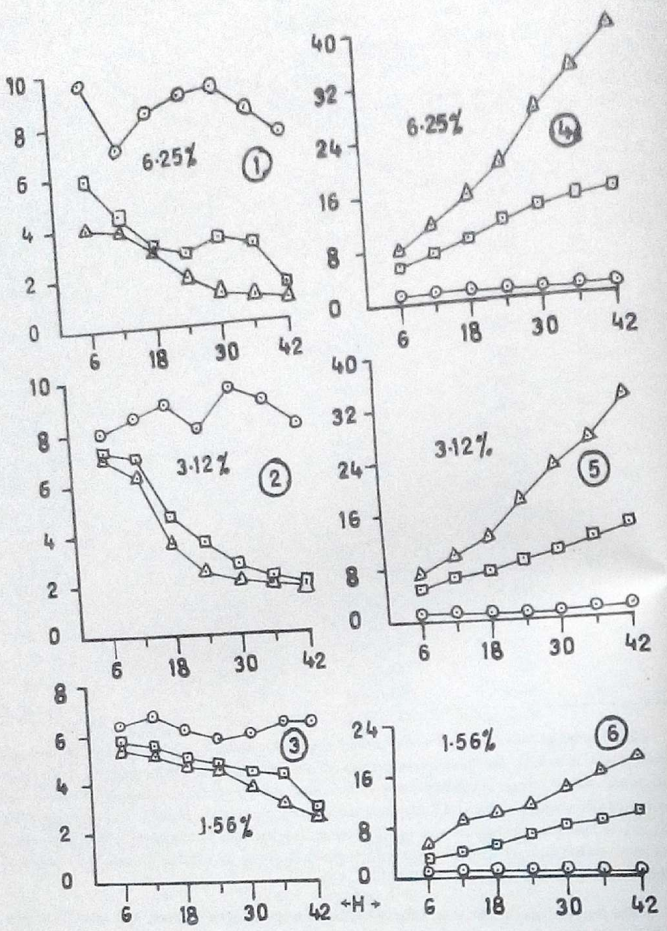


TABLE 2: Non-clastogenic effects recorded in *Allium cepa* root treated with *L. aspera* leaf extract.

| Conc. (%) | Period (h) | TCS | Mitotic index M ± SD | | |
|---|---|---|---|---|---|
| | | | Control | Treatment | Recovery |
| 6.25 | 6 | 2500 | 1.06 ± 0.07 | 8.06 ± 1.12 | 5.45 ± 0.67 |
| | 12 | 2500 | 1.32 ± 0.20 | 11.03 ± 0.77 | 6.97 ± 0.29 |
| | 18 | 2500 | 1.26 ± 0.32 | 15.26 ± 1.14 | 8.77 ± 0.39 |
| | 24 | 2500 | 1.35 ± 0.12 | 19.76 ± 0.79 | 11.03 ± 1.64 |
| | 30 | 2500 | 1.21 ±0.21 | 26.87± 1.23 | 12.87 ± 1.26 |
| | 36 | 2500 | 1.24 ±0.30 | 32.45±1.65 | 13.96 ± 1.03 |
| | 42 | 2500 | 1.35 ± 0.27 | 38.77 ± 2.32 | 15.46 ± 1.33 |
| 3.12 | 6 | 2500 | 1.02 ± 0.03 | 7.12 ± 0.43 | 5.34 ± 0.22 |
| | 12 | 2500 | 1.22 ± 0.06 | 9.15 ± 1.25 | 6.98 ± 0.71 |
| | 18 | 2500 | 1.16 ± 0.04 | 12.45 ± 0.36 | 7.95 ± 0.37 |
| | 24 | 2500 | 1.30 ± 0.10 | 17.50 ± 1.51 | 9.02 ± 1.70 |
| | 30 | 2500 | 1.04 ± 0.09 | 22.88 ± 1.62 | 10.75 ± 1.02 |
| | 36 | 2500 | 1.12 ± 0.11 | 26.42 ± 0.76 | 12.04 ± 0.83 |
| | 42 | 2500 | 1.40 ± 0.21 | 32.33 ±1.67 | 13.77 ± 1.34 |
| 1.56 | 6 | 2500 | 1.04 ± 0.05 | 5.02 ± 0.33 | 3.04 ± 0.15 |
| | 12 | 2500 | 1.20 ± 0.06 | 7.03 ± 0.55 | 4.12 ± 0.24 |
| | 18 | 2500 | 1.35 ± 0.04 | 9.82 ± 0.35 | 5.21 ± 0.87 |
| | 24 | 2500 | 1.15 ± 0.02 | 10.98 ± 0.95 | 6.75 ± 0.27 |
| | 30 | 2500 | 1.25 ± 0.07 | 13.89 ± 0.75 | 8.05 ± 0.47 |
| | 36 | 2500 | 1.02 ± 0.12 | 16.07 ± 1.02 | 8.78 ± 1.01 |
| | 42 | 2500 | 1.12 ± 0.07 | 18.97 ± 1.04 | 10.24 ± 1.21 |

Interestingly, no clastogenic abnormalities could be recorded in any of the concentrations and periods used. However, the three concentrations of the extracts of *L. aspera* induced several non-clastogenic abnormalities including unequal divisions, binucleate cells, clumping, stickiness and thinning of chromosomes, laggard formations and scattered metaphases. In all the concentrations used, while the lowest non-clastogenic abnormalities were recorded for 6 h of treatments, the highest effect was observed for those of 42 h. The total effects gradually increased with the increase in the period of treatments for each concentration (Table 2, Figs. 4-6).

The treated root tips when transferred to Knop's solution for the recovery were found to recover considerably. The pattern of recoveries showed that its percentage gradually decreased with the increase in the hours of treatment and increased with the decrease in the concentration of the extract. In 6.25% of extract 67.72% and 39.88% of recoveries were noted for 6 and 24 h respectively. While




Figs. 1-6 1-3. Mitotic indices. 4-6. Percentages of non-clastogenic abnormalities.

O = Control, Δ = Treatment, □ = Recovery



75.00% and 42.59% of recoveries were calculated for 6 and 42 h in 3.12% of the extract, the lowest concentration of 1.56% showed recoveries of 60.55% and 53.98% in the above two periods of treatments, respectively. Total nonclastogenic effects were always far more higher than the percentage of abnormalities recorded for their respective control sets.

## DISCUSSION

The present investigation carried out on the cytotoxic effects of the leaf extract of *L. aspera* gave rise to certain interesting results. The different concentrations of the extracts were seen to depress the mitotic division appreciably. Its effect on the mitotic index was concentration- and period-dependents. The maximum mitotic depression was observed in 42 h when treated with 6.25% of concentration. On the other hand, the minimum depression was observed for 6 h when treated with 1.56% of concentration. When the mitotic indices of treated root tips were compared with their control sets, it was clear that the aqueous leaf extract of L. *aspera* had the potentiality to decrease the mitotic division to a greater extent.

It was quite significant that no clastogenic abnormality was caused by the leaf extract of this species. The use of the leaves of *L. aspera* as remedial measure to various human and cattle ailments may, therefore, be suggested to be quite safe. The absence of clastogenic abnormality was probably the reason behind the high percentage of recovery met with in the cells carrying induced non-clastogenic abnormalities.

As far as non-clastogenic abnormalities were concerned, except a few, most of the abnormalities were due to spindle disturbances. The total effects were concentration and period-based, that is, the maximum effect (38.77% ± 2.32) was seen in 42 h of treatment when treated with 6.25% of concentration, whereas the lowest effect of 5.02% ± 0.33 was recorded in 6 h for 1.56% of concentration. In all the three concentrations, the effects proportionately increased with the enhancement in the periods of treatment. The total effects for 6.25% varied from 8.06% ± 1.12 to 38.77% ± 2.32, for 3.12% from 7.12% ± 0.43 to 32.33% ± 1.67 and for 1.56% of concentration from 5.02% ± 0.33 to 18.97% ± 1.04.

Like the cytotoxic effects, the patterns of recoveries were also period- and concentration-dependent. While the highest recovery was found for 1.56%, the lowest was exhibited by 6.25% of concentrations. The extent of damage caused by the various periods and concentrations used were expected to be the reason behind this pattern of recovery.

## ACKNOWLEDGEMENT

The first author (M K) is thankful to the CSIR, New Delhi, for the award of Senior Research Fellowship.

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# CROSSABILITY AND MORPHOLOGICAL STUDIES AMONG WILD AND CULTIVATED SPECIES OF *VIGNA*

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(Received 20 March 1993, revised accepted 10 September 1993)

## SUMMARY

The cultivated genotypes of *Vigna radiata* although crossable with their progenitors i.e., *V. radiata* with *V. radiata* var. *sublobata* and *V. mungo* var. silvestris but crossed seeds failed to germinate except in ML 131 x *V. radiata* var. *sublobata* where after germination plants could not survive. It is possible to have a fairly good seed set among cultivated genotypes of *V. radiata* x *V. mungo*. However, crossability, germination and survival percentage of the plants depends upon the parents involved in a cross. Maximum pod setting was achieved in MG125 x UG 473 (75.45%) and minimum in ML 267 x MI-1 (18.8%). The morphological traits scored in hybrids and amphidiploids were observed to have better performances in amphidiploids for most of their characters than their $F_1$ but not their parents.

**Key Words:** *Vigna*, crossability, hybrids, amphidiploids.

## INTRODUCTION

Genetic improvement in *Vigna* has not made much progress, probably due to lack of useful genetic variability. It is, therefore, essential to introgress variable and desirable genes from related wild and cultivated species so as to broaden the genetic base. Restricted gene flow between 2 commonly cultivated species, *V. radiata* (L.) Wilzek (mungbean) and *V. mungo* (L.) Hepper (urdbean) limits the potential for improved production and adaptation of these species in different agroecological systems (Rashid et al. 1987). Interspecific hybridization between compatible species produces at least partially fertile hybrids observed to be greatly enlarged the genetic base of individual agricultural species and helps in broadening the genetic base which may permit to have significant improvement in productivity as well as transfer of desirable genes (Ahuja & Singh 1977). It is, therefore, desired to study crossability of *V. radiata* and *V. mungo* with their progenitors since our experience indicates low crossability of these species with their progenitors. Thus, present study has been extended to study crossability among improved genotypes of mungbean with mash so as to have productive hybrids as well as exploit variability in each species for wider crossability.

## MATERIALS AND METHODS

The experimental materials consisterd of 2 wild species *V. radiata* var. *sublobata* (Roxb). Verdc. and *V. mungo* var. *silvesteris* Lukoki, Marechal and Otoul and 10 cultivars of 2 cultivated species *V. radiata* cvs. MG 125, Pusa 101, ML267, ML131, ML337 and SML32 and *V. mungo* cvs. M218, UG473, MI-I, MI-I-I respectively. Interspecific crosses were made by emasculating and pollinating the flower simultaneously at 8.30 to 11. a.m. or emasculation at 4.30 p.m. and pollination on the next day between 9 and 10 a.m. (Boiling et al.1961). For crossability study, the data was taken on the number of flowers

pollinated, initiation of pods and crossability percentage expressed as the percentage of number of pods set to the number of flowers pollinated.

The morphological characterization had been made by sowing the hybrid seeds in Kharif season in the pots. To induce polypoidy, 10- to 15-day-old hybrid seedlings were treated with 0.25% aqueous colchicine solution at the meristematic region of the terminal buds by keeping the cotton wads for 6 h for 3 consecutive days. Hybrids and amphidiploids so produced scored for the morphological traits such as plant height, leaf texture, leaf shape, leaf size, length of the petiole, colour of the flower, number of pods/plant, pod length, number of full seed/pod and seed colour.

## RESULTS AND DISCUSSION

Crossability percentage among the cultivars of mung (MG 125, Pusa 101, ML 267, ML 131, ML 337, SML 32) and mash (M 218, UG 473, MI-I, MI-I-I) on different combinations along with their progenitors *V. radiata* var. *sublobata* and *V. mungo* var. *silvesteris* is given in Table 1. The maximum pod set was observed in MG 125 x UG 473 (75.45%) and minimum in ML 267 x MI-I (18.75%). The number of pods followed by MG 125 x UG 473 (75.45%) and minimum in ML 267 x MI-I (18.75%). The number of pods followed by MG 125 x UG 473 (75.45%) and minimum in ML 267 x MI-I (18.75%). The number of pods reaching maturity was high in MG 125 x M 218 (11 pods) followed by MG 125 x UG 473 (10 pods) whereas least (1 pod) in Pusa 101 x MI-I-I and ML 276 x UG 473. The number of seeds per pod (normal and shrivelled) also varied and only 38 formed in 11 pods in MG 125 x M 218 and 18-22 seeds were scored in 4 pods in Pusa 101 x MI-I; ML 267 x MI-I; ML 267 x MI-I-I and SML 32 x M 218 respectively. This clearly indicates that the difference in crossability, number of mature pods and number of seeds also depend upon the genotypes of the parents involved.

Among the crosses between wild and cultivated species, the pod percentage in ML 131, ML 267 and *V. radiata* var. *sublobata* ranged from 5.71-26.66% whereas ML 267 x *V. mungo* var. *silvesteris* had 13.33%. Only one pod in these crosses reaching maturity and the number of seeds per pod varied from 2 to 4. As the number of cultivars involved were few, it was suggested that a large number of cultivars should be involved to cross with wild species to identify specific cultivars with high crossability because it is expected that some genotypes of *V. mungo* give better crossability with *V. mungo* var. *silvesteris*, a progenitor of mashbean.

It is generally observed that the hybrid seeds are mostly shrivelled, partially filled and empty (Al-Yasiri & Coyne 1966, Dana 1966, Biswas & Dana 1975) as is evident from Table 2 that very few seeds reach maturity and are viable. These may also be genotype dependent. Crossed seeds were also observed to have wide variation in their germination ability, maximum (72.73%) being in ML 267 x MI-I-I followed by SML 32 x M 218 (70%). Seeds of Pusa 101 x M 218 and ML 267 x MI-I did not germinate. Low seed germination was also reported earlier (Ahn & Hartmann 1978, Biswas & Dana 1975, Shanmugam et al. 1984). Survival percentage also varied and only 105 seedlings survived from variable number of seeds. The variability existed for crossability, germination as well as survival of plants might be dependent upon the compatibility of 2 parents involved in crosses. The present study indicated differences among genotypes which suggested that the large number of genotypes be screened and certain physiological manipulations like growth regulators, embryo culture be restored to



TABLE 1: Crossability and survival percentage of interspecific hybrids of *Vigna* species.

| Combination of female x male | No. of flowers emascu-lated | No. of flowers pollinated | Pod set (%) | Mature pod | | Mature seeds normal + shrivelled (No.) | No. of seeds sown | No. of seeds germinated | Germi-nation (%) | No. of seeds germinated |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No | (%) | | | | | |
| MG 125 x M 218 | 250 | 172 | 70.15 | 11 | 9.0 | 38 | 25 | 5 | 20.00 | - |
| MG 125 x UG 473 | 200 | 154 | 75.45 | 10 | 8.8 | 36 | 29 | 5 | 17.24 | 20.00 |
| MG 125 xMI-I-I | 240 | 160 | 57.50 | 8 | 9.2 | 30 | 23 | 11 | 47.82 | 45.45 |
| Pusa 101 xM 218 | 210 | 65 | 53.84 | 2 | 6.0 | 7 | 4 | 2 | 50.00 | 100.00 |
| Pusa 101 xMI-I | 250 | 50 | 60.00 | 4 | 13.3 | 18 | 4 | - | - | - |
| Pusa 101 x MI-I-I | 200 | 102 | 60.78 | 1 | 1.5 | 4 | 4 | 2 | 50.00 | 50.00 |
| Pusa 101 x UG 473 | 250 | 133 | 67.66 | 3 | 3.3 | 14 | 14 | 3 | 21.43 | 66.66 |
| ML 267 x MI-I-I | 180 | 105 | 33.33 | 4 | 11.2 | 21 | 11 | 8 | 72.73 | 37.50 |
| ML 267 x MI-I | 150 | 80 | 18.75 | 4 | 26.6 | 22 | 16 | - | - | - |
| ML 267 x UG 473 | 200 | 109 | 22.01 | 1 | 4.0 | 2 | 2 | 1 | 50.00 | 100.00 |
| ML 131 x UG 473 | 228 | 168 | 37.50 | 4 | 6.0 | 11 | 10 | 3 | 30.00 | 66.66 |
| ML 337 x M 218 | 225 | 110 | 68.18 | 9 | 12.0 | 15 | 15 | 10 | 66.66 | 20.00 |
| ML 337 x MI-I | 170 | 90 | 66.66 | 7 | 11.6 | 27 | 27 | 15 | 55.55 | 13.33 |
| SML 32 x M 218 | 150 | 30 | 60.00 | 4 | 22.2 | 22 | 10 | 7 | 70.00 | 71.43 |
| ML 267 x *V. mungo* var. *silvesteris* | 100 | 45 | 13.33 | 1 | 6.25 | 4 | 4 | - | - | - |
| ML 267 x *V. radiata* var. *sublobata* | 100 | 70 | 5.71 | 1 | 25.0 | 2 | 2 | - | - | - |
| ML 131 x *V. radiata* var. *sublobata* | 100 | 60 | 26.66 | 1 | 6.25 | 3 | 3 | 1 | 33.33 | - |

TABLE 2: Morphological characteristics of parent hybrids and amphidiploids of *V. radiata* and *V. mungo*

| Genotype/cross | Plant height (cm) | Mean leaflet length (cm) | Mean leaflet width (cm) | Mean leaflet petiole length (cm) | No. of pods/ plant | Mean pod length | No. of fpl seeds/ pod | Seed colour |
|---|---|---|---|---|---|---|---|---|
| ML 337 | 48.50 | 7.78 | 7.74 | 1.34 | 104 | 6.27 | 11.40 | Green |
| Pusa 101 | 62.70 | 11.00 | 9.54 | 1.16 | 44 | 6.70 | 10.20 | Greenish yellow |
| MG 125 | 44.30 | 5.64 | 5.52 | 0.91 | 80 | 5.39 | 10.50 | Greenish yellow |
| ML 267 | 60.00 | 8.42 | 8.19 | 1.70 | 97 | 6.62 | 11.10 | Green |
| M 218 | 30.37 | 6.94 | 2.98 | 0.50 | 32 | 3.60 | 6.70 | Black |
| UG 473 | 31.08 | 7.68 | 3.34 | 0.62 | 20 | 3.49 | 6.50 | Black |
| MI-I-I | 35.26 | 6.92 | 4.25 | 0.95 | 57 | 3.90 | 6.80 | Blackish brown |
| MI-I | 50.20 | 6.50 | 5.63 | 0.94 | 45 | 4.78 | 7.70 | Black |
| Pusa 101 x UG 473 | 48.50 | 7.65 | 4.22 | 1.12 | 10 | 2.26 | 2.37 | Green, Brownish green, Brown |
| MG 125 x MI-I-I | 32.60 | 7.05 | 5.60 | 0.78 | 8 | 3.69 | 3.50 | Green, Brownish green |
| ML 337 x M 218 | 34.06 | 7.79 | 5.83 | 0.68 | 9 | 3.41 | 3.60 | Green, Greenish black |
| MG 125 x UG 473 | 25.90 | 6.82 | 4.09 | 0.75 | 15 | 3.23 | 2.88 | Green, Dark green, Blackish green |
| ML 267 x UG 473 (A1) | 52.40 | 6.69 | 4.67 | 1.50 | 32 | 4.69 | 4.65 | Brown, Brownish green, Black |
| ML 337 x MI-I (A1) | 40.50 | 7.21 | 5.11 | 1.20 | 40 | 5.15 | 5.50 | Brownish black, Brownish green |






Figs. 1-4.: Variation in pod size and seed shape in hybrids and amphidiploids of *V. radiata* (Pusa 101, ML 337) and *V. mungo* (UG 473, MI-I) respectively. 1. Intermediate pod size of a hybrid between Pusa 101 x UG 473. 2. Variation in pod size of an amphidiploid between ML 337 x MI-I. 3. Shrivelled, partially filled and empty seeds of a hybrid between Pusa 101 x UG 473. 4. Bold seed size of an amphidiploid between ML 337 x MI-I.

have better seed set so as to have enough hybrid plants to ensure transfer of genes from wild relatives

Based on morphological studies, the plant height was more in mungbean (44.3-62.7 cm) than mash 30.37-50.20 cm. Hybrid had the plant height intermediate to both the parents and close to urdbean varied from 25.9-48.5 cm except in the hybrid of Pusa 101 x UG 473 where it was 48.5 cm similar to the female parent (Table 2). In 2 amphidiploids ML 267 x UG 473 and ML 337 x MI-1 the plant height varies from 40.5-52.4 cm. The mean leaflet length and width in parents were 5.64-11.0 cm in mung and 2.98-9.54 cm in mash whereas hybrids had leaf width similar to mash parent and leaf length either of mung and mash parent ranging from 4.09-5.83 cm and 6.82-7.79 cm respectively. In amphidiploids both leaflet length and width varied from 6.69-7.21 and 4.67-5.11 cm respectively. Mean leaf petiole length was more in amphidiploids, intermediate in hybrids and nearer to mash type except in Pusa 101 x UG 473 where it was similar to mung parent. Leaf texture varied from subcoriaceous to coriaceous in hybrids, subcoriaceous to smooth in parents and coriaceous in amphidiploids, likewise the leaves were broadly ovate with acute apex in amphidiploids, ovate acute in hybrids and broadly ovate to ovate lanceolate in parents. Same is for colour of the flower which varied from green to greenish yellow to blackish brown (Table 2).

Mean number of pods per plant (32-104) and pod length (5.39-6.7 cm) were higher in mung than in mash (20-57 and 3.49-4.78 cm) and intermediate in hybrids (8-15 and 2.26-3.69 cm) (Fig.1). In amphidiploids, the number increased to $F_1$ hybrids having 32-40 pods and 4.69-5.15 cm pod length (Fig.2). Seed colour in amphidiploids varied from brown to brownish black and green to greenish black in hybrids in comparison to mung and mash parents from green to greenish yellow and black to blackish brown and these were shrivelled, partially filled and empty in hybrids in bold size in amphidiploids (Figs 3,4).

Earlier workers reported that hybrids and amphidiploids carry the morphological characteristics of both or either of the parent (Dana 1966, Subramanian 1980, Dar et al. 1991, Minocha et al. 1991). Satyan et al. (1982) postulated that hybrid plants exhibited a combination of morphological characters of the parents with complete expression of dominance such as colour of epicotyl, plant habit, margin of leaflet, pigmentation of standard petals and sepals irrespective of flower colours. Minocha et al. (1991) indicated that the variable morphological characters increased in amphidiploids compared to hybrids but when compared with parents show the variable trend. It is also indicated that novel and useful recombination can be isolated from segregating generation of interspecific hybrids and by synthesizing amphidiploids which could be valuable material to improve both mungbean and urdbean (Dana & Karamkar 1990). They also suggested that in order to exploit the potential of interspecific crosses, the different species of *Vigna* should be intercrossed extensively with the member, forms and species in their respective primary, secondary and tertiary gene pools with the aid to embryo rescue technique.

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