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CONSANGUINITY STUDIES IN THE MUDUGARS OF ATTAPPADY, KERALA

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SUMMARY

Consanguineous marriages are favoured by most of the hill tribes in Kerala. This paper concerns the results of consanguinity study of one of such tribes, namely the Mudugars of Attappady in Palghat District. The frequency of consanguinity (87.67%) and the mean F (0.06) was found to be very high in the tribe. The most common type of related marriage was the one between first cousins, chiefly the patrilateral cross type. The genetic effects of consanguinity was assessed in terms of various parameters like prenatal mortality (abortions and still births,) postnatal mortality, incidence of various congenital defects and hereditary diseases.

Key Words : Consanguinity, Mudugars

INTRODUCTION

Consanguinity studies in various inbreeding castes and communities from various parts of the world has revealed alarming effects of this phenomenon in most cases. In Kerala, there are innumerable castes and communities and scores of hill tribes in which related marriages are extensively prevalent, most often preferred to and encouraged by social custom and tradition. Consanguinity data on them are very scanty and scattered. (Kumar et al. 1967, Ali 1968, Roychoudhury 1976). Recently, a detailed consanguinity study has been undertaken by us in some of the inbreeding castes and communities and hill tribes in the Kerala State with a view to assess the frequency and genetic effects of inbreeding in them. The paper concerns the results in one of the hill tribes, the Mudugars of Attappady (Palghat District.)

MATERIALS AND METHODS

According to the 1981 census, in Attappady there are only 2590 Mudugars belonging to 531 families living in 22 hamlets. Two hundred and twentysix randomly selected Mudugar families were subjected to the study, and consanguinity data collected by direct interviews. Mortality factor was classified as abortions (foetal loss up to 28 weeks of gestation), still births (after 28 weeks), neonatal mortality (first 28 days after live birth), post-neonatal mortality (28 days to 1 year) and child death (1 to 10 years). The first two types constitute prenatal mortality and the rest postnatal. Z-tests have been used to test the significance of the differences between proportions of the different parameters.

RESULTS AND DISCUSSION

Data of different types of marriages and their frequencies are furnished in Table 1. First cousin marriages constitute the bulk (74. 78%) with the patrilateral cross

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type predominating. The total frequency of consanguinity in the group was 87.61% and the mean coefficient of inbreeding (F) was 0.06. According to Roychoudhury (1976), the tribal groups in Kerala in general are highly inbreeding, and his pooled data showed 63.73% consanguinity and a mean F of 0.04. The strikingly high rate of consanguinity in the Mudugars may be the result of the group being mostly endogamous and illiterate.

TABLE 1 : Frequencies of various types of marrriages in the Mudugars.

Type of marriage	Number	%
Double first cousin	23	10.18
First cousin	169	74.78
a. Patrilateral cross type	103	45.58
b. Matrilateral cross type	66	29.20
One and half first cousin	2	0.88
Second cousin	4	1.77
Total consanguineous	198	87.61
Non-consanguineous	28	12.39

The mortality data at different stages are furnished in Table 2. Prenatal and postnatal mortality in the consanguineous group of families were 16.20% and 24.21% respectively as against 10.83% and 11.21% in the control, though in all the stages the mortality was higher in the former. No significant increase was noted in the prenatal and neonatal mortality, which may be due to the continued practice of consanguinity over several generations which in turn may have resulted in narrowing the differentials of human reproduction and its wastage. Similar situation has been reported in certain other inbreeding groups studied from Tamil Nadu (Rao & Inbaraj 1977, 1979,) and Karnataka (Bittles et al. 1985, 1987) in South India. However, postneonatal, child and total postnatal mortality showed significant increase in consanguineous families. No consistent relationships were observed between degrees of consanguinity and mortality.

The various hereditary diseases and congenital malformations among the children of consanguineous families were diabetes (0.64%), epilepsy (0.16%), mental retardation/diseases (0.32%), squint eye (0.80%), cleft lip (0.16%), polydactyly (0.32%), syndactyly (0.32%) and congenital deafness (0.32%). Most of these conditions were not noticed in the control group except squint eye (1.05%).

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TABLE 2 : Prenatal and postnatal mortality in Mudugar families.

Level of Mortality	Consanguineous					Control	Difference
	Double first cousin	First cousin	One and half first cousin	Second cousin	Total		
Number of pregnancies	159	794	7	20	980	120	–
Prenatal mortality (%)							
a) Abortions	10.69	12.47	0.00	20.00	12.24	7.50	4.74*
b) Still births	5.63	4.03	14.29	0.00	4.52	3.60	0.92*
c) Total	15.72	15.99	14.29	20.00	16.02	10.83	5.19**
Postnatal mortality (%)							
a) Neonatal	7.52	7.50	16.67	6.25	7.54	6.54	1.00*
b) Post-neonatal	13.01	10.06	20.06	20.00	9.61	2.00	7.61**
c) Child	8.41	9.57	25.00	0.00	9.32	3.06	6.26**
d) Total	26.32	23.54	50.00	25.00	24.21	11.21	13.00**

*Not significant ** Significant at 5% level

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INHERITANCE OF FIVE MORPHOLOGICAL TRAITS IN GROUNDNUT

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SUMMARY

The inheritance of 5 morphological characters was analysed using 3 cross combinations. Plant size was found to be governed by a single gene with short type being recessive. The anthocyanin pigmentation of leaf venation and garnet colour of standard petal were controlled by 2 complementary genes, and corduroy leaflet surface by duplicate recessive genes. The intensity of green colour of foliage seemed to be controlled by 2 loci, *Chl 1 chl 1, Chl 2 chl 2* with additive complementary effects. The inheritance of pigmentation of venation and flower colour was reported for the first time in groundnut.

Key Words : Groundnut, morhological traits, inheritance

INTRODUCTION

Since 1910, the inheritance of several traits in groundnut has been reported (see Wynne & Coffelt 1982, Reddy 1988). *Arachis hypogaea L.* is a stabilized allotetraploid and has been reported to have two genomes, A and B (Smartt et al. 1978). Hence, most of the qualitative characters have been reported to be controlled by at least duplicate genes, (Wynne & Coffelt 1982). In this paper, an account on inheritance of 5 morphological traits has been presented.

MATERIALS AND METHODS

The characteristic features of the parents involved in different crosses were as follows :

Chico : Dwarf (15 cm in height), light green colour of foliage, and small pod size (1 to 2 cm long and 0. 5 to 0. 7 cm broad)

BAU 12 : Normal plant height (30 to 35 cm), dark green foliage, bold pods (3 to 4. 5 cm l x 2 to 2. 5 cm b)

J L 24 : Green colour of leaf venation, orange colour of standard petal

NCAc 927 : Red-purple venation, garnet colour of standard petal

G G 2 : Normal leaflet surface

Tetraploid (J 11 x *A. duranensis*) hybrid :Normal leaflet surface

Eight cross combinations were attempted in *Kharif* 1988 and 1989 between cultivars with contrasting features for determining the inheritance of 5 morphological traits.

The F_1 hybrids were space-planted to obtain high pod number. The F_2 generation was grown in *Kharif* 1989 and 1990. The segregation of different traits was recorded on per plant basis and the ratios were fitted to expected genetic ratios using chi-square

test. The number of segregating populations were noted in the F_3 generation for testing the validity of the conclusions made in the F_2.

RESULTS AND DISCUSSION

1. Plant size

The reciprocal cross Chico x BAU 12 resulted in F_1 hybrids having normal plant size, indicating that dwarf is recessive in nature. The χ^2-value for the F_2 ratios in the reciprocal crosses showed a good fit to a monogenic 3:1 ratio for normal versus dwarf plants (Table 1). This conforms to the observations of Patil & Mouli (1975) The F_3 populations from the dwarf plants did not segregate for normal plants, also confirming the monogenic recessive nature of the dwarf plant size. The symbol *Dw dw* is proposed for the locus governing the plant size in groundnut.

2. Pigmentation of leaf venation

The hybrids of the reciprocal cross J L 24 x NCAc 927 showed pigmentation of veins although the pigment was lighter than in NCAc 927. The segregation of plants with purple-red and those with green colour of venation was in agreement with the expected F_2 ratio of 9 : 7. The F_2 plants without pigmentation of veins bred true for the trait in F_3 [Table 1]. These observations confirmed the digenic complementary nature of inheritance of pigmentation of leaf venation in this cross. Genetics of this character was not reported previously in groundnut. The gene symbols *Pig1 pig1* and *Pig 2 pig2* have been proposed for the two complementary loci governing anthocyanin pigmentation of leaf venation in groundnut. The trait is useful as a marker in identification of hybrids at very early stages of development.

3. Colour of foliage

Light green foliage of 'Chico' was found to be dominant to dark green colour in BAU 12, as observed in the F_1 hybrids. In F_2, the segregation of plants with light green and green foliage showed a good fit to the expected digenic ratio of 11:5 (Table 1). The dark green F_2s bred true, whereas the light green ones gave rise to F_3 populations segregating as well as nonsegregating for the trait. Thus it seemed that at least two dominant alleles of one of the two loci *Chl 1 chl 1* and *Chl 2 chl 2* or one dominant allele each at both the loci are necessary for the regulation of chlorophyll production (by perhaps some other independent locus or loci). The previous studies on the foliage colour reported that dark green colour of leaflets is either monogenic or digenic dominant to light green colour (Badami 1923, Datal 1962, Balaiah et al. 1977). Therefore, a different genetic mechanism seems to operate in this cross.

4. Corduroy leaflet surface

The tetraploid interspecific hybrid cv J 11 x *A. duranensis* was backcrossed as a male to a high yielding cultivar GG 2 for introgression of disease resistance genes from *A. duranensis* to *A. hypogaea* background. The parents as well as the BC_1 F_1 hybrids had smooth leaflet surface. But the BC_1 F_2 generation segregated for plants

TABLE 1 : Segregation data for F_2 progenies and F_3 families from different crosses for 5 morphological traits in groundnut

Cross	Segregation in F_2	Total	χ^2	F_3 families (NS and SG) F_2 phenotype	No. of F_3 families
1. Plant size					
Chico (S) x BAU 12 (N)	191 N 73 S	264	0.99 (3:1)	S	30 S
BAU 12 x Chico	66 N 24 S	90	0.13 (3:1)	–	–
2. Foliage colour					
Chico (LG) x BAU 12 (DG)	181 LG 87 DG	268	0.29 (11:5)	DG	35 DG
3. Colour of venation					
JL 24 (G) x NCAc 927 (PR)	139 PR 83 G	222	3.65 (9:7)	G	All G
4. Corduroy leaflets					
GG 2 (N) x (J 11 x *A. duranensis*)	176 N 11 C	187	0.05 (15:1)	C N	11 C 40(NS)+92(SG)
	52 N 4 C	56	0.08 (15:1)		
5. Flower colour					
JL 24 (OR) x NCAc 927 (GA)	40 GA 33 OR	73	0.06 (9:7)	OR	30 OR

N–normal ; S–dwarf ; LG–light green ; DG–dark green ; PR– purple red ; OR–orange ; GA—garnet ; NS–nonsegregating : SG– segregating ; χ^2–nonsignificant at 5% level

with normal leaflets and mutants with corduroy leaflets (the leaflets had ribbed parellel lateral veins, assuming the surface akin to that of a corduroy fabric). The phenotypic ratios of two large BC_1 F_2 families were analysed and the segregation of normal and the corduroy mutants gave a best fit to the expected F_2 ratio of 15:1. This indicated that corduroy leaflets are governed by duplicate recessive genes. The BC_1 F_3 families grown from selfed BC_1 F_2 normal plants contained both segregating and nonsegregating progenies in the expected ratio 7:8 (Table 1). The gene symbols *Cor 1 cor 1* and *Cor 2 cor 2* have been assigned for the two loci governing corduroy leaflets. Since both the parents involved in the backcross were observed to breed true for smooth leaflet surface, it is assumed that these genotypes had one of the duplicate recessive loci. The duplicate recessive nature of X-ray induced corduroy mutants was reported previously by Loesch & Hammons (1968).

5. Colour standard petal

The garnet colour of standard petal in line NCAc 927 was dominant to the orange flower in JL 24. In F_2, the segregation ratio of plants with garnet flowers and those with orange colour of flowers gave a good fit to the expected digenic complementary ratio of 9 : 7. All the F_2 plants bearing orange flowers bred true and those with garnet flowers gave rise to F_3 populations segregating and nonsegregating for the trait. This is the first report on the inheritance of garnet colour of standard petals in groundnut. The complementary genes responsible for garnet colour are designated as *Gfl 1 gfl 1* and *Gfl 2 gfl 2*. The trait is useful as a genetic marker in identification of F_1 hybrids.

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CYTOLOGICAL STUDIES IN TRITICALE

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SUMMARY

Cytological studies were made in 6 hexaploid Triticale genotypes (Bacum, Rohum, DTS-30, DTS-1019, UC-60 and UC-125) in relation to kernel shrivelling. Data on aneuploidy at mitosis were recorded. No correlation between the degree of kernel shrivelling and cytological abnormalities was found.

Key Words : Triticale, kernel shrivelling, cytological stability.

INTRODUCTION

Kernel shrivelling in Triticale is considered as one of the limitations for accepting Triticale as a commercial crop. The seed shrivelling in Triticale has been attributed to metabolic disorders (Klassen 1970), defects at biochemical level (Lorentz 1972) telomeric heterochromatin of rye chromosomes in Triticale (Reddy 1988). The present study involves the role of cytological stability *verses* kernel shrivelling in 6 hexaploid Triticales which markedly differ in the degree of shrivelling.

MATERIALS AND METHODS

Of the 6 hexaploid Triticale genotypes used in the present study, in two, viz. Bacum and Rohum, the kernel shrivelling was low, in DTS–30 and DTS–1019 the shrivelling was medium and in UC-60 and UC-125 the shrivelling was high. The shrivelling was based on visual and other parameters (Reddy 1989).

Standard cytological procedures were employed for the study of mitosis and meiosis. A minimum of 25 cells were used in data recording and 't' test was employed to find out the significance among the 3 groups of Triticales.

RESULTS AND DISCUSSION

The meiotic behaviour was analysed in 6 hexaploid Triticale varieties to understand the possible relationship between meiotic stability and kernel shrivelling (Table 1). For this purpose, 3 groups of Triticale varieties differing markedly in kernel shrivelling i. e., 'low' 'medium' and 'high' were studied cytologically. (Figs. 1 & 2) The data presented in the Table 1 however, suggest that all the 6 varieties examined did not show any significant differences among themselves in cytological parameters such as ring and rod bivalents, univalents, bridges/fragments and micronuclei. This clearly suggests that kernel shrivelling in Triticale may not be associated with meiotic stability.

Kernel shrivelling in Triticale has been shown to occur due to slow growth of endosperm and/or due to formation of internal cavities in the endosperm

TABLE 1 : Data on kernel shrivelling and cytological attributes in 6 hexaploid Triticale cvs

Sl. No.	Cultivars	Kernel shrivelling	Aneuploidy	Open bivalents	Univalents/ cell	Cells with univalents (%)	Cells with bridges/ fragments (%)	Cells with micronuclei (%)
1.	Bacum	Low	6.93 ± 0.28 (3–8)	2.08 ± 0.36 (0–4)	1.76 ± 0.29 (0–4)	31.33	10.23	14.63
2.	Rohum	Low	7.45 ± 0.34 (3–9)	2.10 ± 0.41 (0–3)	1.80 ± 0.31 (0–4)	32.70	9.43	13.64
3.	DTS 30	Medium	6.69 ± 0.37 (4–8)	2.21 ± 0.29 (0–4)	1.64 ± 0.36 (0–4)	36.41	8.62	10.85
4.	DTS 1019	Medium	6.97 ± 0.41 (4–8)	2.18 ± 0.30 (0–4)	1.68 ± 0.31 (0–4)	35.24	11.33	12.87
5.	UC 60	High	7.15 ± 0.29 (4–9)	2.23 ± 0.29 (0–5)	1.72 ± 0.29 (0–4)	33.29	7.49	12.69
6.	UC 125	High	6.88 ± 0.36 (4–8)	2.14 ± 0.31 (0–4)	1.69 ± 0.33 (0–4)	28.46	9.81	13.81

Range whithin brackets

Figs. 1 & 2. Triticale seeds with various degrees of kernel shrivelling. A. 'Bacous' showing 'low' degree of kernel shrivelling. B & C. 'DTS-30' and 'DTS-1019' showing 'medium' degree of kernel shrivelling. D. 'UC-60' showing 'high' degree of kernel shrivelling.

(Bennett 1974). Aberrant endosperm nuclei as a result of faster nuclear divisions in the developing endosperm are also noticed (Kaltsikes et al. 1975). Kernel shrivelling was also found to depend upon the balance between sink and source (Fischer 1973). Variation in shrivelling could also result from different rye genotypes used in the synthesis of Triticales (Darvey 1973). Reddy and Reddy (1981) attributed the kernel shrivelling to the presence of gibberellin–like substances. Lower amounts of carbohydrates and starch due to limitations in the biosynthetic systems and non-complementation between synthetic and structural components are also found to be responsible for kernel shrivelling. Presence of high alpha amylase was suggested as one of the main causes for kernel shrivelling in Triticale (Klassen et al. 1971). At cytogenetic level, in Triticale genome, rye chromosomes were mainly associated with shrivelling since

aberrant nuclei have been noticed in rye endosperm (Kaltsikes et al. 1975). These aberrant endosperm nuclei were found as a result of anaphase bridges (Bennett 1977). The telomeric heterochromatin of rye chromosomes has been suggested to be the main cause of anaphase bridge formation in developing endosperm (Bennett & Gustafson 1982, Heneen & Brismar 1987 and Reddy 1988). The telomeric heterochromatin has shown to contain late replicating DNA, responsible for bridge formation (Lima-de-Faria & Jaworska 1972). These results indicated that kernel shrivelling is a complex character and controlled by many factors including telomeric heterochromatin of rye omosomes.

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PESTICIDE INDUCED CYTOLOGICAL ABNORMALITIES IN *ALLIUM CEPA* L.

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SUMMARY

The mutagenic effects of two organo-phosphorus pesticides, Malathion and Parathion in *Allium cepa* at the morphological level and at both mitotic and meiotic stages of cell division are reported. Mitotic index is decreased due to the effect of pesticides. The latter induced chromosomal aberrations like fragments, stickiness, gaps and anaphasic bridges in the root tip cells and also in pollen mother cells. These abnormalities suggest the mutagenic nature of the pesticides; Parathion has been found to produce more pronounced mutagenic effect than Malathion.

Key Words : Mutagenesis, pesticides, *Allium cepa*.

INTRODUCTION

Pesticides have improved agricultural production world wide. However, they pose risks including the induction of genetic damage (Epstein & Legator 1971, Tomkins & Grant 1972). Therefore, appropriate assays are necessary to determine the genotoxic properties of pesticides and other agro-chemicals.

Among the pesticides, the organophosphates have almost replaced the organochlorine pesticides in agriculture since they do not produce any residue problem. Most of these pesticides e. g., Malathion and Parathion have alkylating properties (Bedford & Robinson 1972, Wooder & Wright 1981). The main primary routes of their metabolism are oxidation and hydrolysis but being alkylating agents, some of them may cause gene mutations too.

The carcinogenicity of pesticides is also suspected in general since they disturb the cell cycle and damage the chromosomes besides producing toxic effects (Tomkins & Grant 1972, Singh et al. 1978, 1979, Grover & Tyagi 1980, Mishra & Sinha 1982, Amer & Farah 1983, Dekergommeaux et al. 1983, Grover & Mittal 1984, Kaur & Grover 1985 and Rao 1987).

Generally, in such studies only short duration assays are conducted, but the fact remains that this particular type of study does not bring about the screening of long duration effects on chromosomes. This can only be done by sowing the treated seeds in the field and screening the meiotic divisions in the flower buds for chromosomal aberrations. Studies conducted at the meiotic level especially the effects of the test chemicals Malathion and Parathion on *Allium cepa* are scarce. Hence it was proposed to undertake the present experiment to test the mutagenic effect of Malathion and Parathion on the *Allium* system both at the mitotic and meiotic levels.

MATERIALS AND METHODS

Root tips were grown by keeping the onion bulbs over jars containing distilled water for 4 days. Different concentrations of the organo-phosphorus pesticides from pure technical grade stock of Malathion and Parathion were prepared with distilled water : 0.25%, 0.5%, 0.75% and 1%. The bulbs with growing roots placed on both the pesticides were kept for 24 h. Triplicates of all the treatments were maintained as also the controls comprising the treatment with 1 % ethyl methane sulphonate (EMS) C_2 and of C_1 i.e. distilled water.

One of the triplicate sets of all treatments and controls was taken, the root tips were thoroughly washed, excised and fixed in Carnoy's fixative at the end of the period of treatment for cytological study. The root tips were later transferred to 70% alcohol. The other set of bulbs of all treatments was left for recovery in Hoagland's nutrient solution (at pH7.0) subsequent to their treatment. After recovery for a period of 24 h, the root tips of the bulbs were thoroughly washed and sown gently in fine soil contained in wooden boxes. The plants were allowed to grow till flowering and the flower buds were fixed for meiotic analysis in Carnoy's fixative and later stored in 70% ethyl alcohol. Aceto-orcein technique was used to process the root tips for mitotic analysis and aceto-carmine technique was used to study the meiotic abnormalities pertaining to pollen mother cells.

Percentage values of recordings of actual number of cells in division against total number of cells observed in ten random microscopic fields were reported as mitotic index. Percentage values of separate recordings of actual number of cells showing abnormal division such as laggards, fragments, bridges, stickiness and multipolar spindles were recorded under respective categories and later the values of percentage abnormalities of all the categories were pooled as values of total percentage abnormalities per treatment. These values are presented in separate tables pertaining to mitosis and meiosis with respect to Malathion and Parathion.

OBSERVATIONS

The pesticide treatments adversely affected the mitotic index and also induced various types of cell abnormalities at both mitotic and meiotic levels like laggards, fragments, bridges, stickiness of chromosomes and multipolar spindles and were similar to the EMS-treated materials (C_2) (Tables 1, 2). The data suggest that Parathion has more pronounced effect than Malathion. There was a decrease in mitotic index in case of both the treatments and also an increase in the percentage of chromosomal abnormalities with increase in concentrations of the pesticides. Material treated with 0.75% and 1% concentrations yielded negligible number of cells in division thereby indiciating that such concentrations might be lethal. Seedlings treated with 1% concentrations did not survive and hence the meiotic studies could not be conducted in them. The percentage of chromosomal aberrations in meiosis increased with increase in the concentration of the pesticides used (Table 2).

TABLE 1 : Types of abnormalities in the root tip cells of *Allium cepa* after treatment with malathion and parathion

Treatment	Dosage	Total No. of cells *	Mitotic Index	Types of abnormalities (%) *					
				Laggards	Fragments	Bridges	Stickiness	Multipolar spindles	Abnormality (%)
Malathion	0.25%	383 ± 3.3	60	3.78 ± 0.2	1.55 ± 0.2	—	—	—	5.3
	0.50%	368 ± 1.5	53	8.23 ± 0.7	3.80 ± 0.4	—	—	—	12.1
	0.75%	372 ± 4.6	46	9.54 ± 0.3	6.04 ± 0.2	0.57 ± 0.03	—	0.09 ± 0.05	16.2
	1%	377.2 ± 2.6	12	8.89 ± 0.5	5.94 ± 0.3	1.53 ± 0.56	0.41 ± 0.4	0.87 ± 0.51	17.6
Control	C_1 Water	382 ± 1.3	92	—	—	—	—	—	
	C_2 EMS 0.25%	383 ± 2.4	80	5.17 ± 0.3	1.53 ± 0.2	—	0.13 ± 0.1	0.04 ± 0.04	6.8
		374 ± 1.4	52	4.89 ± 0.1	1.55 ± 0.1	0.37 ± 0.2	—	0.20 ± 0.12	7.0
Parathion	0.50%	378 ± 2.1	37	7.90 ± 0.6	3.81 ± 0.4	—	0.86 ± 0.3	—	12.5
	0.75%	377 ± 3.7	22	11.14 ± 0.4	6.40 ± 0.3	0.02 ± 0.33	2.71 ± 0.3	—	17.5
	1%	389 ± 3.1	5	9.54 ± 0.3	6.59 ± 0.3	1.79 ± 0.17	—	0.74 ± 0.1	18.6

* All mean values

TABLE 2 : Types of abnormalities in the PMCs of *Allium cepa* after treatment with malathion and parathion

Treatment	Dosage	No. of PMCs	Misoriented division	Fragments	Bridges	Micronuclei	Percentage of abnormality
Malathion	0.25%	450	19	—	—	—	4.222
	0.5%	810	55	8	8	20	11.234
	0.75%	574	75	25	22	40	28.222
Control	C_1 Water	506	—	2	—	—	0.39
	C_2 E M S	601	37	10	1	—	7.986
Parathion	0.25%	529	20	8	3	1	6.153
	0.5%	615	52	15	2	11	13.008
	0.75%	665	98	48	29	65	36.090

DISCUSSION

The inhibitory effect of pesticides on mitosis is a well known phenomenon and majority of them have rightly been called mitotic poisons (Corbett 1974). The reduction in mitotic index suggests that it results due to the disturbances caused by the pesticides in the internal milieu of the cell during interphase (Grover & Mittal 1984). Sufficient literature exists which explains the molecular mode of action of such chemical pollutants and their effect on genetic material (Epstein & Legator 1971). The occurrence of fragmentation, bridges and stickiness are the different manifestations of chromosome damage caused by the pesticides as suggested by Amer & Farah (1976). According to Sharma (1982), clastogenic quality of the chemical is indicated by fragments, gaps, exchanges, micronuclei and bridges which are mainly seen at mitotic metaphase and anaphase and non-disjunction, irregular chiasmata along with those mentioned above in case of meiosis. He further suggested that study of the meiocytes yield more accurate results for chemical effects since the meiotic events have immediate effect on pollen sterility and seed set. Hence, the effects of the test chemical on chromosome structure, pairing, recombination, chiasma frequency, spindle mechanism and aneuploidy can be monitored accurately for genetic consequences. According to Mishra & Sinha (1982), presence of laggards and multipolar spindles as in the present study besides meiotic aberrations suggest that the chemicals have damaged the spindle apparatus also. It can be stated that though the material subjected to lower doses recovered to a certain extent, to be able to grow to maturity, the higher doses inflicted irreversible mutagenic damage as reported by earlier workers (Grover & Malhi 1988, Amer & Farah 1976, 1980, 1983, Singh et al. 1978, 1979, Fishbein et al. 1970, Epstein & Legstor 1971, Tomkins & Grant 1972, Grover & Tyagi 1980, Kaur & Grover 1985, Corbett 1974, Grover & Mittal 1984 and Mishra & Sinha 1982).

However, with respect to the two chemicals, Malathion and Parathion, most of the previous literature was concerned with mitotic abnormatities in various plant and animal systems. The values of various kinds of chromosomal aberrations obtained in the present study as a result of treatment with Malathion are in agreement with the earlier reports of Wuu & Grant (1966) Singh et al. (1979) and Grover & Mittal (1984) on barley and *Allium* at the mitotic level. The present results are also in agreement with the previous reports on meiotic aberrations in other plants (Wuu & Grant 1967, Singh et al. 1978, Grover & Tyagi 1980 and Kaur & Grover 1985).

The inferences drawn in the present investigation are based on the direct exposure of the root tips of *A. cepa* to Malathion and Parathion. In practice, however, they are applied as foliar sprays or through soil which will not cause cytological abnormalities that are recorded in the present study. Therefore, further studies are necessary for in depth analysis.

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INDUCED GENETIC VARIATION IN THE CELLS OF *BRASSICA CAMPESTRIS* L. VAR. SARSON

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(Received 1 July 1991, accepted 25 October 1991)

SUMMARY

Genetic variability in *Brassica campestris* L. var. Sarson has been induced in vitro. Diploid, pentaploid, hexaploid and more than 6n calli are encountered in case of root culture in different media. The segments of leaf and stem produced calli of various ploidy levels. The repeated subcultures of the calli resulted increase in ploidy level up to *ca* 16n. There are some chromosomal aberrations such as, the occurrence of lagging and precocious chromosomes in the cultured cells. Cells with unilateral distribution of chromosomes are also encountered.

Key Words : *Brassica*, callus, polyploidy, chromosomal aberrations.

INTRODUCTION

The cells and tissues of many plants display a greater degree of genetic stability. Generally, it is observed that the cultivated plants and their calli in cultures tend to become progressively polyploid and aneuploid with increase in age (D'Amato 1977). The prolonged culture of those calli loses morphogenetic potentialities. But it may be regained by frequent subcultures (Bayliss 1975, Torrey 1958, 1967, Partanen 1959, Mitra et al. 1960 and Murashige & Nakano 1965). The degree of totipotency and differentiation is governed by genetic factors. The expression of morphogenetic potency is directly correlated with the behaviour of nuclear material. But, in plants in culture, the chromosome complement is usually unstable in comparision to those grown in vivo. The most common change in nuclear materials is the repeated doubling of basic set of chromosomes (Partanen 1963, 1965). The cause of such change is attributed to the presence of growth hormones (Ancora et al. 1977). Auxins like 2,4-dichlorophenoxyacetic acid (2,4-D) accelerates the rate of polyploidization (Shamina 1966). As reported by Landgren (1976) in pea and Narayanswami & Chandy (1971) in *Datura metel*, the cultured cells usually show the various levels of ploidy. Disrupted cytokinin results in the fusion of nuclei as revealed by the work of Mahlberg et al. (1975). Endoreduplication also occurs as the chief cause of polyploidy in cultured cells of polysomatic species (Patau & Das 1961, Partanen 1963, 1965, Murashige & Nakano 1967 and Bennici et al. 1971).

The present work deals with the chromosomes and ploidy levels in the callus cells of *Brassica campestris* var. Sarson in response to various growth hormones.

MATERIAL AND METHODS

Healthy seeds of *Brassica campestris* L. var. Sarson (2n=20) were cultured on Bourgin and Nitsch's (1967) 'H' medium. Different growth hormones like 2,4-D.

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SUMMARY

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The present work deals with the chromosomes and ploidy levels in the callus cells of *Brassica campestris* var. Sarson in response to various growth hormones.

MATERIAL AND METHODS

Healthy seeds of *Brassica campestris* L. var. Sarson (2n=20) were cultured on Bourgin and Nitsch's (1967) 'H' medium. Different growth hormones like 2,4-D.

kinetin (Kin) and indole-3-acetic acid (IAA) were used at the concentration of 1 ppm each in the above medium. For cytological studies, root tips from 7–8 day-old seedlings grown in vitro were excised and pretreated with 0.029M 8-hydroxyquinoline and fixed in acetic-alcohol (1:3). The stains used were 2% acetocarmine and Feulgen. For somatic preparations of calli, materials were fixed directly in acetic-alcohol without pretreatment.

OBSERVATIONS

The somatic chromosome number of *Brassica campestris* var. Sarson (Fig. 1) showed 2n=20 (Fig. 2). Root, stem and leaf segments when cultured produced highly totipotent calli on basal medium (BM) fortified with 2,4-D 3 weeks after implantation. Cytological preparations of the root calli showed diploid set of chromosomes (Fig. 3). The root calli obtained from BM+ 2,4-D when subcultured on BM supplemented with IAA, Kin and IAA + 2,4-D separately showed polyploid conditions increasing the chromosome number up to 60 (Figs. 4 & 5).

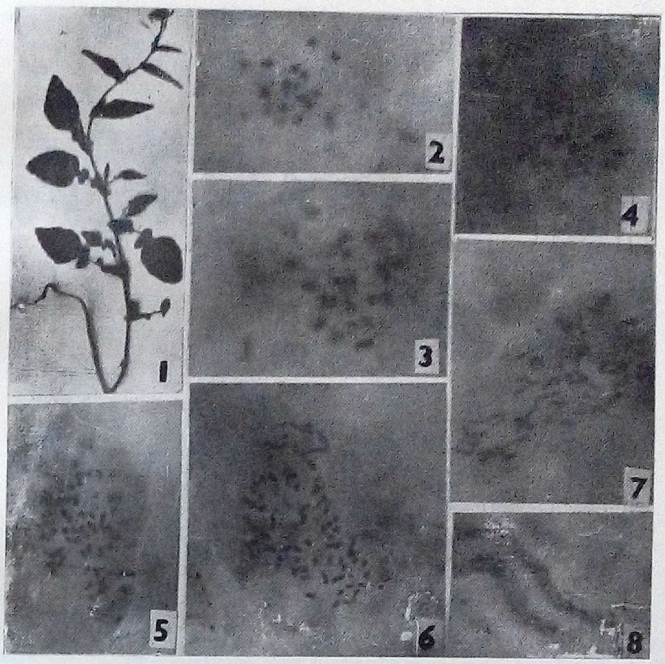
The subcultured stem calli on BM+Kin showed 70 chromosomes (Fig. 6). Cytological preparations of the 9-week-old callus grown on BM+2,4-D+Kin exhibited polyploid condition showing more than 60 chromosomes (Fig. 7). Frequent occurrence of precocious chromosomes have been observed at anaphase stage of polyploid cellsr (Fig 8).

The leaf calli grown on BM+2,4-D when subcultured on BM + Kin, BM + Kin +2,4-D and BM + 2,4-D+ IAA showed variable results. Cytological preparations of 5-and 7-week-old leaf calli on BM + Kin and BM + Kin + 2,4-D showed 70 and 80 chromosomes respectively (Figs. 9 &10). Repeated subculture of the calli on BM+ IAA+2,4-D showed 60 chromosomes (Fig. 11). When IAA was replaced by Kin in the medium, the callus cells showed *ca* 160 chromosomes (Fig. 12). Anomalous anaphase stage with unilateral distribution of chromosomes were also encountered in these cells (Fig. 13).

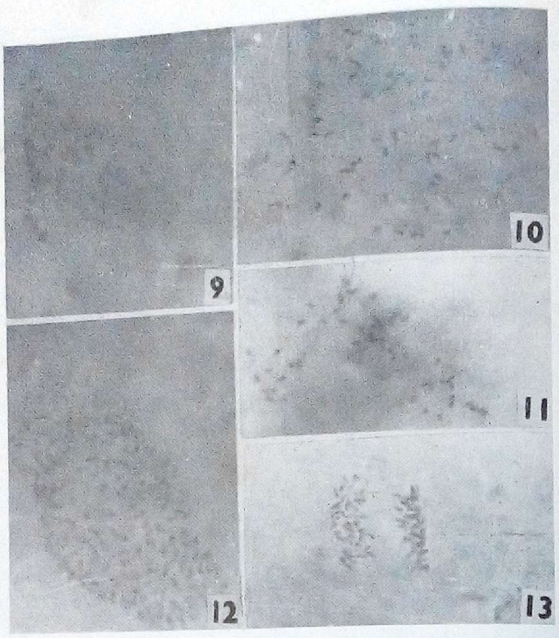
DISCUSSION

The chromosome number or the ploidy level in callus cultures are usually unstable. Polyploidy could be induced in callus cultured in the nutrient medium as revealed by the works of Mitra et al. (1960) and Mitra & Steward (1961). Prasad & Das (1977) working on *Vicia faba* found that 2,4-D inhibited cell division causing the chromosome breaks and other abnormalities. Landgren (1976) investigating on pea root protoplasts inferred that the occurrence of mitotic figures of diploid, tetraploid, octoploid and 16-ploid nuclei might be due to hormonal supply in the culture medium.

In the present investigation, root, stem and leaf calli grown on BM supplemented with 2, 4-D showed no change in the basic set of chromosomes. However, subculture of the root calli on the medium with IAA and 2, 4-D or Kin separately showed polyploid conditions.



Figs. 1-8. *Brassica campestris* var. Sarson. 1. Herbarium specimen (×0.19). 2. metaphase of root tip cell from seeding grown in vivo showing chromosome number 2n=20. 3. 'Metaphase of 3-week-old root callus cells on BM+ 2,4-D. 4. Metaphase of 4-week-old callus cell on BM + Kin + IAA showing more than 60 chromosomes. 5. Metaphase of 8-week-old root callus cell on BM + 2,4-D + IAA showing 60 chromosomes. 6. Metaphase of 8-week-old stem callus cell on BM + Kin showing 70 chromosomes. 7. Metaphase of stem callus cells on BM + 2,4-D + Kin showing 80 chromosomes. 8. Anaphase of stem callus cell on BM + 2,4-D + Kin showing numerous chromosomes. (all X 1167).



Figs. 9-13. *Brassica campestris* var. Sarson. 9. Late metaphase of 5-week-old leaf callus cell on BM + Kin showing 70 chromosomes. 10. Metaphase of 7-week-old leaf callus cell on BM + Kin + 2,4-D showing 80 chromosomes. 11. Metaphase of 5-week-old leaf callus cell on BM + 2,4-D + IAA showing 60 chromosomes. 12. Metaphase of 5-week-old leaf callus cell in repeated subcultures on BM + 2,4-D + Kin showing *ca* 160 chromosomes. 13. Anaphase of 5-week-old leaf callus cell on BM + 2,4-D + IAA showing unilateral distribution of chromosomes. (all × 1167)

The callus derived from stem explant implanted on BM+2,4-D when subcultured on BM+Kin showed 70 chromosomes. On BM+2, 4-D+Kin polyploid conditions with more than 60 chromosomes were also observed and it was found predominant in the culture. Increase in ploidy level was found corresponding to the increase in age of the culture and repeated culture of the calli. Similar

results were observed by Prasad & Das (1977) in *Crepis capillaris*, Mitra et al. (1960) and Evans (1979) in *Nicotiana* species.

In comparision to the callus derived from the root and the stem segments, the leaf callus revealed most variable chromosome numbers. While subculturing the calli in the medium with Kin alone or Kin+2, 4-D or 2, 4-D+IAA, calli with varying ploidy were obtained. After repeated subcultures, most of the cells exhibited more than 70 chromosomes. Some of the cells were found to have *ca* 16n chromosomes on the medium with 2, 4-D and Kin. It showed that the repeated subcultured calli tend to become progressively polyploid with the increase in age of the culture and declined in morphogenetic response resulting failure in differentiating embryoids by the calli with polyploid cells as revealed by the work of Negrutiu et al. (1978) in *Arabidopsis thaliana*.

From the present study, it is indicated that the calli before subculture were mostly diploid. All the subcultured calli showed polyploidy at various levels ranging from 4n to *ca* 16n. Vegetative parts like root, stem and leaf segments produced calli with diploid number in the medium containing 2, 4-D. This callus subcultured in the medium without hormonal supplements remained unchanged in its ploidy level. But, calli subcultured on media with either 2, 4-D or Kin or IAA or their combinations, the ploidy level changed. Repeated subcultures of these calli induced polyploidy at higher level and frequency indicating that the maintenance of culture without any change in their ploidy level is difficult in the presence of hormones for longer time. However, in the absence of hormones, the age of callus did not affect its ploidy level.

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MUTAGENIC EFFECTS OF GAMMA RAYS, EMS, SODIUM AZIDE AND THEIR COMBINED TREATMENTS IN LENTIL

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SUMMARY

Mutagenic effectiveness, efficiency and coefficient of interaction were calculated in M_2 mutagenic populations of three lentil varieties LL-19, P-332 and PL-639. Combined mutagenic treatments were found highly effective. Effectiveness was decreased with increase in mutagenic level. Efficiency was slightly decreased at higher mutagenic level. Individual treatments of EMS and sodium azide were found highly efficient. Coefficient of interaction showed that both additive and synergistic effects occured in combined mutagenic treatments.

Key Words : Lentil, induced mutagenesis.

INTRODUCTION

The response of plant genotype to different physical or chemical mutagens appears as modification of phenotypes. For the plant species these modifications may be neutral or beneficial, others may be detrimental and may cause lethality to the organism. Therefore, the effectiveness and efficiency of the mutagen, and the utility of induced variability depends to a large extent, on induced lethality. For any induced mutation experiment, the selected mutagen must have specificity to act on genes, so that high mutation rate could be achieved. Efficient mutagens are essential for the economical use of mutagens as a tool for direct improvement or for the induction of mutations for certain quantitative or qualitative traits. In the present study, information pertaining to effectiveness, efficiency and coefficient of interaction of different mutagens in three lentil varieties are given. Data on chlorophyll mutation frequencies used in this study are discussed in detail elsewhere.

MATERIALS AND METHODS

Seeds of 3 lentil varieties (LL-19, P-332 and PL-639) were treated with 3 doses of gamma rays (GR) (20, 30, 40 Kr) 3 durations of 0.5% EMS (10, 12, 14 h); 3 concentrations of sodium azide for 4 hours (1, 1.5, 2%) and combined treatments of gamma rays + EMS (20 Kr + 10 h; 30 Kr + 12 h; 40 Kr + 14 h) and gamma rays + sodium azide (20 Kr + 1% ; 30 Kr + 1.5%; 40 Kr + 2%). Data on pollen sterility in M_1 generation and chlorophyll mutation frequencies in M_2 generation were used to determine the following mutagenic parameters :

a) Mutagenic effectiveness $= \frac{M}{Ct}$

Where,

M = Chlorophyll mutation frequency in M_2 generation on M_2 plant basis.

C = dose or concentration of the mutagen
t = duration of the treatment (applicable for only chemical mutagens)

b) Mutagenic efficiency = M/L

Where,

L = pollen sterility in M_1 generation

c) Coefficient of interation (K) = $\frac{(a+b)}{(a)+(b)}$

Where,

(a+b) = chlorophyll mutation frequency produced by the two mutagens in combination treatments

(a)+(b) = chlorophyll mutation frequency produced by the two mutagens applied singly.

RESULTS AND DISCUSSION

Konzak et al. (1965) defined effectiveness of mutagen as a measure of frequency of mutations induced by a unit dose of mutagen. In the present experiment, the mutagen effectiveness was high in combined treatment over individual treatments (Table 1). Among combined treatments both GR + EMS and GR + sodium azide showed equal effectiveness. Among individual treatments, the effectiveness was high in EMS treatments and were followed by sodium azide. These findings were in agreement with Walther (1969) and Gupta & Yashvir (1975). In all the mutagenic treatments, the effectiveness was decreased with increase in dose/duration and concentration of mutagen either in individual or in combined treatments. These results are supported by earlier reports in rice (Siddique & Swaminathan 1968, Reddy & Smith 1984), foxtail millet (Gupta & Yashvir 1975) and lentil (Kumar et al. 1988).

Ehrenberg (1960) described the efficiency as a parameter which gives highest mutation rate due to mutagenicity. According to Konzak et al. (1965), the efficiency gives the proportion of mutations in relation to undesirable effects. In the present study, the efficiency was decreased with increase in GR treatments in all the three lentil varieties (Table 1). Similarly, in individual treatments of EMS and sodium azide, except 12 h, and 14 h treatments of lentil variety P-332 the efficiency was decreased with increase in duration and concentration of mutagens. In combined treatments, the efficiency did not change much in all the three lentil cultivars with the elevation of mutagenic level. Among different mutagens, the efficiency was more in individual treatments of EMS and sodium azide and were followed by combined treatments. The results suggest that although in combined treatments the frequency of mutations is high, the efficiency is decreased. Yadav & Singh (1988) noted a reduction in effectiveness and efficiency with increase in GR treatments in mungbean. They concluded that the biological damage (survival and pollen sterility) increased with increase in dose of GR at a faster rate than increase in the rate of mutations induced. Therefore,

TABLE 1: Different mutagenic parameters in M2 generation in three lentil varieties

Treatment	LL-19			P-332			PL-639			
	Effectiveness	Efficiency	Coefficient of interaction	Effectiveness	Efficiency	Coefficient of interaction	Effectiveness	Efficiency	Coefficient of interaction
1	2	3	4	5	6	7	8	9	10
Gamma rays									
20 Kr	0.046	0.023	—	0.047	0.022	—	0.023	0.011	—
30 Kr	0.032	0.020	—	0.034	0.023	—	0.017	0.011	—
40 Kr	0.025	0.020	—	0.027	0.018	—	0.014	0.011	—
EMS (0.5%)									
10 h	0.438	0.083	—	0.284	0.047	—	0.420	0.089	—
12 h	0.401	0.072	—	0.426	0.081	—	0.438	0.090	—
14 h	0.385	0.068	—	0.407	0.073	—	0.407	0.085	—
Sodium azide (4h)									
1%	0.532	0.081	—	0.392	0.085	—	0.472	0.103	—
1.5%	0.378	0.075	—	0.291	0.059	—	0.400	0.096	—
2%	0.356	0.078	—	0.237	0.052	—	0.381	0.096	—
GR + EMS									
20 Kr + 10 h	0.122	0.056	0.98	0.102	0.043	1.08	0.142	0.065	1.38
30 Kr + 12 h	0.097	0.057	1.04	0.074	0.40	0.74	0.116	0.068	1.33
40 Kr + 14 h	0.080	0.055	1.01	0.064	0.042	0.77	0.109	0.078	1.51
GR + SA									
20 Kr + 1%	0.122	0.058	0.96	0.095	0.044	0.90	0.099	0.050	1.00
30 Kr + 1.5%	0.088	0.058	0.93	0.078	0.051	1.01	0.086	0.062	1.06
40 Kr + 2%	0.077	0.061	0.099	0.061	0.46	0.98	0.067	0.059	1.00

it is concluded that chemical mutagens particularly, EMS was highly efficient than other mutagens, thereby confirming earlier studies of Ehrenberg (1960), Goud (1968) and Gupta & Yashvir (1975). Mutagenic effectiveness and efficiency of physical and chemical mutagens have been compared in small seeded lentils (Sharma & Sharma 1979 and Dixit & Dubey 1986) and in large seeded lentils (Shrama 1990). Sharma (1990) observed that in macrosperma (large seeded) lentils, NMU is found to be more effective and efficient than GR, EMS and sodium azide. In *Phaseolus* (Milkov 1919), highest mutation rate was obtained in combined treatments of GR and EMS. Ignacimuthu & Babu (1988) noted that in urd and mungbean, of the various mutagens tested, the combination of EMS + GR was the most potent both in terms of effectiveness and efficiency, and this has been earlier explained by synergistic effect (Singh et al. 1978 and Bahl & Gupta 1982).

The coefficient of interaction in combined treatments suggest that both additive and synergistic effects occurred (Table 1). Additive effects were noted in 20 Kr + 1% and 40 Kr + 2% treatments of GR + sodium azide, while synergistic effects in the remaining treatments. In the synergistic effects, positive synergism was observed in all the treatments of lentil variety PL-639, and 20 Kr + 10 h (GR + EMS) and 30 Kr + 1.5% (GR + sodium azide) treatments of lentil variety P-332 and 30 Kr + 12 h and 40 Kr + 14 h (GR + EMS) treatments of lentil variety LL-19. Earlier, Siddique & Swaminathan (1968) working with rice noticed additive effects of mutagens for chlorophyll mutations. Both additive and synergistic effects for several other characters were also reported in lentil (Sinha & Godward 1972-73, Sharma & Sharma 1981 and Dixit & Dubey 1984).

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CHLOROPHYLL MUTANTS IN LENTIL – FREQUENCY AND SEGREGATION

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SUMMARY

Using gamma rays, EMS, sodium azide and their combinations, four types of chlorophyll mutants namely albina, chlorina, xantha and virescens were induced in two varieties of lentil LL-19 and P-332. The frequencies were calculated as per cent M_1 plants and per cent M_2 seedlings. The frequencies were increased with increase in mutagenic level. Combined treatments produced slightly higher number of mutants than individual treatments of EMS and sodium azide. The frequency of all the four chlorophyll mutants were lower in gamma rays treatments compared to chemical mutagens. Xantha chlorophyll mutant was found in high frequency. Segregation pattern of the mutants revealed that they are monogenic and recessive in nature.

Key words : *Lens culinaris*, mutagens, chlorophyll mutants.

INTRODUCTION

Chlorophyll mutations have been used as an index in evaluating the mutagenic action of different mutagens in several crops. Chlorophyll mutations, in the past, studied in lentil to examine the response of large and small seeded lentils to various mutagens (Sharma & Sharma 1979, 1981). The present study was undertaken to evaluate different mutagens and their combination with respect to frequeucy of mutation and to study their segregation, in two lentil varieties.

MATERIALS AND METHODS

One hundred seeds each of two lentil varieties namely LL-19 and P-332 were subjected to three doses of gamma rays (20, 30, 40 Kr), three durations of 0.5% EMS (10, 12, 14 h), three different concentrations of sodium azide (1, 1.5, 2%–4 hours each), combined treatments of gamma rays + EMS for three treatments (20 Kr + 10h; 30 Kr + 12h; 40 Kr + 14h) and combined treatments of gamma rays + sodium azide for three treatments (20 Kr + 1%; 30 Kr + 1.5%, 40Kr +2%). Each M_1 plant was harvested separately and plant progenies (plant to row) were grown to raise M_2 generation.

Chlorophyll mutants were recorded following Gustafsson (1940) and their frequencies were calculated as per cent M_1 plants and per cent M_2 seedlings. Chlorophyll mutants recorded in segregating rows at seedling stage. The chi-square test was applied for goodness of fit for segregation of normal to mutant plants.

RESULTS AND DISCUSSION

Chlorophyll mutations were studied in segregating progenies in M_2 generation in two varieties of lentil. Four types of chlorophyll mutants were recorded and

classified according to Gustafsson (1940). They are albina, chlorina, xantha and virescens. The mutagenic frequencies were calculated as per cent M_1 plants and per cent M_2 seedlings (Tables 1-4). Although both the methods gave similar results, the latter method was found more reliable as mutation frequencies calculated as per cent M_1 plants would give relatively underestimated values in most of the cases due to reduction of seeds at higher mutagenic levels (Gaul 1964). Irrespective of the method used, the frequencies increased with the elevation of mutagenic level which confirms most of earlier reports in barley (Gaul 1964), mung bean (Khan 1981, Bahl & Gupta 1982), lentil (Dixit & Dubey 1983) and *Arabidopsis* (Bhatia 1967). Combined mutagenic treatments produced slightly more mutants than individual mutagenic treatments. EMS either alone or in combination induced high frequency of chlorophyll mutants. This confirms that EMS is a more potent chemical mutagen in induction of chlorophyll mutants and supports the earlier reports in a number of crops (Rao & Reddy 1985, Ehrenberg 1960, Heslot et al. 1959, Ramanna & Natarajan 1965, Nerker 1976). Reddy & Gupta (1989) suggested that high frequency of chlorophyll mutations in EMS treatments is perhaps due to preferential action of EMS on genes for chlorophyll development. Earlier,

TABLE 1: Frequency of chlorophyll mutations on the basis of M_1 plant progeny, M_2 plant population in different mutagenic treatments in M_2 generation in lentil cultivar LL-19

Treatment	Population size		Mutation frequency (%)	
	M_1 plants	M_2 seedlings	M_1 plants	M_2 seedlings
Gamma rays				
20 Kr	73	4307	5.47	0.92
30 Kr	70	4160	5.71	0.96
40 Kr	66	3876	6.06	1.03
EMS				
10 h	63	3643	6.43	2.19
12 h	58	3319	6.80	2.41
14 h	50	2954	8.00	2.70
Sodium azide				
1%	67	3742	5.97	2.13
1.5%	61	3521	6.55	2.27
2%	52	2804	7.69	2.85
GR + EMS				
20 Kr + 10 h	55	3268	9.09	3.05
30 Kr + 12 h	50	2842	10.00	3.51
40 Kr + 14 h	46	2651	10.86	3.77
GR + SA				
20 Kr + 1%	58	3412	8.62	2.93
30 Kr + 1.5%	53	3163	9.43	3.16
40 Kr + 2%	49	2648	10.20	3.71

TABLE 2: Frequency of chlorophyll mutations on the basis of M_1 plant progeny, M_2 plant population in different mutagenic treatments in M_2 generation in lentil cultivar P-332

Treatment	Population size		Mutation frequency (%)	
	M_1 plants	M_2 seedlings	M_1 plants	M_2 seedlings
Gamma rays				
20 Kr	72	4216	5.55	0.94
30 Kr	67	3869	5.97	1.03
40 Kr	54	3644	7.40	1.09
EMS				
10 h	61	3498	6.55	1.42
12 h	56	3116	7.14	2.56
14 h	51	2804	7.84	2.85
Sodium azide				
1%	67	3812	4.47	1.57
1.5%	62	3428	4.83	1.75
2%	55	3142	5.45	1.90
GR + EMS				
20 Kr + 10 h	60	3128	8.33	2.55
30 Kr + 12 h	53	2986	9.43	2.67
40 Kr + 14 h	48	2621	10.41	3.05
GR + SA				
20 Kr + 1%	65	3519	7.69	2.27
30 Kr + 1.5%	56	2843	8.92	2.81
40 Kr + 2%	51	2698	9.80	2.96

Freese (1963) noted that EMS is more specific to guanine and cytosine and thus causes alkylation of chloroplast DNA.

In the present study, all the four chlorophyll mutants were recorded in all the treatments with high frequency at higher doses/durations/concentrations of the mutagens. In contrast to the above, Tyagi (1988) recorded a wider spectrum at higher doses in one lentil variety and lower spectrum in another lentil variety. These results clearly indicating varietal variation which was attributed to differences in mutagenic sensitivity, or to number of genes controlling the chlorophyll development in different varieties (Bhatia & Swaminathan 1962).

In the present study, among four chlorophyll mutants, xantha mutant was found in highest frequency in both the varieties, suggesting that genes of xanthophyll development are readily available for mutagenic action. Albina mutants were comparatively less in gamma rays treatments than chemical mutagenic treatments which is in contrast to earlier reports of Gustafsson (1963) and Heslot et al. (1961). Ehrenberg

TABLE 3: Frequency of chlorophyll mutants (per cent M_2 plants) in various mutagenic treatments in M_2 generation in lentil cultivar LL-19

Treatment	Total	Spectrum of chlorphyll mutations (%)			
		Albina	Chlorina	Xantha	Virescens
Gamma rays					
20 Kr	0.92	0.21	0.16	0.37	0.18
30 Kr	0.96	0.22	0.16	0.39	0.19
40 Kr	1.03	0.24	0.19	0.40	0.20
EMS					
10 h	2.19	0.49	0.41	0.68	0.61
12 h	2.41	0.57	0.43	0.74	0.67
14 h	2.70	0.61	0.47	0.91	0.71
Sodinm azide					
1%	2.13	0.41	0.39	0.76	0.57
1.5%	2.27	0.44	0.41	0.83	0.59
2%	2.85	0.55	0.47	1.20	0.63
GR + EMS					
20 Kr + 10 h	3.05	0·51	0.43	0.40	0.71
30 Kr + 12 h	3.51	9.59	0.53	1.56	0.83
40 Kr + 14 h	3.77	0.62	0.57	1.73	0.85
GR + SA					
20 Kr + 1%	2.93	0.39	0.51	1.56	0.47
30 Kr + 1.5%	3.16	0.44	0.59	1.64	0.49
40 Kr + 2%	3.71	0.61	0.63	1.92	0.55
Total	37.59	6.90	6.35	16.09	8.25

et al. (1961) observed high frequency of albina in physical mutagenic treatments and other chlorophyll mutants in chemical mutagens. However, lentils which are not yet fully utilized for mutation breeding experiment and particularly reports of induction of chlorophyll mutants in lentil were almost negligible, therefore, the present results are may not be surprising. A few reports are available in other pulse crops like mung bean (Khan 1981, Bahl & Gupta 1982), where it has been reported that all types of chlorophyll mutants including albina were high in chemical mutagenic treatments.

The data on segregation pattern of the four chlorophyll mutants (Table 5) revealed that they are monogenically controlled and recessive in nature as chi-square values gave a good fit to 3 : 1 ratio in M_2 generation with probability range of 0.05-0.70.

TABLE 4: Frequency of chlorophyll mutants (per cent M_2 plants) various mutagenic treatments in M_2 generation in lentil cultivar P-332

Treatment	Total	Spectrum of chlorphyll mutations (%)			
		Albina	Chlorina	Xantha	Virescens
Gamma rays					
20 Kr	0.94	0.14	0.11	0.56	0.13
30 Kr	1.03	0.16	0.14	0.56	0.17
40 Kr	1.09	0.18	0.15	0.57	0.19
EMS					
10 h	1.42	0.17	0.14	0.80	0.31
12 h	2.56	0.41	0.49	1.21	0.45
14 h	2.85	0.49	0.52	1.35	0.49
Sodium azide					
1%	1.57	0.24	0.19	0.96	0.18
1.5%	1.75	0.28	0.23	1.03	0.21
2%	1.90	0.29	0.25	1.08	0.28
GR + EMS					
20 Kr + 10 h	2.25	0.29	0.33	1.46	0.47
30 Kr + 21 h	2.67	0.31	0.35	1.50	0.51
40 Kr + 14 h	3.05	0.39	0.41	1.61	0.64
GR + SA					
20 Kr + 1%	2.27	0.26	0.29	1.34	0.38
30 Kr + 1.5%	2.81	0.38	0.49	1.53	0.41
40 Kr + 2%	2.96	0.41	0.51	1.60	0.44
Total	31.42	4.40	4.60	17.16	5.26

Lower probability in some cases is due to proportionate reduction in mutant plants. This may be due to the following reasons: (a) The mutation rate increased with increase in mutagenic dose/duration/concentration, but the surviving rate of treated embryos decreased. Moreover to vitality and the seed production of M_1 plants also decreased. Further, the population is too small to be satisfactory for the test of goodness of fit. (b) Because of the chimeras in M_1 plants, a deficit of recessives occurs so that even in large M_2 plants, the mutant genes may not be discernible. (c) Another deficit of recessives due to certation, is a characteristic feature of many mutant genes, due to which the proportion of mutants is reduced in the segregating families.

TABLE 5: Segregation pattern of four chlorophyll mutants in M_2 generation (pooled data is given) in lentil cultivars (first line is the number of segregating rows and ratio of normal to mutant seedlings; second row is the probability of goodness of fit for 3 : 1 ratio)

Mutant	LL-19	P-332
Albina	15, 891 : 263 (0.05–0.10)	15, 816 : 253 (0.30–0.50)
Chlorina	15, 864 : 274 (0.50–0.70)	15, 903 : 287 (0.50–0.70)
Xantha	15, 798 : 249 (0.30–0.50)	15, 791 : 254 (0.50–0.70)
Virescens	15, 869 : 268 (0.20–0.30)	15, 814 : 264 (0.50–0.70)

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COLCHICINE INDUCED TETRAPLOIDY IN *CAJANUS CAJAN* (L.) MILLSP. VAR. ICPL 87

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SUMMARY

Colchicinization in *Cajanus cajan* was achieved in its variety ICPL 87. Successful induction of polyploidy was obtained through cotton swab method with 0.2, 0.25, 0.3 and 0.4 % aqueous solutions of colchicine. The colchiploids showed distinct increase in size of different vegetative and floral parts. The presence of quadrivalents in all the colchiploids confirmed their origin through autotetraploidy.

Key Words : Colchicine, Polyploidy, *Cajanus cajan* var. ICPL 87.

INTRODUCTION

Cajanus cajan (L.) Millsp. var. ICPL 87 is a determinate, short statured but large seeded plant. It tolerates 'Fusarium wilt' and yields over 2000 kg/ha in 120 days at high density when sole cropped with normal and high levels of input. This variety was selected for the induction of tetraploidy because of its superior agronomic traits and also for further utilization of its genome in cytogenetic investigations and in genetic improvement programmes.

MATERIALS AND METHODS

Viability and germination percentage of the seeds were determined before its use as experimental material. Seedlings were raised in experimental plots and were treated with colchicine at 2-leaf stage. The cotton swab method adopted was that of Biswas & Bhattacharya (1971). Treatments with 0.1, 0.2, 0.25 0.3 and 0.4% aqueous colchicine solutions for 6, 12 and 18 hr durations were carried out between 6.30 a.m. and 12.30 p.m. Treatments of 12 hr was completed in 2 and 18 hr in 3 consecutive days with 6 hr treatment per day. Seedlings treated with cotton swabs soaked in distilled water for the same durations were considered as controls. During the treatments, the seedlings were covered with large earthen pots so as to maintain humidity. After completion of the treatments the seedlings were washed thoroughly with distilled water and cotton swabs soaked in distilled water were placed on the apical buds for 6 hr per day during the days of treatment to leach out the excess colchicine. Periodic observations were carried out and data collected with reference to a) length and width of mature leaves, b) leaf thickness, stomatal size and their frequency per unit area, c) approximate date of flowering and the number of flowers per inflorescence, d) size of floral parts, e) meiosis, f) breeding behaviour, g) size variations of the pollen grains and pollen fertilty and h) per cent pod-set and seed-set.

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To study meiosis, flower buds of suitable size were collected in the forenoon between 10 a. m. and 12 noon and were fixed in 1 : 3 acetic acid : ethanol mixture for 24 hr at room temperature. The acetic acid fraction of the fixative was earlier saturated with ferric acetate. The flower buds after fixation were washed with distilled water and were preserved in 70% ethanol at 4°C. Anthers were squashed in 2% acetocarmine and observations were made from freshly prepared slides, pollen grains at the time of anthesis were stained with 1 : 1 mixture of acetocarmine and glycerine and the pollen fertility and size variations were determined. Healthy flowers in tetraploids were selfed and observations on pod-set were made after 5-6 days.

OBSERVATIONS

The plant treated with 0.1, 0.2, 0.25 and 0.3% were more or less normal in their morphological characters. Some of them were more sturdy, with larger leaves and flowers. Those treated with 0.4% colchicine were by and large dwarf, bushy and with smaller and fleshy leaves. Treatments with 0.2, 0.25, 0.3 and 0.4% colchicine resulted in induction of tetraploidy (Table 1). The ideal dose of colchicine to induce autotetraploidy in *C. cajan* var. ICPL 87 was observed to be 0.25% for 12 hr.

TABLE 1 : Percentage frequency of tetraploids obtained with different treatments of colchicine in *C. cajan* var. ICPL 87

Concentration (% w/v)	Duration in hours	Number of seedlings treated	Number of seedlings survived	Number of polyploids obtained	% of polyploids in treated plants	% of polyploids in survived plants
Set-1 0·10	6	50	50	–	–	–
0.10	12	50	48	–	–	–
0.10	18	50	48	–	–	–
Set-2 0.20	6	50	48	2	4	4.2
0.20	12	50	48	6	12	12.5
0.20	18	50	48	8	16	16.7
Set-3 0.25	6	50	48	6	12	12.5
0.25	12	50	46	20	40	43.5
0.25	18	50	46	16	32	34.8
Set-4 0.30	6	50	48	8	16	16.7
0.30	12	50	46	14	28	30.4
0.30	18	50	46	12	24	26.1
Set-5 0.40	6	50	48	4	8	8.3
0.40	12	50	46	6	12	13.0
0.40	18	50	44	4	8	9.1

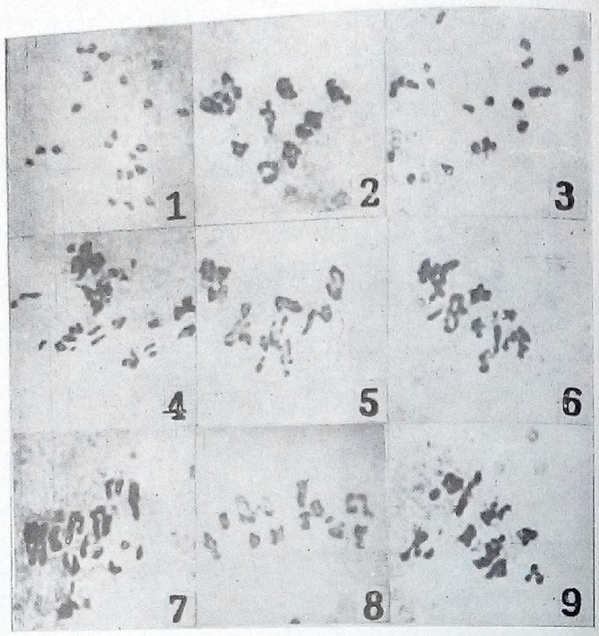
The induced tetraploids of *C. cajan* var. ICPL 87 were distinct from the diploids and were characterized by sturdy habit, broader and thicker leaves, slow initial growth, larger stomata, larger and variable pollen grains, high sterility and lesser pod-setr (Table 2).

TABLE 2 : Details of variation in the morphological characters between diploid and induced tetraploid *C. cajan* var. ICPL 87

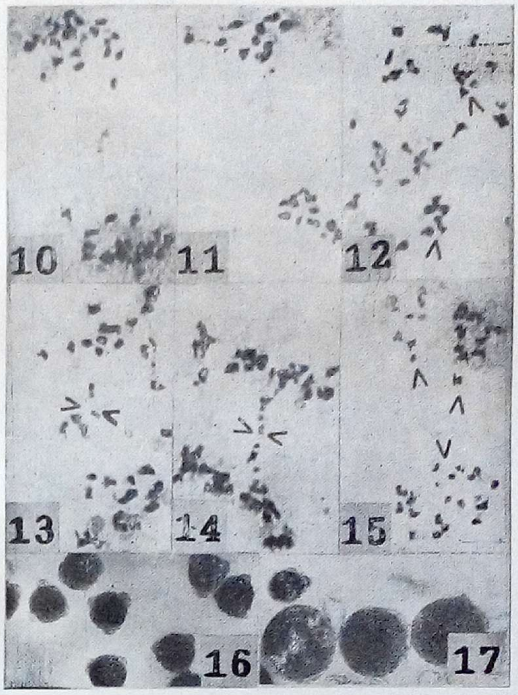
Characters	Diploid Mean ±S.E.	Tetraploid Mean ± S.E.
Length of petiole (mm)	39.18±0.29	41.00±0.30
Length of terminal leaflet (mm)	126.00±0.39	133.50±0.72
Breadth of terminal leaflet (mm)	40.00 0.31	49.30±0.96
Length of lateral leaflet (mm)	94.10±0.29	106.00±0.39
Breadth of lateral leaflet (mm)	43.89±0.75	96.64±0.86
Leaf surface area (mm^2)	1305.70±0.98	2357.30±0.96
Thickness of leaf (μm)	336.30±5.30	562.50±7.60
Number of leaves on main stem	86.94±1.31	49.45±1.43
Number of stomata per unit area on upper epidermis	24.90±0.12	18.00±0·18
Length of guard cells (dorsal) (μm)	22.60±0.10	30.20±0.47
Breadth of guard cells (dorsal) (μm)	15.00±0.38	19.00±0.26
Length of guard cells (ventral) (μm)	19.50±0.50	26.00±0.35
Breadth of guard cells (ventral) (μm)	12.00±0.16	16.70±0.15
Length of pedicel (mm)	9.84±0.74	13.70+0.50
Length of corolla (mm) Standard	19.00±0.69	22.50±0.39
Wings	16.60±0.21	19.00±0.31
Keel	7.82±0.51	9.00±0.31
Breadth of corolla (mm) Standard	17.68±0.05	25.72±0.05
Wings	15.53±0.05	19.70±0.04
Keel	6.72±0.06	8.67±0.04
Lergth of anther (cm)	0.98±0.02	1·21±0.01
Diameter of pollen (μm)	47.80±0.08	56.00±0.29
Pollen fertility (%)	95.50	30.27
Length of pod (mm)	62.00−0.99	33.40±0.64
Thickness of pod (mm)	17.60±0.51	7.85±0.03
Number of pods/plant	44.40±1.36	8.72±0.12
Number of seeds/pod	4–5	1–3

Meiosis in the diploids was regular. At diakinesis, 11 bivalents could be observed. The second division was also regular resulting in normal tetrads and high pollen fertility. In tetraploids the meiosis was very irregular. A wide range of chromosomal associations between the 2 extremes of 22 bivalents and 11 quadrivalents

were observed. The anaphase I segregation of the chromosomes were either equal or unequal. Aggregation of the chromosomes into groups at the equator, laggards and bridges at anaphase I and anaphase II, multipolar segregation etc. were some of the abnormalities observed frequently in the meiosis of the induced tetraploids of *C. cajan* var. ICPL 87.



Figs. 1-9. Chromosomal distribution at diakinesis in the induced tetraploids of *C. cajan* var. ICPL 87. 1. 22_{II}. 2. 11_{IV}. 3. $18_{II} + 2_{IV}$. 4. $I + II + III + IV$. 5. $1_I + 14_{II} + 1_{III} + 3_{IV}$. 6. $3_I + 5_{II} + 1_{III} + 7_{IV}$. 7. $2_I + 7_{II} + 6_{IV}$. 8. $10_{II} + 6_{IV}$. 9. $1_I + 6_{II} + 1_{III} + 7_{IV}$.



Figs. 10-17. 10. Equal distribution of chromosomes at anaphase I (22 : 22) in the induced tetraploids of *C. cajan* var. ICPL 87. 11. Unequal distribuuion at A I (10_{II} : 12_{II}). 12. Aggreation of chromosomes into groups (arrows). 13. Laggards at A I (arrow). 14. A I bridge (arrow). 15. Multipolar segregation at A I (arrow). 16. Pollen grains in diploids. 17. Pollen grains in induced tetraploids.

Detailed study of the earlier stages of meiosis such as pachytene and diplotene was not possible due to the highly ailed condition of the chromosomes. The mean frequencies and the ranges of the univalents, bivalents, trivalents and quadrivalents are presented in Table 3. Ring, chain and Y-shaped multivalents were frequently observed. The percentage frequencies of PMCs with different types of chromosomal

associations and the mean chiasma frequency per PMC in the induced tetraploidsin comparison with the diploids are depicted in Table 4. The anaphase I chromosomal distribution is presented in Table 5. The initiation and timing of the meiosis in the induced tetraploids were found to be upset. Different stages of meiosis varying from early prophase to anaphase II or even telophase II were observed in the same

TABLE 3 : Frequencies of pollen mother cells showing different types of chromosome associations in the induced tetraploids of *C. cajan* (L.) Millsp. var. ICPL 87

Chromosome associations	Number of PMCs	Percentage frequency
$0_I + 22_{II} + 0_{III} + 0_{IV}$	5	4.62
$1_I + 20_{II} + 1_{III} + 0_{IV}$	4	3.70
$0_I + 18_{II} + 0_{III} + 2_{IV}$	8	7.40
$0_I + 16_{II} + 0_{III} + 3_{IV}$	10	9.25
$1_I + 14_{II} + 1_{III} + 3_{IV}$	12	11.11
$0_I + 14_{II} + 0_{III} + 4_{IV}$	20	18.51
$0_I + 12_{II} + 0_{III} + 5_{IV}$	24	22.22
$0_I + 10_{II} + 0_{III} + 6_{IV}$	12	11.11
$0_I + 8_{II} + 0_{III} + 8_{IV}$	8	7.40
$0_I + 6_{II} + 0_{III} + 11_{IV}$	5	4.62

TABLE 4 : Mean per cell frequencies of different chromosomal associations and chiasmata in the diploids and induced tetraploids of *C. cajan* var. ICPL 87

Chromosome number	Number of plants analysed	Number of cells scored	Uni-valents	Bi-valents	Tri-valents	Quadri-valents	Mean number of chiasmata per cell
2n=2×=22	25	100	0	11	0	0	17.50
2n=4×=44	25	108	0.15	18.13	0.15	4.38	41.56

TABLE 5 : Anaphase I segregation of chromosomes in the induced tetraploids of *C. cajan* var. ICPL 87

Distribution of chromosomes	Number of PMCs	Percentage frequency
22–0–22	56	48.69
21–0–23	14	12.17
20–0–24	9	7.82
19–0–25	8	6.95
Laggards, bridges lagging bivalents	36	26.08

anther. In some of the PMCs more than 4 polar groups of chromosomes were noticed resulting in the formation of polyads.

The induced tetraploids in *C. cajan* var. ICPL 87 when self-pollinated, the pod-set was observed to be 7.5 %.

DISCUSSION

Attempts to induce autotetraploidy in different cultivars of *C. cajan* were made earlier by a very few workers (Singh 1947, Bhattacharjee 1956, Shrivastava et al. 1972).

Our observation of seedling treatment with 0.25 and 0.3% of aqueous colchicine solutions for 12 hr and 18 hr as highly effective in inducing tetraploidy in *C. cajan* var. ICPL 87 is in agreement with earlier reports in different crop plants. Colchicine solutions of 0.2 and 0.25% have been reported as optimum concentrations for induction of polyploidy in soybean (Sen & Vidyabhusan 1960a), *Zebrina pendula* (Venkateshwarulu & Narasimha Rao 1963), *Capsicum frutescence* (Raghuvanshi & Joshi 1964), *Momordica charantia* (Kadir & Zahoor 1965), rye grass (Alhoowalia 1967), grape (Das & Mukherjee 1967), *Sorghum vulgare* (Saddiq 1967), *Cicer arietinum* (Phadnis & Narkhede 1972), and *Nicarda physaloides* (Gupta & Roy 1986). Lower concentrations in the present study (0.1%) appear to be ineffective whereas higher concentrations (0.4%) proved to be toxic but not lethal.

Observation of some of the tetraploids as whole polyploids whereas some as chimeric may be due to the fact that when apical buds are treated with colchicine, not all cells are equally affected and some deep seated cells are unaffected. The latter may divide at a faster rate than the tetraploid cells. Similar observations were made in *Trigonella foenum-graecum* by Shambulingappa et al. (1965).

Although Bhattacharjee (1956) observed only slight differences in the leaf size between the diploids and tetraploids in *C. cajan* var. ICPL 87 leaf size variation was found to be a reliable criterion for the identification of tetraploid shoots. Increase in the diameter of the guard cells and low frequency of stomata on both the sides of the leaf appear to be a characteristic associated with polyploidy. Similar observations were reported by Siskovic (1957), Monge et al. (1963) and Sheopuria & Tiwari (1970) in *Phaseolus vulgaris*.

Observation of fewer branches and leaves on the tetraploids of *C. cajan* var. ICPL 87 is in agreement with the report of Bhattacharjee (1956). However, slower initial growth but taller plants towards maturity in tetraploids of the ICPL 87 variety of *C. cajan* is contradictory to the report of Bhattacharjee (1956) of initial lead but shorter tetraploid plants towards the concluding phases.

Low frequency of flowers is reported in blackgram also (Sen & Chedda 1958). However, increase in the number of flowers in tetraploids has been reported in *Dolichos biflorus* (Sen & Vidyabhusan 1960b) and *Glycine javanica* (Singh 1968). The high frequency of flower drop observed in *C. cajan* var. ICPL 87 may be due to physiological imbalance.

Occurrence of pollen grains of varying sizes and polyads with the formation of 5-13 microspores observed in tetraploids of *C. cajan* var. ICPL 87 is comparable to reports of Biswas & Bhattacharya (1976) in *Phaseolus vulgaris* and Dnyansagar & Sudhakaran (1970) in *Vinca rosea*.

Though the per cell multivalent association in the tetraploids was only 4.53% the frequency of pollen mother cells with multivalent associations was 95.38%. Existence of approximately 82% of the genome in the bivalent state indicate the possibility of stabilizing the tetraploids.

The high incidence of irregularities during the first and second meiotic divisions may be the cause of high sterility in this variety of *C. cajan*. The irregular distribution of chromosomes upsets the genome balance resulting in the non-viability of the pollen grains. This irregular distribution might have been influenced by several factors including absence of well organized spindle and the presence of multivalents in high frequencies. Aggregation of chromosomes in group occuring at anaphase I and irregular synchronization of further stages in this groups might have resulted in the abnormal distribution of chromosomes at the poles. Bhattacharjee (1956) also attributed high sterility to multivalent configuration and irregular distribution. In autotetraploids of *C. cajan* var. ICPL 87, since 48.69% of PMCs exhibited regular chromosomal distribution, the involvement of some additional factors causing sterility cannot be ruled out.

Magoon et al. (1957) and Shambulingappa et al. (1965) have suggested the involvement of some genetic, physiological or environmental factors in pollen sterility, in addition to meiotic abnormalities. Parthasarathy (1953) is of opinion that chromosome doubling might upset the balance of polygenes and/or modifying genes controlling sterility resulting in no correlation between sterility and meiotic abnormalities.

Inspite of regular distribution of chromosomes in 48.69% of PMCs during androgenic meiosis, poor natural seed-set in tetraploids might be due to a lesser degree of tolerance to abnormal meiosis in the gynoecium resulting in non-viable eggs or due to the production of non-viable zygotes.

High frequency of flower drop after selfing may be due to the fact that artificial pollination is less successful in *C. cajan*.

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Editor

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SOMATIC INCOMPATIBILITY IN HIGHER FUNGI - A REVIEW

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SUMMARY

Genetically different individuals in ascomycete and basidiomycete populations often can be delimited by self- non-self recognition reaction referred to as somatic incompatibility. The basis of somatic incompatibility is that adjacent mycelia will reject one another if they differ genetically at their polygenic or mult allelic somatic incompatibility loci. The rejection is expressed as a reaction zone between mycelia. Somatic incompatibility is due to the interaction between different nuclear and cytoplasmic genetical determinants. Since somatic incompatibility is due to different genes, it can also be termed as "heterogenic incompatibility". The genetic basis of somatic incompatibility has been studied in a relatively few fungi. A review of the work done on somatic incompatibility in higher fungi is presented here.

Key Words : Somatic incompatibility, mating systems, heterogenic incompatibility.

INTRODUCTION

The neglection of population studies with higher fungi (including wood rotting fungi) held the view that it is difficult to define an "individual". This was partly because it was thought that as a general rule, separate genetically different individuals of the same species could fuse on meeting via hyphal anastomoses, to produce a new functional unit (Rayner & Boddy 1988). The formation of hyphal fusions allowing cooperation between separate colonies was proposed by Buller (1933) to account for a large size of fruit bodies of *Coprinus sterquilinus* on balls of horse-dung. Burnett & Partington (1957) accepted this theme to suggest that the same mechanism could also operate between thalli of different genotypes. This was based on the evidence of mating factor distribution in fruit bodies of *Coriolus versicolor* and *Piptoporous betulinus*. The results were interpreted including the occurrence of a simultaneous mating between monokaryons and mono– and dikaryons before establishing a population of dikaryons. The two workers did not examine the structure of the mycelial population in wood which turned to be a critical omission. Secondly, work with certain Ascomycotina brought into light, the parasexual cycle. This process was dependent upon initial production of a heterokaryon via mutation or anastomosis (Pontecorvo et al. 1953), followed by diploidization. Heterokaryosis and parasexuality were then used to explain variation in a wide range of fungi especially in pathogens where sexual state was unknown. However, Caten & Jinks (1966) noted that much of the supposed evidence for heterokaryosis was enforced between complementary auxotrophic mutants or was observed between strains differing at one or few loci. These views of widespread heterokaryosis and somatic collectivism between genetically different mycelia of the same species became established against the evidence that meeting of different genotypes resulted not in physiological unification but in rather antagonisitic interactions. It is now

clear that in genetically variable populations of Ascomycotina and Basidiomycotina these reactions are due to self-and non-self rejection mechanisms, which can be termed as *somatic incompatibility* or *vegetative incompatibility*. This operates to delimit individual genotypes from one another and reduces the scope for genetic and physiological collectivism between them (Rayner et al. 1984). It also provides an important tool in studying the structure of natural populations of wood inhabiting higher fungi (Rayner & Todd 1982a,b).

Occurrence and timing

In a nutritionally rich medium, the most typical observation of somatic incompatibility at the mycelial level is the formation of a narrow demarcation zone between colonies in which hyphae are sparse or aerial and/or pigmented. The reaction varies considerably in form of intensity, within and between species with respect to width, presence and amount of pigment within and outside the hyphae, presence of aerial mycelium or depressen zone, etc. At the level of interaction between individual hyphae expression of somatic incompatibility is again variable. In some cases, rejection mechanisms are pre-fusion, while in some, it is not brought about until after hyphal fusion or post-fusion mechanism.

Whatever the pattern of expression of somatic incompatibility, in order to understand the significance of this phenomenon, it is important to appreciate its timing in relation to life cycle stages (Rayner et al. 1984). In many Ascomycotina, it is expressed directly between homokaryons This is because of the vegetative prominence of the homokaryotic phase in them and the presence of specialised organs through which sexual exchange can be channeled. By contrast, in sexually outcrossing Basidiomycotina, somatic incompatibility is typically expressed between genetically different secondary mycelia which is the dominant phase in these fungi.

In wood

The numerous observations of zone lines between decay columns in wood and their connection with the results of somatic incompatibility in culture was hinted at by several workers (Childs 1963 and Adams & Roth 1967). But, this was brought into light by the work of Rayner & Todd (1977) on a natural population of *C. versicolor*. Now it is clear that narrow decay zone lines are often a result of somatic incompatibility between adjacent mycelial thalli. These zone lines consist of relatively undecayed regions darker in shade than the adjacent decayed regions and bounded by pseudo-sclerotial plates and longitudinally continuous in adjacent columns of decay. The fact that somatic incompatibility is seen so directly in natural substratum (wood) enables the direct analysis of the natural substratum i.e. three dimensional structure of decay populations.

Genetic mechanisms

Somatic incompatibility is due to the interaction between different nuclear and perhaps exceptionally different cytoplasmic genetic determinants. In a very general way, incompatibility is defined as a genetically determined prevention of karyogamy which is not caused by sterility factors. Therefore, it concerns the sexual and parasexual cycle. On the basis of their genetic action, two different systems are recognized : (a) Homogenic and (b) Heterogenic, each of which is caused by various genetic mechanisms.

Homogenic incompatibility is present when karyogamy is prevented by the presence of identical incompatibility factors in the two strains. So for as is known, it concerns only the sexual cycle.

Heterogenic incompatibility is present when karyogamy is prevented by the presence of non-identical incompatibility factors in the two strains. This system concerns both the sexual and vegetative phases. It occurs between geographical races and is widely distributed in all living beings. Thus, heterogenic incompatibility is the consequence of interaction between genetic elements which cannot coexist in close proximity to each other. In fungi, heterokaryon incompatibility prevents heterokaryon formation (Esser 1974) and does not prevent outcrossing and should not be interpreted as a sexual incompatibility system.

The genetic basis of somatic incompatibility is studied in very few fungi and so it is not proper to make any general statement. In Ascomycotina, there is evidene for polygenic multiallelic, non-allelic control and one gene with biallelic locus (Vaidya 1990). It is often stated that somatic incompatibility is dependent on the identicality of somatic incompatibility loci or vegetative compatibility loci or heterokaryon compatibility loci (Rayner & Boddy 1988).

In Basidiomycotina, there is some evidence for control by multiallelic major genes (Rayner 1986), These can be expressed independently of the mating type loci in some fungi e.g. *Piptoporus betulinus* and *Stereum hirsutum* and hence result in rejection responses between mating incompatible homokaryons. However, absence of strong rejection responses between mating incompatible homokaryons in *Phanerochaete velutina* (Ainsworth & Rayner 1986) suggest that the mating factor alleles themselves may act as or be linked to major genes conferring somatic incompatibility between heterokaryons.

It is also common in a wide variety of fungi to find a gradation in interaction intensity between strains related to their overall genetic similarity and dissimilarity, implying control by polygenic systems.

Physiological mechanisms

These mechanisms underlying somatic incompatibility are unclear. However, it is supposed that this phenomenon is due to the advancement of protoplasm into a premature senescence cycle. This is evident from observations of "vacuolation" by the senescent pattern of growth of subcultures from interaction zones and activation of protease and phenoloxidase systems which are normally repressed until ageing allows the onset of natural cell death (Boucherie & Bernett 1977, Hiroth 1985, Li 1981 and Rayner & Coates 1987).

Population and community structure

In general, the inhabitants of a place are often referred to as a "population". This term is imprecise and terms developed for other organisms may not always be appropriate for fungi because of their heterotrophy and their unusual growth forms. Hence, to describe ecological groupings of organism, a hierarchy of terms is used to refer to systems with progressively complex levels of organization. The basic units of such systems are "individuals". Assemblage of individuals of the same species in proximity in space and time are called "populations". An assemblage of diverse species occupying the same, functionally discrete environment or ecosystem is termed a community. A feature of importance of these populations, individuals and communities is that they represent discrete functional units.

Relationship of somatic incompatibility to colonization patterns

The expression of somatic incompatibility will obviously exert a controlling influence over and directly reflect patterns of colonization. Two things are important in this aspect. (1) The extent to which somatically incompatible individuals invade wood simultaneously from a colonization court and (2) the form and longevity of the homokaryotic thallus in outcrossing Basidiomycotina.

Generally, if colonization is affected by genetically different ascospores or basidiospores, then the chances are that somatically incompatible individuals may develop side by side and restrict each other's domain. This will be most important in cut or detached wood, where a large surface is available for colonization. However, in intact trees, the colonization may be restricted, but if an individual is successful in establishing itself, it provides a very large domain. The relatively small fruit bodies produced by decay fungi colonizing cut or detached wood compared with those in intact trees is readily understandable in terms of "domain" which they occupy.

Colonization strategies

Patterns of colonization are correspondingly diverse. Rayner & Boddy (1988) described five distinct colonization strategies. These are, heart-rot, active pathogenesis, specialized opportunism, dessication tolerance and unspecialized opportunism.

The first four involve some form of tolerance or overcoming of selective stress conditions, including unfavourable aeration, lack of assimilable nutrients, presence of mechanical barriers and allelopathic chemicals. Unspecialised opportunism depends on enrichment disturbance and consequent alleviation of stress conditions.

(a) Heart-rot : The basic strategy of heart-rot is to grow and cause decay within the inner core of the tree where living cells are absent or rare and a relatively extensive gaseous phase may exist. However, this strategy imposes its own stringencies and highly selective conditions can result from a gaseous regime with the presence of allelopathic extractives. Therefore, many heart-rot fungi have slow growth rates, lack combative abilities and strong selectivity for tree taxa.

Commonly, it is assumed that entrance of a heart-rot fungus must be through trunk wounds of the host exposing heartwood or through wounded or diseased roots or branches large enough to contain heartwood.

(b) Active pathogenesis : Some decay fungi gain access to intact sapwood as a result of active pathogenic mechanisms. They produce pectinolytic enzymes which destroy pit membranes and hence gain access in the host. They also exhibit necrotrophic behaviour whereby living cells of the host are killed associated with colonization.

(c) Specialized opportunism : Here, the fungus capitalized as a result of microenvironmental stress other than its own activities and having first established itself under these stress conditions. Such a strategy gives an advantage in primary resource capture over potential competitors.

(d) Dessication tolerance : In this case, the wood of above ground parts of the trees can become subject to considerable dessication or to fluctuations in moisture content. In such situations, the ability to tolerate low or fluctuating water potential becomes of selective advantage and helps colonization by the fungus.

(e) Unspecialized opportunism : This strategy involves colonization of the normally nonsusceptible sap-wood when it is made accessible by injury or rapid death of bark.

Work on somatic incompatibility

In the fungi studied so far, barriers to heterokaryon formation have been detected. Esser (1962) described a complex type of incompatibility in *Podospora anserina* strains of different geographical origins. Four unlinked loci with 2 alleles appeared to be involved and incompatibility resulted from the interactions of specific alleles at different loci. Juxtaposition of mycelia carrying incompatible alleles resulted in the formation of a "barrage" between them meaning a depressed zone of sparse and abortive heterokaryotic hyphae. Later, Blaich & Esser (1971), studied the biochemical

characterization of heterogenic incompatibility on cellular level and found that the breakdown of cellular structure in the barrage was associated with the release of certain catabolic enzymes.

Studies carried out by Grindle (1963) in *Aspergillus nidulans* using complementation of white and yellow conidial colour mutants as a criterion of heterokaryon formation, proved that independently isolated wild type strains were usually mutually incompatible. Compatibility was more often found between strains isolated from the same locality but even isolates from the same soil sample were sometimes found to belong to different compatility groups. Sexual crosses between heterokaryon incompatible isolates were frequently fertile, but the progeny were often found to be less vigorous than the parents, possibly because of the segregation into the same meiotic products of mutually incompatible alleles. By means of a heterokaryon test, Jinks & Grindle (1963) established that partial incompatibility between two isolates had a nuclear rather than a cytoplasmic basis. More extensive analysis revealed that at least five incompatibility loci were involved in the inter-isolate differences.

In another fungus, *Neurospora crassa*, studies have revealed that heterokaryon will be formed only between closely related genotypes. Hyphal fusion with the formation of a stable heterokaryon only occurs readily between mycelia of the same mating type. Garnjobst (1955) showed that two loci in *N. crassa* with respect to which the two strains must be similar if they are to be heterokaryon compatible. If paired mycelia differ at either locus or both, a lethal incompatibility follows hyphal anastomosis, thus preventing formation of a heterokaryon.

More recent work at Stanford University has shown that the loci identified by Garnjobst (1955) represented only a small part of the incompatibility present in the species as a whole. A broader survey was made possible by the extensive collections of *N. crassa* by Perkins et al. (1976) for the ready generation of viable duplications of various chromosome regions. Crosses made by Mylyk (1976) led to the identification of at least 10 incompatibility loci in various parts of the genone. Secondly, there was much variation within population (Mylyk, 1976).

Sonoda et al. (1982) while studying mycelial interactions between ascospore isolates of *Monilinia fructicola* observed interaction zones which indicated a possibility of a vegetative incompatibility system existing in the fungus. Similar reaction was observed between certain field isolates of the coprophilous fungus *Ascobolus immersus* and vegetative incompatibility was supposed to act as a barrier to prevent genetic exchange between races of the fungus (Meinhardt et al. 1984).

Other notable work on somatic incompatibility in Ascomycotina includes that on *Cryphonectria parasitica* (*Endothia parasitica*), the cause of chestnut blight

(Anagnostakis 1984) and *Ophiostoma ulmi (Ceratocystis ulmi)* causing the dutch elm disease (Brasier 1984). The system of somatic incompatibility in these fungi is similar to that of *A. nidulans*. Field isolates of these fungi fall into different vegetative compatibility (VC) groups, within which isolates are compatible and between which they are incompatible.

From 258 North American field isolates, 73 VC groups of *C. parasitica* have been found (Anagnostakis 1984). Among 141 European strains that were tested, most of them fell into 22 VC groups (Grente 1981). A comparison of diversity of VC groups in Connecticut and Europe revealed that VC groups were more diverse in Connecticut than in Europe (Anagnostakis et al. 1986).

In an aggressive strain of *O. ulmi*, 40-60% of isolates from a worldwide sample of North American race (NAN) and Eurasian race (EAN) respectively, belonged to the same VC group; the remaining isolates each belonged to a different VC group. Such VC groups are termed as "super VC groups". The super VC groups of the NAN and EAN are believed to arise as a result of the chance dispersal and spread of a particular group during the current epidemic of the Dutch elm disease (Brasier 1984).

Jamil et al. (1984) found 18 VC groups in 31 isolates in *Gaumannomyces graminis* var. *tritici* of wheat fungus from a single field.

Many studies on somatic incompatibility among the Basidiomycotina have been carried out. Some recent works include those of Aylmore & Todd (1966) on the cytology of non-self hyphal fusions and somatic incompatibility in *P. velutina* May (1988) on somatic incompatibllity and individualism in *Coprinus cinereus*, Adaskaveg and Gilbertson (1987) on vegetative incompatibility between intraspecific dikaryon pairings of *Ganoderma lucidum* and G. *tsugae*, Stenlid (1985) on the population structure of *Heterobasidion annosum* by studying somatic incompatibility, sexual incompatibility and isozyme patterns. Studies on population structure in an aspen plantation of *Peniophora rufa* was also studied by the analysis of sexual and somatic incompatibility behaviour by Chamuris & Stuart (1987).

Extensive work by Reyner and his co-workers has been done since 1976 on population structure and mycelial interactions at the University of Bath, England, in the following fungi :

Basidiomycotina

Bjerkandera adusta (Willd. ex Fr.) Karst. *Clitocybe nebularis* (Batsch ex Fr.) Kummer., *Coniophora puteana* (Schum. ex Fr.) Karst., *Coriolus versicolor* (L. ex Fr.) Quel., *Hymenochaete corrugata* (Fr. ex Fr.) Lev., *Hypholoma fasciculare* (Huds. ex Fr.) Kummer, *Phallus impudicus* (L.) Pers., *Phanerochaete velutina*

(DC. ex Pers.), Parmasto, *Phlebia radiata* Fr. et *P. rufa* (Fr.) M.P. Christ, *Stereum hirsutum* (Willd ex Fr.) S. F. Gray, *S. insignitum* Quel, *S. remeale* (Pers. ex Fr.) Fr., *S. rugosum* (Pers. ex Fr.) Fr. and *S. sanguinolentum* von Alb. & Schwein.

Ascomycotina :

Biscogniauxia nummularia (Bull ex Fr.) O. Kuntze, *Colpoma quercinum* (Fr.) Wall, *Cryptostroma corticale* (Ell. et Everh.) Gregory & Waller, *Daldinia concentrica* Ces et de Not., *Hypoxylon* spp. (Pers.) Fr., *Rhopalostroma hawksworthii* Vaidya, Rayner & Whalley, *Rosellinia aguila* (Fr.) de Not., *R. desmazieresii* (Berk. & Br.) and *Xylaria hypoxylon* (L. ex Hooker) Grev.

Role of somatic incompatibility

Genetic exchange and gene flow are prevented by vegetative incompatibility and promoted by a variety of mating systems. When a cell undergoes vegetative fusion with a genetically different cell, its genome may be changed. Nuclei containing genes enabling them to replace other nuclei in a heterokaryon could be introduced, so also, mitochondria capable of normal replication but defective in their respiratory performance or plasmids or viruses with deleterious effects. The vegetative incompatibility may be a protection against such alien genetic material.

Nuclei and genetic material cannot be transmitted between cells without cell fusion and plasmids. DNA fragments or viruses cannot pass through intact fungal cell walls. Hence, fusion incompatibility gives complete protection against alien material. Post-fusion reactions prevent transmission of nuclei but the rate at which virus movement is prevented varies. In *Endothia parasitica,* a virus that reduces the growth rate and pathogenicity of the fungus spreads readily between strains of the same VC groups by hyphal anastomosis (Anagnostakis 1984) Similar results have also been obtained in *O. ulmi* (Brasier 1984).

Although vegetative incompatibility is widespread in fungi, there is a possibility that it my be absent in some species capable of vegetative fusion. This may occur where a new area is colonized or a new host is infected, as the initial colonists might be of a single haploid genotype and the resulting population of a single VC group. However, later, genetic diversity would arise and the individuals will need to face competition with others and also to defend against alien nucleic acid.

Though the role of somatic incompatibility may be protection of individuals, its occurrence may have consequences for the species. In fungi, fusion iucompatibility will prevent genetic exchange and thus, delimit a genospecies. Post-fusion incompatibility will restrict the transmission of nuclei and prevent heterokaryon formation and parasexual recombination (Carlile 1987).

Inspite of these plausible suggestions as to the possible advantages of preventing heterokaryosis, it is difficult to believe that heterokaryosis has never been advantageous to fungi. There is a delicately poised balance between genetic mixing and genetic isolation in relation to sexual reproduction.

Prospects

Till todate, a lot of work as been done on somatic incompatibility systems in Basidiomycotina and a few Ascomycotina. Our results on these aspects in *Diatrype* and *Diatrypella* show a great deal of similarity with the members of Xylariaceae.

Further studies in this direction are necessary especially in wood decaying members because the role of wood decomposition is widely appreciated as an integral process in ecosystem functioning.

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INHERITANCE OF GROWTH HABIT IN BENGAL GRAM

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SUMMARY

This paper describes the inheritance of growth habit in 8 crosses of gram involving 8 parents. The inheritance of erect, semierect and spreading growth habit was studied and the genes Segr Spgr (semi-erect), Spgr, Segr (erect) and segr, spgr (spreading) were symbolized.

Key Words : Inheritance, *Cicer arietinum*, growth habit.

INTRODUCTION

In gram (*Cicer arietinum* L.), inheritance of several characters have been studied by Ayyar & Balsubrahmanyam (1936), Patil (1959), Argikar & D'Cruz (1965) Sandha & Athwal (1971), Singh & Dahiya (1974), Patil & Deshmukh (1975), Rao et al. (1980), Pandey & Tiwari (1981), Pawar and Patil (1982) Kidambi et al. (1988). The present investigation was undertaken to gather additional information on inheritance of growth habit in gram.

MATERIAL AND METHODS

Eight parents used in the present investigation were selected from a collection of 135 gram types maintained in the Botanical Garden of College of Agriculture, Pune. These parents were differing from each other in respect of number of morphological characters. The growth habit was erect, semi-erect aud spreading in different parents. The crossess were made during 1978-79 and their F_1, F_2 and F_3 generations were raised and studied during subsequent years.

The growth habit was studied in six triangular crosses involving eight parents in F_2 generation and the results obtained were confirmed in F_3 generation.

The chi-square test was applied for testing goodness of fit in individual as well as joint segregation (Fisher 1941). The genes governing the characters were symbolized as suggested by Tanaka (1957).

RESULTS

Table 1 indicates the dominance of erect growth habit over semi-erect in 6 crosses viz. TL x BL, TL x GB, TL x N-31, TL x PDP-83, TL x WFWG-II and TL x Chy (bl). However, semi-erect growth habit was found to be dominant over spreading in Sp x WFWG-II. It was observed that the appearance of growth habit was due to interaction between the factors governing these characters. The segregation

of this character in F_2 generation of various crosses studied in the present investigations indicated that the growth habit is governed by two pairs of genes having supplementary gene action (Table 2). The results obtained in F_2 generation of various crosses were confirmed from breeding behaviour of selected families in F_3 generation (Table 3).

TABLE 1 : Growth habit of parents and their F_1

Name of parent cross	Growth habit
Tiny leaf (TL)	Erect
Green bold (GB)	Semi-erect
Bronze leaf (BL)	Semi-erect
White flower White grained-II (WFWG-II)	Semi-erect
Spreading (Sp)	Spreading
Pusa-83 DP (PDP-83)	Semi-erect
N-31	Semi-erect
Chrysanthifolia (b1) [Chy (b1)]	Semi-erect
TX × BL	Erect
GB × TL	Erect
TL × N-31	Erect
TL × PDP-83	Erect
TL × WFWG-II	Erect
TL × Chy (b1)	Erect
Sp × WFWG-II	Semi-erect
TL × Sp	Erect

DISCUSSION

Monogenic dominance of erect growth habit over spreading was reported by Ayyar & Balsubrahamanyam (1936), Patil (1959), and Argikar & D'Cruz (1965). Similarly, Rao et al. (1980) reported monofactorial recessive inheritance of prostrate growth habit.

In the present Investigation, erect growth habit was found to be dominant over spreading but supplementary ratio of 9 erect : 3 semi-erect : 4 spreading was recorded in a cross between TL x Sp for the first time. A monogenic ratio of 3 erect : 1 semi-erect was observed in crosses viz., TL x BL, TL x GB, TL x N-31,

TABLE 2 : Segregation of F_2 population of 8 crosses for growth habit.

No.	Cross	Ratio	Growth habit			X^2	P	Gene Symbol
			Erect	Semi-erect	Spreading			
1.	TL × BL	3 : 1	363	125	—	0.3934	0.50–0.70	(Segr) Spgr
2.	GB × TL	3 : 1	337	109	—	0.0560	0.70–0.80	(Segr) Spgr
3.	TL × N-31	3 : 1	467	159	—	0.0532	0.80–0.90	(Segr) Spgr
4.	TL × PDP-83	3 : 1	386	132	—	0.0643	0.80–0.90	(Segr) Spgr
5.	TL × WFWG	3 : 1	215	69	—	0.0751	0.70–0.80	(Segr) Spgr
6.	TL × Chy (b1)	3 : 1	396	136	—	0.0902	0.70–0.80	(Segr) Spgr
7.	Sp × WFWG	3 : 1	—	280	92	0.0143	0.90–0.95	Segr (Spgr)
8.	TL × Sp	9 : 3 : 4	259	87	112	0.0765	0.95–0.98	Segr Spgr

TABLE 3 : Breeding behaviour of F_3 families in 8 crosses for growth habit

No.	Cross	No. of families	F_3 Behaviour						
			B.T. E.	Segregn. 3E : 1Se.	B.T. Sp.	Segregn 3. Se : 1 SP.	Segregn 3E : 1SP.	Segregn 9E:3Se: 4Sp.	B.T. Sp.
1.	TL × BL	58	16	25	17	—	—	—	— 0.50–0.70
2.	GB × TL	63	17	30	16	—	—	—	— 0.90–0.95
3.	Sp × WFWG	56	—	—	16	27	—	—	13 0.80–0.90
4.	TL × WFWG	61	17	26	18	—	—	—	— 0.50–0.70
5.	TL × Sp.	52	5	8	4	5	6	12	12 0.90–0.95
6.	TL × Chy(bl)	64	15	31	18	—	—	—	— 0.80–0.90
7.	TL × N-31	63	17	26	20	—	—	—	— 0.30–0.50
8.	TL × PDP-83	62	14	31	17	—	—	—	— 0.80–0.90

TL x PDP-83, TL x WFWG-II, and TL x Chy (bl) which confirmed the earlier findings. In another cross Sp x WFWG-II dominance of semi-erect growth habit over spreading was observed giving a monogenic segregation of 3 semi-erect : 1 spreading in F_2. Patil & Deshmukh (1975) reported a segregation of 3 burchy (basal) : 1 non-bunchy (umbrella type) branching.

The genes governing the growth habit character in different parents under study were symbolized. The behaviour of the 8 parents in triangular crosses for growth habits, the segregation in F_2 generation and the genes governing the character in different parents are depicted in Fig. 1.

Fig. 1 : Triangular crosses showing the segregation of growth habit with F_2 phenotypic ratios and genes involved in gram.

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FIRST REPORT ON CYTOLOGY OF TWO ANEUPLOIDS OF *CAPSICUM ANNUUM* L.

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SUMMARY

Two new aneuploids with 2n = 28 and 34 chromosomes with low fertility were isolated in the first and third generation progenies of an autotriploid of *Capsicum annuum* respectively. On the basis of cytology, the first one with 2n = 28 was identified as a multiple trisomic for 4 different chromosomes since at meiosis, the highest association observed was a trivalent and the maximum number of trivalents observed per cell being 4. The second aneuploed was proved to be a hypotriploid with 2n = 34 forming higher associations. Presence of associations higher than a trivalent indicates the occurrence of structural repatterning. The probable origin of aneuploids, their altered phenotypic appearance and their cytological behaviour were described and discussed.

Key Words : *Capsicum annuum*, aneuploid, multiple trisomic, hypotriploid.

INTRODUCTION

Although aneuploids have been extensively used for cytogenetic studies and hromosome manipulation in serveral crops (Khush 1973) and are a valuable source for obtaining more aneuploids, reports on aneuploids of *Capsicum*, an important cash and condiment crop are fragmentary. Radiation induced aneuploids with 2n = 30 and 31 chromosomes were reported in *Capsicum* by Subhash & Nizam (1975) and Rao & Lakshmi (1984). Aneuploids have been obtained in several species either from natural populations or from autotriploids (Tsuchiya 1954, Rick & Barton 1954 Price & Ross 1955, Vari & Bhowal 1982 and Sapre & Barve 1983). In the present investigation, the morphology and meiotic behaviour of two aneuploids with 2n = 28 and 34, obtained in the progeny of an autotriploid *Capsicum* were reported for the first time.

MATERIALS AND METHODS

Two aneuploids with 2n = 28 and 34 were located in the progeny of an autotriploid in first and third generations respectively and these constituted the meterial for the present study. Usual acetocarmine technique was followed for meiotic studies. Pollen fertility was estimated by using the vital stain 4% iodine potassium iodide solution.

RESULTS AND DISCUSSION

Morphology

The two aneuploids were phenotypically distinct from the diploid in showing less vigour and fertility and can be reckoned as inferior to diploids since they

exhibit a general reduction in size of the plant parts (Table 1, Figs. 1 & 5). But, the first aneuploid (2n = 28) was bushy with an increase in the number of branches while the second had big flowers with 6 petals in contrast to the disomic pentamerous condition. In both the aneuploids, there was delayed and extended flowering as in colchiploids of *Capsicum* (Raghuvanshi & Joshi 1964, Lakshmi et al. 1987) and aneuploid of *Cyamopsis* (Biswas & Bhattacharya 1971) which can be attributed to the slower rate of metabolic activities. In both stomatal abnormalities such as paired stomata (Fig. 6) and stomata surrounded by single guard cell were present. Pollen fertility and fruit setting were low in both the aneuploids (Table 1).

TABLE 1 :Comparative morphometrics of the two aneuploids along with the sibling disomic

Parameters	Normal plant	Aneuploid (2n=28)	Aneuploid (2n=34)
Plant height (cm)	65.0	50.0	54.0
Plant spread (cm)	94.0	120.0	81.0
Number of branches	5	7	4
Length of the leaf (cm)	4.5	3.0	4.4
Width of the leaf (cm)	2.8	1.1	2.7
Flower length (cm)	2.5	2.4	2.7
Flower width (cm)	1.8	1.7	2.0
Pollen fertility (%)	92.6	32.4	14.8
Number of fruits per plant	122	24	6
Number of seeds per fruit	87	13	2

Similar observations have been made in aneuploids of pearl millet (Vari & Bhowal 1982, Sai Kumar et al. 1983), *Trigonella* (Singh & Singh 1975), *Coix* (Sapre & Barve 1983) and *Capsicum* (Subhash & Nizam 1975, Rao & Lakshmi 1984 and Sadanandam & Subhash 1985). The reduced vigour and low pollen and ovule fertility of the 2 aneuploids can be traced to the presence of extra 4 and 10 chromosomes respectively.

Cytology of aneuploid with 2n=28

Meiotic studies revealed the chromosome number as 2n = 28 in contrast to the normal diploid complement of 2n = 24 (Fig. 2) and the highest association possible was a trivalent, the frequency of which was 2-4 per cell (Fig. 3). The association of $^{3}_{III} + ^{6}_{II} + ^{7}_{I}$ was more prevalent. The extra chromosomes were involved either in trivalent formation (chain) or remained as a univalent but never formed associations higher than a trivalent (Table 2). Since the highest association observed

TABLE 2 : Mean frequency, types of chromosome associations and chiasma frequency in PMCs of aneuploids

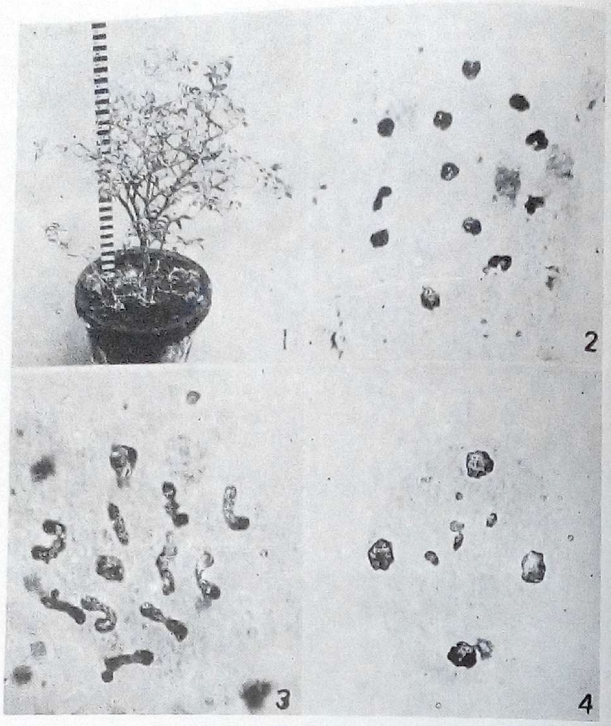
Aneuploids	Mean frequency of chromosome associations								Chiasmata per cell
	VIII	VII	VI	V	IV	III	II	I	
I (2n=28)	—	—	—	—	—	3.16 ± 0.07	6.68 ± 0.28	5.375 ± 0.43	15.1 ± 0.46
II (2n=34)	0.096 ± 0.037	0.032 ± 0.022	0.145 ± 0.050	0.27 ± 0.073	0.983 ± 0.14	3.90 ± 0.31	6.12 ± 0.28	2.12 ± 0.25	24.45 ± 0.127

was a trivalent and the maximum number of trivalents observed per cell being 4, the aneuploid was identified as a multiple trisomic for 4 different chromosomes. Univalents were observed in 96% of the PMCs observed, the number ranging from 1–13 indicating the presence of partial asynapsis. The occurrence of univalents in addition to 4 trivalents indicates that other bivalents also remain as univalents. Obviously, they result from failure of pairing or loss of chiasmata by terminalization or by simple separation of homologues without chiasmata. Significant decrease in the chiasma frequency was observed as a result of formation of univalents.

During metaphase I, 47.01% of cells exhibited nonorientation of 1–2 chromosomes and anaphase segregations were mostly irregular with 2-8 laggards in 44.68% of cells. These abnormalities lead to the formation of 1–4 micronuclei in 70.37% of cells along with 4 groups in telophase II (Fig. 4).

Cytology of aneuploid with 2n=34

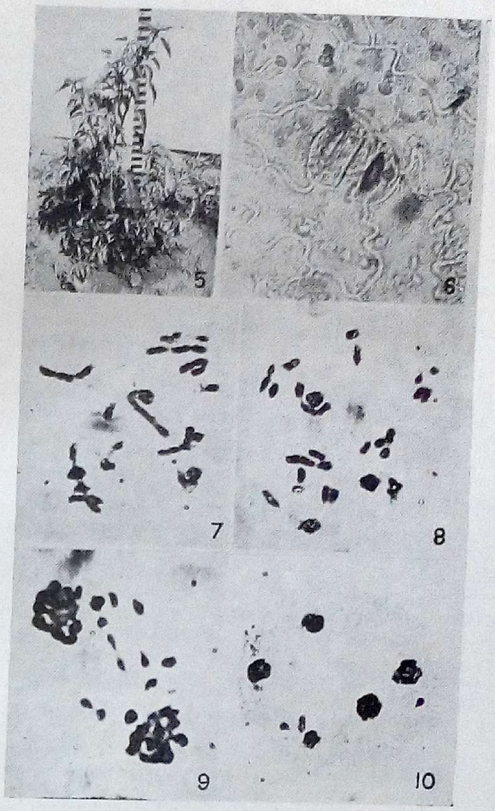
The second aneuploid was proved to be a hypotriploid with 2n=34, 2 chromosomes less than the triploid number 2n=36. Meiosis in this was highly irregular with the formation of higher associations of 8, 7, 6, 5, 4 and 3 chromosomes at diakinesis and metaphase I. The different chromosome associations formed and their frequency are given in Table 2. Of all the multivalents, trivalents were observed to be in higher frequency The higher associations were either of chain, frying-pan, Y- or of ring type (Figs. 7 & 8) and the type of configuration depends upon the extent of pachytene pairing and the number and position of chiasmata. Of all these, chain type was more frequent than frying pan, Y- and ring types. The presence of multivalents higher than a trivalent indicates the existence of structural repatterning in the species. The chiasma frequency per cell was 24.45 whereas it was 19.97 in diploid which was significantly less than that of the diploid. It may be due to a small number of multivalents formed and more number of univalents.



Figs. 1-4 : Morphology and cytology of aneuploid I (2n=28) 1.Photograph of the plant. 2. Normal diakinesis of sibling disomic showing 12_{II}, X 2000. 3. Diakinesis with $3_{III} + 9_{II} + 1_{I}$, X 2500. 4. Telophase II with 4 micronuclei, X 2500.

Distribution of chromosomes at anaphase I was irregular and 1-i4 laggards were observed in 20.9% of cells (Fig. 9). Formation of 1-3 micronuclei along with the polyads at telophase I and II was observed in 38.12% of cells (Fig. 10). Increase in size of the pollen grains was observed in both the aneuploids which may be due to an increase in chromosome number (Biswas & Bhattacharya 1971).

Triploids, asynaptics and desynaptics were proved to be a good source of primary and multiple trisomics (Khush 1973). These have been recorded in *Capsicum*



Figs. 5–10 : Morphology and cytology of aneuploid II (2n=34) 5. Photograph of the plant. 6. Epidermal peel of leaf showing paired stomata, X 2950. 7. Diakinesis showing the association $1_{V} + 1_{IV} 5_{III} + 4_{II} + 2_{I}$, X 2000. 8. Diakinesis with $3_{III} + 9_{II} + 7_{I}$, X 2000. 9. Early telophase I showing 10 laggards and division of a univalent, X 2500. 10. Telophase II exhibiting 5 nuclei and 3 micronuclei, X 2500.

annuum (Pal & Ramanujam 1940) and *Lolium* (Ahloowalia 1982). The same holds good for the two aneuploids of the present investigation also since the first with 2n=28 chromosomes originated from an autotriploid probably due to the chance union of 'n+4' gamete with an 'n' gamete or n+3 gamete with n+1 gamete or as a result of union of two n+2 gametes while the second aneuploid with 2n=34 chromosomes originated from an asynaptic disomic showing fragmentation. The extreme unequal separation of this disomic might have led to the formation of aneuploid gametes such as n=10, n=7, n=6, n=5 n=4, n=3 etc. and by the chance union of any two of the gametes with appropriate number could have contributed to the origin of the hypotriploid.

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CHROMOSOMAL INSTABILITY IN CALLUS CULTURES OF *NIGELLA SATIVA* L.

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SUMMARY

Cytological studies performed on the callus cultures of *Nigella sativa* revealed numerical variation in chromosomes such as haploid, diploid and tetraploid cell lines in older cultures. From the observations made, it is clear that plant tissues grown indefinetly in vitro result in mixoploidy.

Key Words : *Nigella sativa*, callus cytology, chromosomal variation.

INTRODUCTION

Keeping in view the medicinal value of *Nigella sativa* an attempt was made to standardise the cultural conditions for callus induction. The growth regulators 2,4-D and kinetin were found to be essential in the induction of callus. However, a wide range of structural and numerical variations of chromosomes were observed in the callus. Knowledge about the ploidy levels of callus cells is helpful in carrying out regeneration studies, if uniformly normal individuals are to be recovered.

MATERIAL AND METHODS

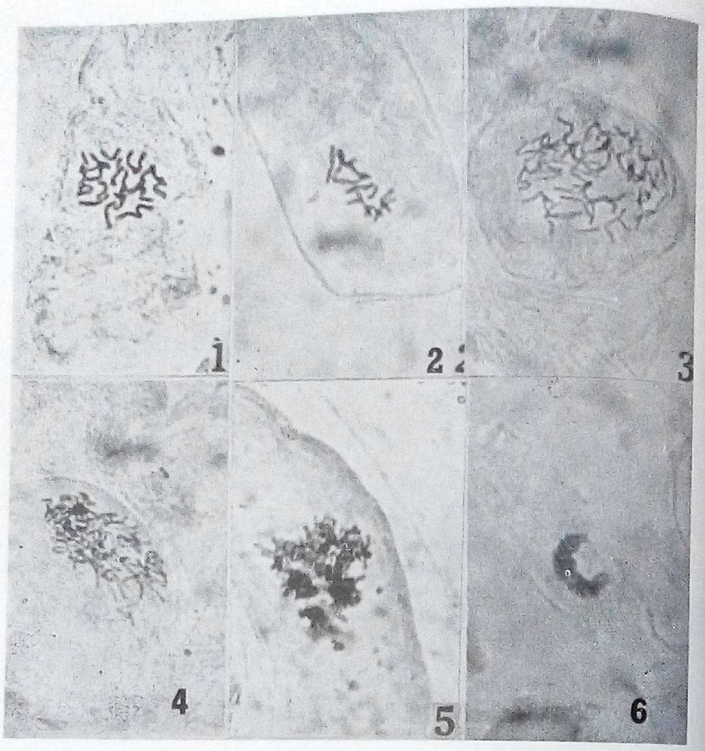
The seeds of *Nigella sativa* were germinated aseptically following surface sterilization with 0.1% mercuric chloride for 5 min. Callus cultures were initiated from hypocotyl and leaf segments in Murashige and Skoog's medium supplemented with 2 mg/l of 2,4-D and 1.0 mg/l of kinetin. After the growth of the callus tissue for 20 days, the tissues were subcultured to a fresh medium at an interval of 30 days and maintained in a culture chamber.

For cytological studies, small portions of callus tissue were taken during maximum phase of growth for four successive subcultures and fixed in acetic-ethanol (1 : 3) and stained with acetocarmine.

OBSERVATIONS

Cells at metaphase with unoriented chromosomes and those with different types of mitotic anomalies were observed after one month of culturing. Formation of irregular or C-shaped nuclei was also observed very frequently in 60 day-old cultures (Figs. 1-6).

Diploid cells were predominantly observed in cultures initially, the frequency of which decreased during subsequent subculturing with concomitant increase in the ploidy level. The frequency of tetraploid and higher polyploid cells showed an increasing trend with the increase in age of the callus.



Figs. 1–6 : Cytology of callus in *Nigella sativa*. 1. Normal diploid cell at metaphase showing 2n=24. 2. A haploid cell showing 12 chromosomes at metaphase. 3. A cell at prometaphase showing enhanced chromosome number (4n). 4. A polyploid cell showing irregular condensation of chromosomes. 5. A higher polyploid cell showing stickiness of chromosomes. 6. Cell showing C-shaped nucleus. All X 1500.

DISCUSSION

The occurrence of chromosomal instability and karyotype changes in older callus cultures has been shown in the present investigation. The in vitro studies revealed an inverse relationship between the frequency of dividing cells and the age of the callus

with concomitant increase in chromosomal variations. A change from initial diploid cell line to tetraploid line has been reported by Singh (1976) and Ghosh & Sharma (1979). Chand & Roy (1980) and Datta et al. (1983) reported increased frequency of polyploid cells due to the increased age of the callus. The occurrence of polyploid cells may be due to failure of cytokinesis with subsequent nuclear fission or a combination of both. Naylor et al. (1954) and Bayliss (1973) suggested spindle irregularities to account for the occurrence of polyploid and aneuploid cells in callus cultures of *Daucus carota*.

The present investigation emphasises the fact that plant tissues grown indefinetly on agar medium result in mixoploidy. It is, therefore, necessary to attempt regeneration in freshly cultured callus tissue in order to recover cytologically stable individuals. The cell lines with numerical variation in chromosomes on the other hand could be utilised for further studies on regeneration and selection of desirable variants.

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SOMATIC REDUCTION IN THE DIFFERENTIATED TISSUE OF *ALLIUM* SPECIES

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SUMMARY

In the differentiated root tissue of *Allium cepa* and *A. sativum*, 2,4-D induces division. Frequently, equal as well as unequal chromosomal groupings are observed. These groupings might by generating genomic inconstancy produce new genotypes.

Key Words : *Allium*, somatic reduction, differentiated tissue.

INTRODUCTION

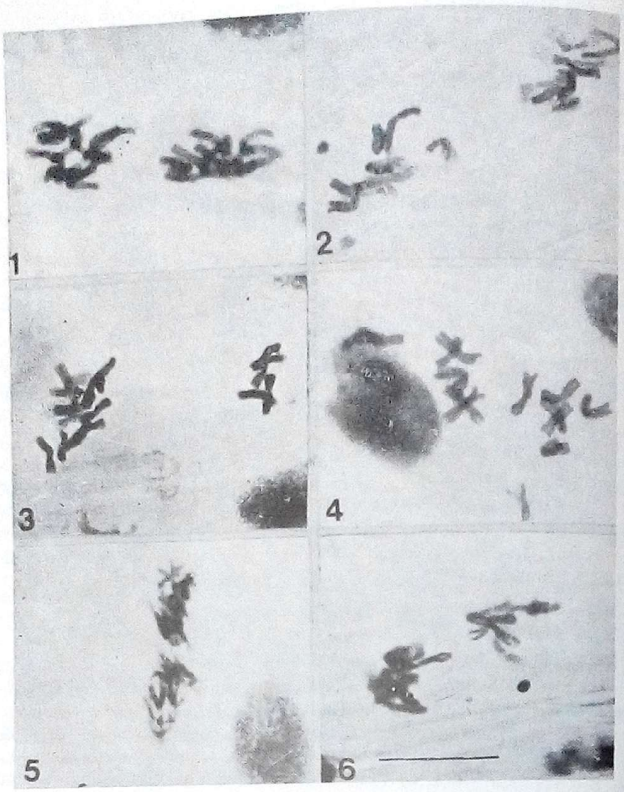
Somatic reductional division or 'somatic meiosis' is the spontaneous or induced reductional segregation of chromosomes in tissues other than those that are involved in meiosis. It was first described in cottony cushion scale insect *Icerya purchasi* (Hughes-Schrader 1925). Since then it has been shown to be occurring in plants (Huskins 1948, Huskins & Cheng 1950, Sharma 1959, Mita & Steward 1961 and Dyer 1976) as well as in animals (Berger 1938, Grell 1946, Risler 1959 and Sinha 1967) and thought to be useful to plant breeders (Huskins 1948). The present investigation deals with the study of reductional groupings of chromosomes in differentiated regions of the roots of *Allium cepa L.* and *A. sativum L.* Divisions were induced in differentiated region by 2, 4-dichlorophenoxyacetic acid (2, 4-D) (Bansal & Sen 1985).

MATERIAL AND METHODS

Germinating bulbs of *Allium cepa* and *A. sativum* with roots about 20-30 mm long were separately kept on tubes containing 2, 4-D (0.01%) so that the roots remained immersed in the solution. Parallel control experiments were set up. After 24 h, roots about 10 mm long were cut both from treated and control sets and were fixed in acetic acid : ethanol (1 : 2) for 1 h. To soften the root tissue hydrolysis for 10–12 min was done with 1 N HCl at 60°C and staining was performed with the usual aceto-orcein schedule (Sharma & Sharma 1980). Before squashing, each root was cut into two parts, viz the meristematic part (the tip) and the differentiated part (5 mm away from the tip). Both were squashed separately in 45% acetic acid. A minimum of of 2500 cells were examined for meristematic as well as differentiated regions of control and treated roots.

RESULTS AND DISCUSSION

Chromosome complement in the meristematic region of the treated roots was observed to be normal with 2n=16 whereas in the differentiated region it showed the



Figs. 1-6 : 1 & 6. *Allium sativum*. 2-5. *A cepa*. 1. Reductional (8 : 8) grouping in differentiated tissue 2. Reductional (8 : 8) grouping. 3. 12 : 4 segregation. 4. Somatic pairing. 5. Somatic grouping (16 : 16) in a polyploid cell. 6. 9 : 7 segregation. Bar=0.1 mm.

TABLE 1 : Mitotic index (MI) and reductional groupings (Data are Mean ± S E)

Species	Treatment	Tissue	MI	Reductional grouping	
				most frequent groupings	frequency
Allium cepa					
	Control	t_1	4.08 ± 0.14	—	—
		t_2	No division	—	—
	Treated	t_1	4.11 ± 0.23	—	—
		t_2	4.70 ± 0.08	8 : 8, 9 : 7, 12 : 4	1.70 ± 0.08
A. sativum					
	Control	t_1	3.87 ± 0.20	—	—
		t_2	No division	—	—
	Treated	t_1	3.67 ± 0.18	—	—
		t_2	3.97 ± 0.30	8 : 8, 9 : 7, 10 : 6	1.42 ± 0.06

t_1 = meristematic region, t_2 = differentiated region

occurrence of reductional groupings of somatic chromosomes (Table I). It, however, did not reveal any strict regularity in the segregation of chromosomes in both the species studied. Thus both *A. sativum* and *A. cepa* frequently showed a separation of 16 chromosomes that look like meiotic univalents into two perfect groups of 8 (Figs. 1 & 2) as well as into unequal groups of 9 : 7 (Fig. 6) and 12 : 4 (Fig. 3). Often, paired chromosomes were also observed along with unpaired ones (Fig. 4). Control roots, on the other hand, lacked division in the differentiated regions due to non induction of division but showed normal division in the meristematic region.

The occurrence of equal as well as unequal chromosomal groupings in both *A. sativum* and *A cepa* emphasizes that the somatic groupings in the materials attempted do not necessarily involve separation into equal halves. That somatic pairing occurs between chromosomes in induced dividing cells in both species in the present study indicates a stage similar to meiotic pairing without involving crossing over. In addition to the somatic groupings in the diploid cells even the polyploid cells showed chromosome groupings in the present study (Fig. 5) which was rather peculiar.

The somatic reductional groupings in the differentiated cells of species in the present study might have possibly arisen due to separation of the whole chromosomes by suppression of centromere division (Sharma & Mookerjea 1954, Sinha 1967). Alternatively, an increase in the amount of DNA in differentiated cells (Bansal & Sen 1985) while inducing divison in polyploid cells might possibly have caused reduction in diploid cells (Sharma & Mookerjea 1954). Whatever the reason, such somatic groupings in the present study generate an inconstancy in the chromosome complement which together with non-disjunction and partial endomitosis is a potential candidate for producing new genotypes (Sharma 1956).

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EFFECT OF HERBICIDE 'SATURN' ON *ALLIUM CEPA* L.

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SUMMARY

The herbicide 'Saturn' was studied for its cytotoxic effects on root tips of *Allium cepa* L. The herbicide induced mitostatic effects on root meristem cells ; the mitotic index decreased with increasing concentrations. Chromosomal abnormalities such as stickiness, clumping, chromosome breakage, bridges, laggards, multipolarity, polyploidy and multinucleate conditions were observed. These observations demonstrate the mutagenic property of the herbicide.

Key Words : 'Saturn', *Allium cepa* L., mitotic index, mutagenic property.

INTRODUCTION

Improvement of food production and minimization of crop yield due to pests and herbs have been the major concern in agriculture. In the past 30 years many new herbicides have been commercially introduced. Their application in plant protection has resulted in complex problems because of the side effects they produce. Excessive and indiscriminate use of herbicides have many undesirable secondary consequences on higher plants and cause environmental pollution and ecological imbalance. Several cytological studies have been carried out to detect the harmful effects of different herbicides on different plants (Liang & Liang 1972, Skorupska 1975, Reddy & Rao 1982, Badr & Ibrahim 1987, El-Khodary et al. 1990) but not much work is done on the actions of herbicide 'Saturn' (Benthiocarb 50% w/v). This work was, therefore, undertaken with a view to study its action on roots of *Allium cepa* L.

MATERIAL AND METHODS

The bulbs of *A. cepa* were allowed to grow in moist saw dust at 25° C. When the emerged roots reached 2 cm long, the bulbs were immersed in various concentrations of 'Saturn' such as 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05% and 0.06% for 8 h and then excised and fixed in Carnoy's fluid. After 6 h, they were transferred to ethyl alcohol. Squash preparations were made using acetocarmine stain by following standard cytological procedure.

OBSERVATIONS

The various concentrations of 'Saturn' clearly affected the percentage of mitotic index (Table 1). Wide spectrum of chromosomal abnormalities were recorded such as C-mitosis, clumping, stickiness, destruction of nucleus, non-disjuncture,

diagonal metaphase and anaphase, laggards, fragmentation, bridges and tripolarity (Table 1, Fig. 1). These abnormalities increase with increasing concentration. In some treatments, trinucleate condition and elongated nuclei were also seen.

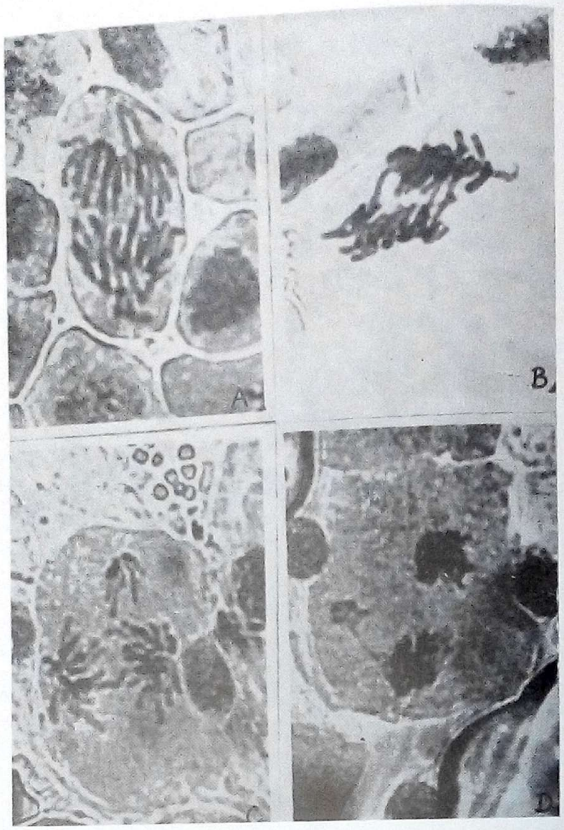

Fig. 1 : Chromosomal abnormalities caused by 'Saturn'.
A. Laggards B. Bridges C. Tripolarity D. Tripolarity with a bridge.

TABLE 1 : Types and frequency of chromosomal abnormalities during different phases at varying concentrations of 'saturn'.

CONCENTRATION%	Total No. of cells observed	Total No. of cells in diva.	Mitotic index %	PRO-PHASE	METAPHASE			ANAPHASE				TELOPHASE			
				Clumping	Stickiness	C-metaphase	Nondisjuncture	Laggards	Bridges	Nondisjuncture	Multipolarity	Multinucleate	Elongated nucleate	Micronucleate
0.0	5152	1532	29.73				0.13			0.06				
0.005	8252	1112	13.48	0.27	1.08	1.08	1.62		0.27	1.89		0.54	0.55	
0.01	8458	905	10.71		0.88	1.88	0.33		0.55	2.66		0.33	2.21	1.99
0.02	11647	912	7.83		1.84	3.29	3.18	0.55	0.55	2.63		0.99	2.41	
0.03	13581	914	6.73	2.10	1.51	4.05	9.08			5.80		1.20	0.66	0.55
0.04	14928	836	5.60	1.43	1.80	4.19	8.85	1.31		5.26	0.12	2.99	3.96	1.08
0.05	15028	807	5.37	3.58	4.84	5.08	11.89	1.24	0.25	4.21	1.73	1.12	2.35	0.37
0.06	19851	800	4.03	0.87	8.49	2.12	17.59	0.63	0.38	7.50	1.50	0.90		1.99

DISCUSSION

The reduction in mitotic index may be due to the arrest of cells in G_0 phase or a retardation in the pace of events during S or G_2 phases (Cummins 1969). There seems to be a direct relationship between decrease in mitotic index and increase in herbicide concentration. This may indicate that the herbicide interferes with the normal sequence of cell cycle to reduce the number of cells starting to divide at interphase (Adam et al. 1990). It is also possible to suggest that herbicide 'Saturn' interferes in the normal sequence of mitosis inhibiting DNA synthesis (Beu et al. 1976). Disturbed metaphase, anaphase and telophase may have arisen due to the disturbance of spindle apparatus. In some cases, even multipolar spindle seems to have operated (El-Khodary et al. 1990). The chromosomal abnormalities seen in the present species indicate the mutagenic property of 'Saturn'.

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INHERITANCE PATTERN OF POLYDACTYLY/SYNPOLYDACTYLY AMONG SEVEN FAMILIES FROM KURUKSHETRA (HARYANA)

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SUMMARY

Seven families, 2 each belonging to Rajput Jhimar and Khatri and 1 Brahmin castes were observed to be affected with polydactyly/synpolydactyly. All these families except the Jhimar are migrants from other places but now settled at Kurukshetra. A total of 15 individuals were found to be affected with these traits in all the 7 families. The pedigrees of each affected family were analysed up to a minimum of 3 generations. In most of the families the pattern of inheritance was observed to be recessive. However, in some cases the environmental stress could also affect the gene responsible for the trait.

Key Words : Polydactyly, synpolydactyly, pedigree, trait, inheritance.

INTRODUCTION

Environmental changes are capable of modifying the effect of genes. The expression of almost all the genes in man (except some blood groups and a few others) is altered by environment. Human limb abnormalities are rare. However, a considerable number of families have been observed with serious abnormalities like complete absence of hands or feet. Polydactyly and synpolydactyly are types of congenital malformations easily detectable at the time of birth. Polydactyly is characterised by presence of extra digits and the synpolydactyly is characterised by both the presence of extra digits and webbing of adjacent digits. Such traits mostly occur singly but rarely may occur together. Analysis of family records is the only substitute for controlled breeding experiments that help in knowing wether a particular trait is inherited or not. It also helps in the transmission of a trait through generations. Polydactyly and synpolydactyly are usually inherited as autosomal dominant traits (Gates 1946) but other factors also play an important role for their appearance.

Cases affected with polydactyly, synpolydactyly and syndactyly were observed during a genetic survey of various communities of Haryana. The present report relates to 7 families belonging to different castes affected with polydactyly and synpolydactyly. A detailed study of each affected family was made and possible mode of inheritance of these traits was analysed with the help of pedigree charts.

MATERIALS AND METHODS

The present investigation relates to 7 families belonging to different families (2 each from Rajput, Jhimar and Khatri and one from Brahmin caste) of Kurukshetra.

The data on polydactyly and synpolydactyly were obtained by interviewing the affected as well as normal individuals of these families. The data pertaining to a minimum of 3 generations of a family were compiled in the form of pedigree charts (Figs. 1-7). Photographs of the affected individuals were taken for record (Figs. 8–13).

RESULTS

The details of polydactyly and synpolydactyly among the seven families have been shown in Tables 1 & 2.

Pedigree I : The extra digit is present in the left foot of individual III-9 and III-16 only. The extra toe was postaxial and the webbing was present in the fifth and the extra toe (Tables 1 & 2). Individual III-9 had a normal daughter, 2 normal sisters and cousins. The parents and other relatives of the individuals III-9 and III-16 were also normal.

TABLE 1 : Types and distribution of extra-digits in the polydactylus subjects

Name of the affected Individual		Type-III							
		Palm				Foot			
		Pre-axial		Post-axial		Pre-axial		Post-axial	
		L	R	L	R	L	R	L	R
Pedigree I	III–9			—	—	—	—	+	—
(Fig. 1)	III–16			—	—	—	—	+	—
Pedigree II	III–3	—	+	—	—	—	—	—	—
(Fig. 2)	III–10	—	+	—	—	—	—	—	—
Pedigree III	III–1	—	—	—	+	—	—	—	+
(Fig. 3)									
Pedigree IV	II–2	—	—	—	—	—	—	—	+
(Fig. 4)									
Pedigree V	III–5	—	—	+	+	—	—	—	+
(Fig. 5)	III–6	—	—	+	+	—	—	+	+
Pedigree VI	III–1	—	—	+	+	—	—	—	—
(Fig. 6)									
Pedigree VIII	II–5	+	—	—	—	—	—	—	—
(Fig. 7)	II–10	+	—	—	—	—	—	—	—
	II–2	+	+	—	—	—	—	—	—
	II–4	+	+	—	—	—	—	—	—
	II–5	—	+	—	—	—	—	—	—
	II–6	+	+	—	—	—	—	—	—

TABLE 2 : Polydactyly/Synpolydactyly among the affected subjects in the present pedigrees

Affected individual		PALMS				FEET			
		Number of fingers		Finger Involved in webbing		Number of toes		Toes involved in webbing	
		L	R	L	R	L	R	L	R
Pedigree I	III–9	5	5	—	—	6	5	5th & 6th	—
	III–16	5	5	—	—	6	5	5th & 6th	—
Pedigre II	III–3	5	6	—	—	5	5	—	—
	III–10	5	6	—	—	5	5	—	—
Pedigree III	III–1	5	6	—	—	5	6	—	—
Pedigree IV	II–2	5	5	—	—	5	6	—	5th & 6th
Pedigree V	III–5	6	6	—	—	5	6	—	—
	III–6	6	6	—	—	6	6	—	—
Pedigree VI	III–1	6	6	—	—	5	5	—	—
Pedigree VIII	II–5	6	5	—	—	5	5	—	—
	II–10	6	5	—	—	5	5	—	—
	II–2	6	6	—	—	5	5	—	—
	II–4	6	6	—	—	5	5	—	—
	II–5	6	6	—	—	5	5	—	—
	II–6	6	6	—	—	5	5	—	—

Pedigree II : Individuals III–3 and III–10 are having hexadactyly in the right hand only (Fig. 2). Webbing of the digit was absent. Parents of III–3 and III-10 were normal. Both the affected individuals are married to normal individuals and had all normal children. No other affected individual was traced in the pedigree, neither on maternal side nor on paternal side. The extra finger of the affected individuals was preaxial in position (Tables 1 & 2).

Pedigree III : It comprises of a small family in which individual III-1 was affected with hexadactyly in the right hand and the right foot (Fig. 3). Webbing of the dight was absent. The position of extra digits in both the hand and the foot was postaxial (Table 1 & 2). The parents, sisters and other relatives of affected individual were found to be normal.

Pedigree IV : It has a single affected individual II-2 (Fig.4). The affected individual has 6 toes in the right foot which was fused with the fifth toe. The position of the extra digit was postaxial in position (Tables 1 & 2). Affected individual had normal parents and have 2 daughters and a son. No other relative of the individual II-2 was found to be affected.

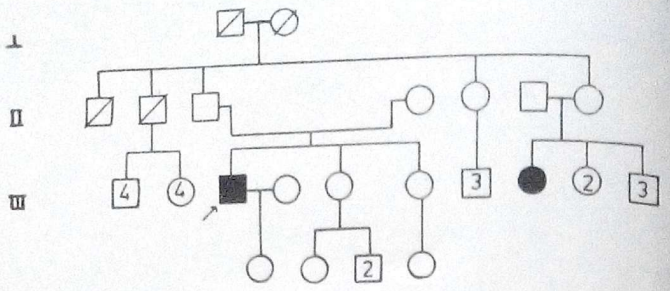


Fig. 1

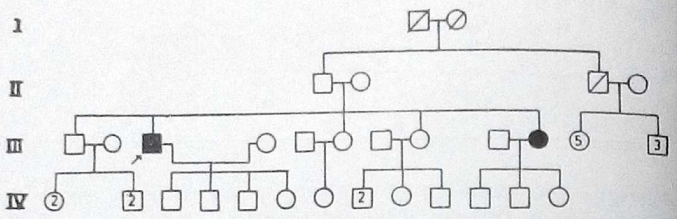


Fig. 2

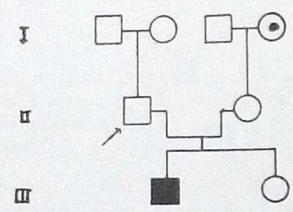


Fig. 3

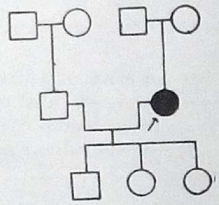


Fig. 4

Figs. 1-4 Pedigrees 1-4

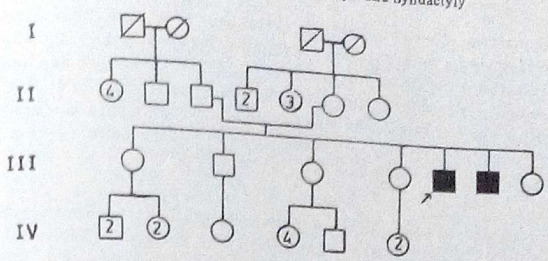


Fig. 5

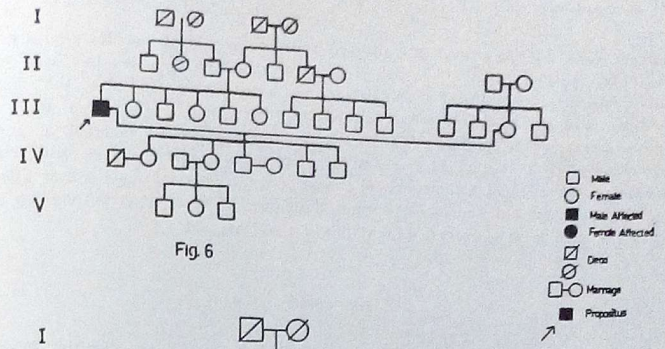


Fig. 6

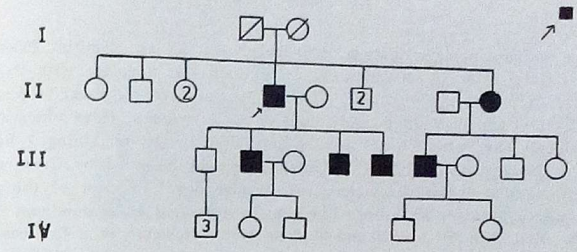


Fig. 7

Figs. 5-7 : Pedigrees 5-7

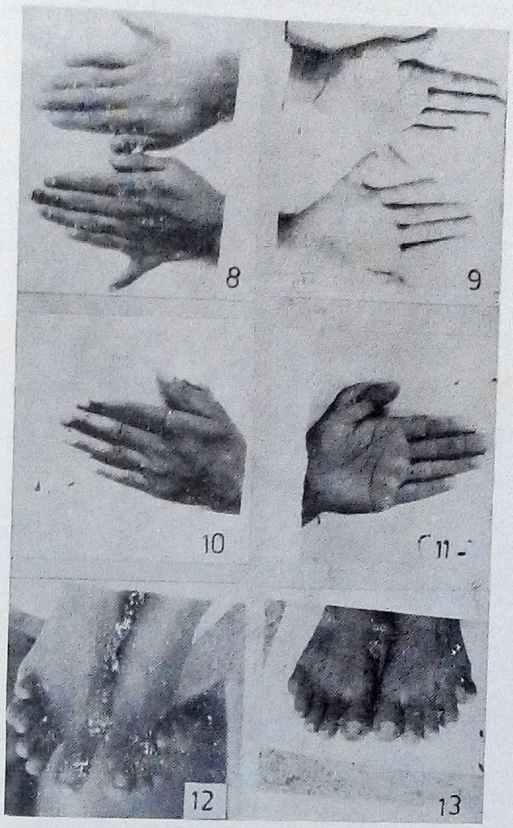
Pedigree V : It shows two affected individuals, III-5 and III-6. Individual III-5 is affected with hexadactyly in both the hands and only the right foot. Individual III-6 has 6 digits in both the hands and the feet (Fig. 5). Webbing of digit was absent in both the individuals. Position of the extra digit was postaxial (Tables 1 & 2). The parents, a brother and 4 sisters of the affected individuals were normal. No other case of polydactyly/synpolydactyly was observed on either the maternal side or the paternal side of the proposita.

Pedigree VI : It comprises a single affected individual III-1 having hexadactyly in both the hands (Fig. 6). Position of the extra finger is postaxial (Tables 1 & 2). Affected individual married a normal spouse and all his children and grandchildren are normal. Moreover no other relative either on maternal or paternal side is found to be affected.

Pedigree VII : It has a total of 6 affected individuals having hexadactyly in hands only (Fig. 7). Individuals II-5 and II-10 have extra fingers in the left hand only. Individuals III-2, III-4, III-5 and III-6 have extra fingers in both the hands. Webbing of digit is absent in all the affected individuals. The extra digit was always preaxial in position (Tables 1 & 2). Individual II-5 married a normal individual and has a normal son and 3 affected sons (III-2, III-4 and III-5). Similarly, the affected female (II-10) married to a normal male has an affected son (III-6), a normal son and 2 normal daughters. The affected individulas III-2 and III-6 each have a normal daughter and a normal son.

DISCUSSION

During the present investigation polydactyly in all the families (Table 2) confirmed to Gates type III, in which the extra dight was complete with its own metacarpals or metatarsals (Gates 1946). The position of extra digit can be preaxial or postaxial. Out of 15 affected individuals in the 7 families under observation, only 8 individuals have preaxial position of extra digits, the remaining 7 having extra digit in the postaxial position. Synpolydactyly was observed by Cross et al. (1968) and found that it was a less frequent phenomenon. In most of the cases polydactyly and syndactyly have been found to be inherited as a dominant trait (Gates 1946, Bell 1953, Stein 1960 and Mathew 1988). However, in a few cases a recessive mode of inhertiance has also been suggested (Gates 1946, Yadav et al. 1991). Stein (1960) is of the view that a dominant gene D controls the formation of number of bony rays in embryonic buds of palm and feet and further suggested that among dd genotype, a few rays of metacarpals or metatarsals and phalangeal bones are formed leading to normal digital conditions.



Figs. 8-13 : 8,9 Hexadactyly in the individual III-5 of pedigree V, extra digit postaxial. 10,11. Hexadactyly in individual II-5 of pedigree VII, extra digit preaxial. 12. Hexadactyly in individual III-6 of pedigree V, extra digit postaxial. 13. Synpolydactyly in the left foot of the individual III-9 of pedigree I.

The pedigrees (Figs. 1-6) contain either a single or 2 affected individuals with polydactyly synpolydactyly. The trait was not transmitted or expressed in the next generations and was confined to a single generation only. The possible reason for this could either be a complete lack of expressivity or incomplete penetrance of the defected gene/genes. Further, exposure to certain environmental conditions could also be responsible for such mutations. These traits could also be inhertied as autosomal recessive in these families. In pedigree VII (Fig. 7), 2 generations, II and III, had affected individuals of both sexes. The possible mode of inheritance may be autosomal dominant. However, the usual 1:1 ratio of the affected to normal individuals is lacking. The X-linked recessive hypothesis in which only males are affected and the trait skips over generations frequently can be eliminated since in this pedigree a female is also affected. The trait could not be inherited as X-linked dominant as females are more likely to inherit the trait than males and it cannot be inherited from father to sons also. In case of autosomal recessive inheritance, both males and females are affected but in the present case the recessive trait appears only in siblings and not in parents and frequently appears in case of consanguinity. But in the present pedigree we did not observe any consanguinous marriage. The data is insufficient to permit any definite conclusion regarding mode of inheritance in the present pedigrees. Whether the position (pre- or post-axial) and webbing of the digits are under the influence of the same gene or are governed by separate loci needs further investigation.

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SEED PROTEIN PROFILE AND ORIGIN OF *PANICUM SUMATRENSE* (GRAMINEAE)

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SUMMARY

Water soluble seed proteins of *Panicum sumatrense* and *P. psilopodium* involving 6 collections were fractionated by disc gel electrophoresis and their protein profiles were compared. Seed protein profiles of *P. sumatrense* (sama millet) and *P. psilopodium* from several collections are identical. The average similarity index of protein homology between these two species is high. It is concluded that genomes of these 2 species are homologous and *P. sumatrense* might have originated through selection and further cultivation from wild grass *P. psilopodium*.

Key Words : *Panicum sumatrense*, *P. psilopodium*, seed proteins.

INTRODUCTION

Seed protein profiles obtained by electrophoresis has been widely utilized in taxonomic studies, genome analysis, cultivar identification, origin and evolution of crop plants. These and other aspects have been reviewed by Ladizinsky & Hymowitz (1979), Kapse & Nerkar (1985) and Smith (1986). Seed proteins are mainly storage proteins and are unlikely to be changed in dry mature seeds. Thus mature seeds of various age groups will possess same protein profile (Ladizinsky & Hymowitz 1979). Among cultivated plants, where a large number of accessions are available from different geographic regions still possess the same basic protein profile (Ladizinsky 1975, Ladizinsky & Adler 1975a, and Johnson 1975). Recently, even the specific cultivars and hybrid varieties of crop plants have been identified by protein profile. This knowledge is likely to be used in future for testing genetic purity of hybrid seeds (Kapse & Nerkar 1985, Smith 1986). Further chromosomal rearrangements or even doubling of chromosome numbers have no effect on the seed protein profile (Moustakas et al. 1986).

Genetically related species have similar protein profile and unrelated species possess different protein patterns. This basic tenet of protein homology has been amply verified in several cases (Ladizinsky & Hymowitz 1979). Thus seed protein profile is a stable character and has been widely used to assess the genome relations and elucidate the origin and evolution of crop plants.

Panicum sumatrense Roth or sama millet is cultivated in Indian subcontinent and forms an important part of tribal agriculture in eastern ghats of India. *P. psilopodium* Trin. is a wild grass and is distributed in Indian sub continent and

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islands of Indonesia. Morphological (De Wet et al. 1983) and cytogenetical (Hiremath et al. 1990) evidences suggest that *P. psilopodium* is the progenitor of sama millet *P. sumatrense*. These 2 species are allotetraploid (2n=4x=36) based on base number of x=9 (Chennaveeraiah & Hiremath 1991). Hiremath et al. (1990) from chromosome pairing data of *P. sumatrense* × *P. psilopodium* hybird suggested that genomes of these 2 species are similar and fully homologous. They proposed that *P. sumatrense* has originated from *P. psilopodium* through selection and further cultivation.

Purpose of this study was to verify the hypothesis formulated by Hiremath et al. (1990) about the origin and evolution of *P. sumatrense* through seed protein profiles. This is a preliminary step to our molecular approach to the study of origin, evolution and domestication of minor millets.

MATERIALS AND METHODS

Seed material of cultivated taxon *P. sumatrence* was obtained from ICRISAT, Hyderabad and University of Agricultural Sciences, Bangalore. The wild species *P. psilopodium* was collected around Pune and Aurangabad. Identification of these species were confirmed by the Royal Botanic Gardens, Kew. One set of vouchered specimens are filed at Kew Herbarium.

Mature seeds of *P. psilopodium* and *P. sumatrense* involving 6 accessions were randomly collected and surface sterilized with 0.2% mercuric chloride and washed thoroughly with water. They were ground in agate mortar and pestle and flour was mixed with cold 0.2M phosphate buffer pH 7.0 containing 5mM mercaptoethanol. The homogenate was centrifuged for 15 min and supernatant was used for electrophoresis. The proteins thus extracted are largely albumins. Folin Ciocalteu method (Lowry et al. 1951) was used to estimate the protein content in the above supernatant.

Polyacrylamide disc gel electrophoresis was performed (Davis 1964) in 5×60 mm glass tubes using 7.5% acrylamide gel electrophoresis apparatus consisted of 12 vertical tubes. A suitable aliquot of supernatant containing 100–150 μg protein was layered on the gel column. Proteins were fractionated in anodic system using tris-glycine buffer pH 8.3 with bromophenol blue as tracking dye. An initial regulated current of 2 mAmp per tube was applied for 20 min after which an amperage of 3 mAmp per tube was maintained till the tracking dye reached 5.5 cm mark on glass tube. In this anodic system negatively charged acidic proteins migrate towards the anode. After electrophoresis, gel rods were removed from the glass tubes and proteins were fixed in 12.5% trichloracetic acid for 1 h. Later, gels were stained in freshly prepared 0.25% aqueous solution of coomassie brilliant blue-R 250 for 20 min. Destaining was done by diffusing out excess stain in 7% acetic acid overnight (Chrambach et al. 1967).

Variations in the number and position of bands were expressed by relative mobility (Rm) and similarity index (SI) values (Vaughan & Denford 1968). These values were calculated as follows :

$$Rm = \frac{\text{Distance travelled by the protein}}{\text{Distance travelled by the tracking dye}}$$

$$SI = \frac{\text{No. of pairs of similar bands}}{\text{No. of different bands} + \text{No. of pairs of similar bands}}$$

RESULTS AND DISCUSSION

Water soluble seed proteins of *P. psilopodium* and *P. sumatrense* involving 6 collections were separated by disc gel electrophoresis. The resolved protein profiles are shown in Fig.1 and their Rm values are presented in Table 1. Similarity index values as a parameter of protein homology between these two species are summarised in Table 2.

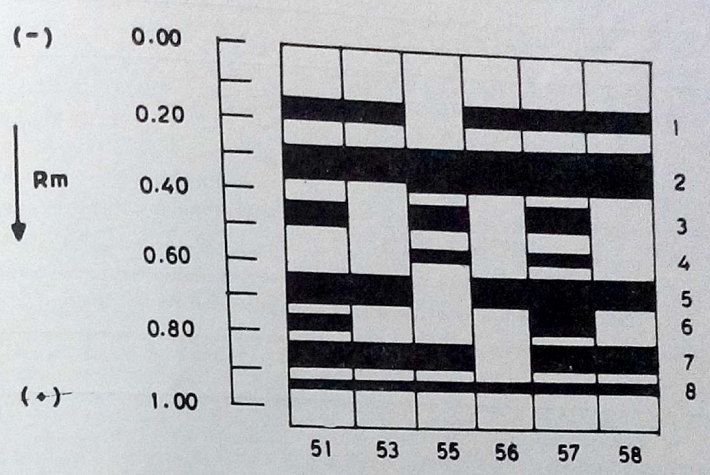

FIG. 1

Electropherogram of seed proteins in *Panicum* species. *P. psilopodium* Coll. Nos. 51, 53. *P. sumatrense* Coll. Nos. 55, 56, 57, 58.

In *P. psilopodium*, seed protein profiles of two collections analyzed are quite similar with 71.4% protein homology, However, Coll. No. 51 is distinct in having bands 3 (Rm 0.45) and 6 (Rm 0.78). These two additional bands are absent in Coll. No. 53. The number of protein bands varies from 4 to 7 in 4 collections of *P. sumatrense* (Fig. 1, Tables 1 & 2) The similarity index values range from 28 to 80% in this species. Protein pattern is similar but not identical in Coll. No. 55 and 57. These collections have 5 bands with similar Rm values. However, Coll. No. 57 has 2 additional bands, 1, 5 and 6. Similarity index value among these 2 collections is 71.4%. Seed protein pattern in collections 56 and 58 are nearly identical except band No. 7 (Rm. 0.87) found in latter collections The protein homology among 2 collections is 80%. Seed protein profiles of P. *psilopodium* and *P. sumatrense* are indentical (Fig. 1, Tables 1 & 2). There is no band unique to any of these above two species. The similarity index values in these two species vary from 42 to 100%, with an average of 67% (Table 2).

TABLE 1 : Rm values for seed protein profile of *Panicum* species

Sl. No.	Species	Coll. No.	Bands 1	2	3	4	5	6	7	8
1.	*P. psilopodium*	51	0.16	0.33	0.45	—	0.70	0.78	0.87	9.1
2.	*P. psilopodium*	53	0.16	0.33	—	—	0.70	—	0.87	0.91
3.	*P. sumatrense*	55	—	0.33	0.45	0.6	—	—	0.87	0.91
4.	*P. sumatrense*	56	0.16	0.33	—	—	0.70	—	—	0.91
5.	*P. sumatrense*	57	0.16	0.33	0.45	0.6	0.70	—	0.87	9.1
6.	*P. sumatrense*	58	0.16	0.33	—	—	0.70	—	0.87	9.1

TABLE 2 : Similarity index values among various collections of *Panicum* species

Sl. No.	Species	Coll. No.	1	2	3	4	5	6
1.	*P. psilopodium*	51	X					
2.	*P. psilopodium*	53	71.4	X				
3.	*P. sumatrense*	55	50.0	42.8	X			
4.	*P. sumatrense*	56	57.1	80.0	28.6	X		
5.	*P. sumatrense*	57	71.4	62.5	71.4	66.6	X	
6.	*P. sumatrense*	58	71.4	100.0	42.8	80.0	71.4	X

The seed protein electrophoresis is a powerful tool in elucidating genome relations, origin and evolution of crop plants. Cultivated plants and their wild progenitors have the same gene pool and from genetic point of view, they are members of the same species. Inspite of morphological differences between them, they still share the same protein profile (Ladizinsky & Hymowitz 1979). *P. psilopodium* is a weed in the cultivation of sama millet, *P. sumatrense*. In nature, gene exchange between them is not uncommon. Seed protein profiles of *P. psilopodium* and *P. sumatrence* from several collections are identical. There is no unique band specific to any of the 2 species. Average similarity index between them is 67%. Thus it is concluded that genomes of these 2 species are homologous and sama miliet *P. sumatrence* might have originated from wild grass *P. psilopodium*. Hiremath et al. (1990) reached a similar conclusion based on chromosome pairing of *P. sumatrense* x *P. psilopodium* hybrids. Cultivated *P. sumatrense* and its progenitor *P. psilopodium* are tetraploid (2n=4x=36) with AABB genomes. It is worthwhile to compare the seed protein profiles of all the diploid species of *Panium* with these 2 tetraploid species to discover A and B genome donors to this sama millet.

Similarity between seed protein profiles of progenitor wild species and their cultivated counterparts has been well documented in wheat (Johnson 1967), cotton (Johnson & Thein 1970), soybean (Mies and Hymowitz 1973), and corn (Paulis & Wall 1977). Employing these principles, *Cicer reticulatum* was identified as the wild progenitor of chickpea and was later supported by breeding experiments (Ladizinsky and Adler 1975 b).

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STARCH GEL ELECTROPHORETIC METHODOLOGY FOR POPULATION GENETIC ANALYSIS

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SUMMARY

The starch gel electrophoretic technique is currently being applied in the population genetic analysis of natural populations of diverse taxa. The present report deals with a detailed account of the electrophoretic apparatus, buffer systems, support media, preparation of samples, types of staining systems involved for detection of genic variability at several loci coding for soluble proteins as well as enzymes. Empirical as well as statistical methods used in the transformation of elctrophoretic banding patterns into indices of genic diversity have also been discussed.

Key Words : Starchy-gel electrophoretic methodology. Allozymic variation, Genetic structure.

INTRODUCTION

The application of gel electrophoresis to population genetic analysis constitutes a major thrust of the evolutionary genetics and systematics (Hedrick 1983, Karlin & Nevo 1986 and Mac Intyre 1986). The electrophoretic analysis of parents and progeny of genetic crosses enables the transformation of electrophoretic banding patterns into genetic variation (Oxford & Rollinson 1983). The technique permits the characterisation of amounts and types of genetic variability in populations of diverse types of organisms and to provide estimates of the extent of genetic divergence among closely related species (Karlin & Nevo 1986). Thus the technique has enormous potential in elucidating the evolutionary biology of diverse taxa. Although an account of electrophoretic techniques occurs in some standard sources (Harris & Hopkinson 1976), there is a complete lack of comprehensive account of the utility of the technique in evolutionary studies. Thus, the aim of the present report is to discuss the details of the techniques involved, collection and interpretation of electrophoretic data in terms of genetic variability indices and their utility in various evolutionary studies.

MATERIAL AND METHODS

Samples :

Tissue fluids such as blood plasma, hemolymph etc. can be obtained from live organisms of several populations and can be directly subjected to electrophoretic analysis. However, aequous or buffer extracts of tissue proteins (1 gm/5 ml) are made by homogenisation and centrifugation in a cold room. The co-enzymes NAD/NADP and/or activators are included in the resultant extracts so as to stabilise the activity of

some unstable enzymes. If required, the enzyme extracts are stored at —20°C in a deep freezer or at —190°C in liquid nitrogen. The homogenisation of plant tissues (leaves, cotyledons, stems etc.) is carried out in a suitable buffer of pH 8.0 plus 0.1% of cysteine hydrochloride and ascorbic acid, 1% polyethylene glycol and 1 mM 2-mercaptoethanol.

Buffers :

Electrophoresis involves a supporting medium (starch gel/acrylamide gel/agar gel/cello-gel), a buffer to control and conduct electric current and a stablised power supply. Electrophoretic resolution depends upon the pH and ionic strength of the buffers employed. The gel buffers are generally 10 times diluted as compared with the electrode buffers. Furthermore, the buffer systems are required to counter the pH change occurring at cathodal and anodal ends due to electric power applied across the gel. Therefore, the general buffer systems, in the range of pH 8-9, are used because most proteins become negatively charged and thus migrate towards anode and their buffering capacity is optimum (Table 1). The cathodal and anodal

TABLE 1 : Gel and electrode buffer systems used for electrophoretic analysis of different enzyme systems.

Type of Buffer system & pH	Chemical composition	To be used for enzyme systems
A. Gel Buffer, 8.9	0.706 M tris (9.21 g/1) + 0.005 M Citric acid (1.05 g/1)	Acph, Est, Lap, Aph, Pgm, Adh,
Electrode Buffer, 8.7	0.3 M borate (18.5 boric acid/l) + 0. 1 M, NaoH ,4g NaoH/l)	Odh, AO etc.
B. Gel Buffer, 8.5	0.015 M tris (6.2 g/1)+ 0.008 M Citric acid. (1.68 g/1)	Amylase, G-6-pdh, Pgi, general proteins etc.
Electrode Buffer, 8.1	0.029 M LiOH (1.2 g/1) +0. 192M boric acid (1.89 g/1)	
C. Gel Buffer, 8.5	0.74 M tris (9gm / 1) +0.008 M citric acid (1.68g/1)	Mdh, Me, Adh, Idh, G-3-pdh, pgm, α-Gpdh
Electrode Buffer, 8.1	0.687 M tris (86.2 g/1) + 0.157 M Citric acid (33 g/1)	

buffer solutions are mixed after every electrophoretic run and their pH are checked. The electrode buffer is discarded after 3-4 electrophoretic runs. For some of the enzymes, co-enzymes (NAD/NADP) and/or activators are included in the buffer system so as to chance the separation, stability and staining intensity of the isozymes e.g. NAD/NADP in case of dehydrogenases; Mg++ in case of phosphatases and PGI.

Gel preparation :

Starch gels are preferred for population genetic studies because of their superior molecular sieving property easy preparation, detection of several enzyme systems in the gel slices and the accurate detection of mobility differences in several samples included in the slab gels. The starch gels are prepared by continuous heating coupled with mechanical shaking the 12% (W/V) mixture of starch in gel buffer till the solution turns nearly transparent. The resulting solution is degassed to remove air bubbles and is poured in a suitable gel mould of glass plates (20 X 12 X 1 cm) and is covered with a glass plate. The gel is allowed to set at room temperature. Sample wicks containing tissue extracts are inserted, either in the middle or near the cathodal end of the gel, into well spaced slits made in the gel. The end slits are generally loaded with wicks soaked in bromophenol blue (dye indicator) so that the moving front may be tracked during total running period of 3-4 hrs. The cathodal/anodal and Rt/Lt ends of the gel are marked and the gel is sliced into three layers of equal thickness.

Staining :

After electrophoretic run, the cut surfaces of the gel slices are stained for ditferent enzymes by following the standard staining methods. Several dehydrogenases which are capable of reducing co-enzymes (NAD or NADP) are stained by tetrazolium method. Th's involves reduction of colourless soluble tetrazol um salt (MTT, methyl th'oazolyl tetrazolium) into an insoluble formazan. All such reactions are photosensitive and hence are carried out in the dark. Tetrazolium method somet'mes/produces pseudo bands or nothing dehydrogenases due to endogenous substrates and/or chemical contaminat'ons. The hydrolases (APH, ACPH, EST etc.) act on an artificial substrate (α-naphthyl derivatives) and the product binds with a diazo dye (fast blue RR/BB salt) to give rise to coloured bands which are quite stable. However, enzymes such as hexokinase, phosphoglucomutase, glucose phosphate isomerase etc. are unique in the sense that these involve enzyme linked staining methods. The exogenous dehydrogenase acts on the product of such enzyme and is stained by the usual tetrazolium method. For most of dehydrogenases, it is desirable to apply enzyme stains to agar overlay on the starch gel as this enhances the resolution of the isozymes bands (Smith 1976).

RESULTS AND DISCUSSION

The patterns of bands which result after staining a gel for enzyme is called a zymogram or electropherogram (Fig.1). The electrophoretic mobility variation in the

banding patterns are interpretable in terms of variation in the gene coding for the variant proteins. The pattern of bands is treated as a phenotype and investigated by genetic tests that determine which bands are coded by allelic and/or nonallelic loci. The segregation patterns of bands can be correlated with segregating alleles if the enzyme phenotypes are under monogenic control. Fig. 1 depicts enzyme phenotypes in single wild caught individuals. The patterns include either of segregating single band variants of faster mobility (Fast band) and slower mobility (Slow band) and both fast and slow bands together in individuals 1 to 3. If such electrophoretic phenotypes in parents and progeny of genetic crosses find fit to 1 : 1 or 1 : 2 : 1 ratios characteristic of monogenic inheritance, then the single band variants FF or SS and two banded patterns (FS) represent homozygous and heterozygous genotypes. Such patterns result from monomeric enzymes. The occrrence of 0, 1, 2 and 3 hybrid bands in heterozygotes refer to monomeric, dimeric, trimeric and tetrameric enzymes (Fig.1). An array of single band

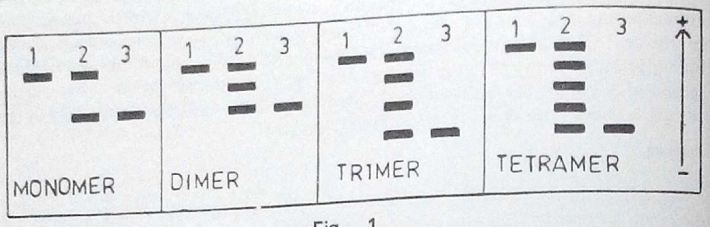

Fig. 1

Schematic diagrams of electrophoretic patterns of enzymes under monogenic control. Samples (1 & 3) refer to homozygotes and the single band variants (fast = F ; slow = S) represent two co-dominant alleles. The heterozygous isozyme patterns (sample No. 2) differ with respect to 0, 1, 2 & 3 hybrid bands in case of monomeric, dimeric, trimeric and tetrameric enzymes under monogenic control. Enzymes include : monomers-PGM, EST & PEP etc. ; dimers-ADH, ODH' AO, AOPH, APH, MDH,&-GPDH etc. ; tetrameric-LDH, SOD & ME etc. Arrow indicates the direction of current flow.

(Fast, Medium & Slow) often results due to multiple allelic variation patterns at a locus. The nomenclature patterns of allelic isozymes (allozymes) are quite diverse e.g. allelic variants may be designated as A_1, A_2, A_3 and A_4 in order of decreasing electrophoretic mobility towards the origin; the most common electromorph (allozyme) is given a mobility value of 100 and other variants are referred by mobility values less or more than the most frequent one. Sometimes rare alleles result in the production of nonfunctional proteins i.e. null alleles (Parkash 1987).

Some gene-enzyme systems are under the control of multiple loci. If a band appears in the same position on the gel in all the individuals, it can be infered that the

gene controlling the enzyme also does not vary i.e., monomorphic. On the contrary, segregating banding patterns are often under the control of a polymorphic locus. The gel electrophoretic technique thus enables to count the loci that vary and those which do not vary and results in the calculation of polymorphic loci in a species population (Hartl 1980)

A variety of statistics is used to denote the amouut of genetic variation in a population. The most extensively used measures are the polymorphism (P) and the heterozygosity (H). A locus is defined as polymorphic when the frequency of the most common allele is less than a set value usually 0.95 or 0.99 since above these values, the loci are said to be monomorphic. The average frequency of heterozygotes over all loci examined per individual is denoted as H. The number of alleles per locus are calculated by averaging over all polymorphic and monomorphic loci.

TABLE 2 : Formulae used for calculation of different genetic indices from electrophoretic data for polymorphic gene-enzyme systems in species populations.

S. No.	Parameter	Formulae
1.	Allele frequency	$\frac{2 \times D + H}{2N}$ (D-homozygous individuals ; H-heterozygous individuals ; N-total number of individuals)
2.	Heterozygosity	$H_{exp.} = 1 - \Sigma xi^2$ (xi^2 is square of frequencies of alleles) $H_{obs.}$ = observed number of heterozygotes/total number of individuals.
3.	Effective number of alleles (n_e)	$\frac{1}{\Sigma xi^2}$
4.	Hardy-Weinberg equilibrium	$p^2 + p^2 + 2pq$ (p-frequency of one allele ; q-frequency of second allele, for diallelic locus)
5.	Wright's inbreeding coefficient (F)	$1 - \left(\frac{H_{obs.}}{H_{exp.}}\right)$
6.	Log-likelihood X^2 test (G-test)	4.60517 ($F_i \log F_i + \frac{}{} F_i \log f_i$) ($F_i$-observed genotypes ; f_i-expected genotypes)
7.	Percentage of similarity (electromorphs)	$\frac{\text{Number of pairs of similar bands}}{\text{Number of different bands + Number of pairs of similar bands}} \times 100$

The data on allelic frequencies at polymorphic loci in several geographical populations are tested for heterogeneity by using contingency X^2 test. The genotypic frequencies at polymorphic loci are tested for fit to Hardy–Weinberg expectations using log-likely hood X^2 test or G–test. The extent of genetic differentiation in populations are measured by using Nei's coefficients of genetic identity I (allelic similarity) and genetic distance D (average number of allelic substitutions per locus which have accumulated since two or more populations diverged). The values of I range from 0 to 1.0, (i.e. no allelic differences between populations) D may range from zero to infinity, and can be used to estimate the period of evolutionary time involved since the populations diverged (Nei 1975). The data on I or D values are used to construct phylogenetic dendrograms which help in assessing phylogenetic relationships between species groups or taxa. The electrophoretic data permit genetic variability to be compared between different populations or species in relation to their evolutionary histories, and for taxonomic phylogenetic relationships (Milkman 1983). The present report includes data compiled on the use of gel electrophoretic technqiue in population genetic studies applicable to plants, animals and human beings.

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KARYOLOGICAL AND MEIOTIC PECULIARITIES IN NATURALLY OCCURRI TETRAPLOID HYBRID *GLORIOSA MASTERPIECE* (2n=4x=44)

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SUMMARY

Detailed karyological and meiotic studies have been carried out in the naturally occurring tetraploid species of *Gloriosa* L. with somatic number 2n= 4x= 44 chromosomes. This taxon is morphologically distinct in colour of the petal brown and yellow with entire lanceolate perianth. It is distinct in its cytology with the karyotypic formula $2n= 4x = 44= B'_2 + G'_2 + I_2 + M_2 + M'_2 + N'_{12} + 0_8 + T'_{12} + U_2$ which supports its tendency of isolation as natural allotetraploid. Meiosis is highly irregular with first and second division stages showing large number of laggards. Univalents and multivalents are of common occurrence with high frequency of quadrivalents.

Key Words : *Gloriosa* Karyotype, meiotic analysis, allotetraploid.

INTRODUCTION

The genus *Gloriosa* L. belonging to the tribe Uvularieae of the family Liliaceae has great relevance in both horticulture and pharmaceutical sectors due to its dual importance as ornamental plant as well as colchicine yielding one. Percy-Lencaster & Percy – Lancaster (1966) provided detailed records of speciations in *Gloriosa*. Euploids with 2n = 22, 44, 66, 77 and 88 chromosomes have been recorded (Karihaloo 1985, Lemattre 1986 Narain 1986 and Lugade 1987). *G. masterpiece* a naturally occurring hybrid recognised by the authors is taken up in the present investigation for its karyological and meiotic studies.

MATERIALS AND METHODS

The tubers of the present material were collected from Darjeeling of West Bengal and the plants were raised in pots as well as soil beds in Botanical garden of the department. Young growing root tips pretreated with 0.2% colchicine for $4\frac{1}{2}$ h at 10° ± 2°, were fixed in modified Farmer's fluid where acetic acid is replaced by lactic acid (Fernandez 1973) for 24 h. Mitotic metaphase chromosome plates were studied from squash preparations of root meristems using 2% propiono-orcein stain.

Karyotypic analysis was done based on the data after examining atleast 10 metaphase plates. For evaluation of chromosome morphology, the terminology proposed by Abraham & Prasad (1983) is followed. Photomicrographs were taken from temporary preparations and photoidiogram was prepared as per the method of Macgregor et al. (1983). For constructing the karyotype formula chromosomes with range in length from 2.6 to 16.1 μm were arbitrarily classified under four size groups such as very long (12.2 to 16.1 μm), long (8.2 to 12.1 μm), medium (4.2 to 8.1 μm) and short (0.2 to 4.1 μm).

RESULTS AND DISCUSSION

The somatic number was consistently found to be 2n=4x=44 in all the root tips examined (Fig. 1). The karyotype formula is $2n=4x=44=B'_2+G'_2+I'_2+M_2+M'_2+N'_{12}+O_8+T'_{12}+U_2$. One pair ($B'_2$) of chromosomes is largest in size (16.1μm) with nearly median centromere and other 2 pairs (G'_2 and I_2) are appreciably larger (11.8 and 8.6 μm) where in 1 pair (G'_2) with secondary constriction and other (I_2) with nearly submedian (–) centromere could be seen. Twelve pairs of chromosomes (M_2, M'_2, N'_{12}, O_8) are of medium size (4.2 to 7.7 μm) and 7 pairs (T'_{12}, U_2) are short (2.6 to 4.1 μm). A pair of medium chromosomes M (5.3μm) possesses satellite on short arm whereas other pair M' (7.7μm) has secondary constriction. Six pairs each of medium size (N'_{12}) (4.2 to 6.0 μm) and short (T'_{12}) chromosomes (2.6 to 4.1 μm) are with nearly median centromere. Four pairs of medium sized chromosomes O_8 (5.1 to 7.1 μm) and a pair of short chromosomes U_2 (4.1μm) are with nearly submedian (–) centromere. Absolute chromosome length of 131μm and average chromosome length of 5.95 μm (Table 1) reflect on increasing quantity of redundant DNA. Ratio of the shortest to the longest chromosomes of the complement is 0.16.

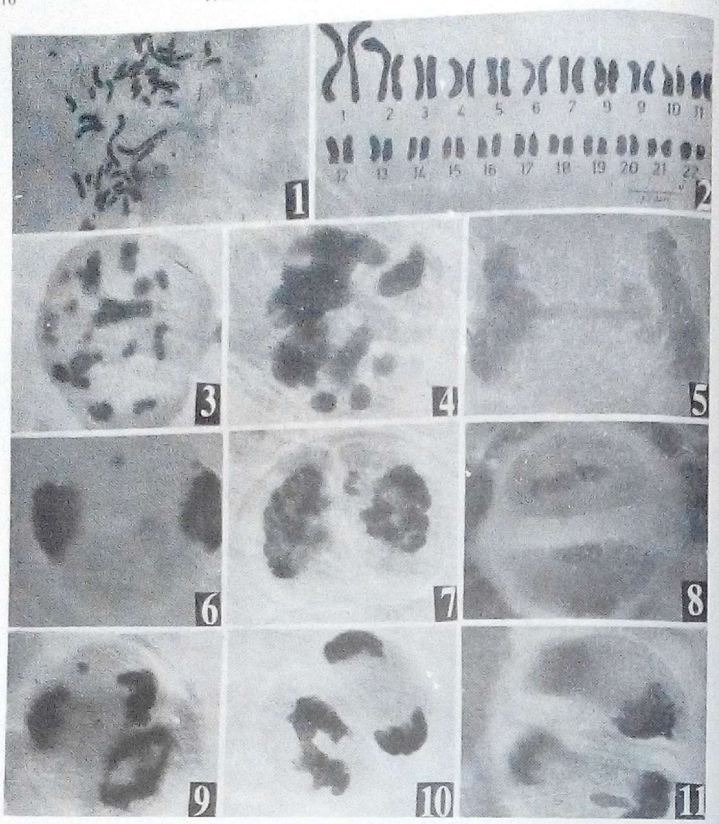
Meiosis is highly irregular (Figs. 3–11). Chromosomal associations at diakinesis are analysed covering average per PMC and percentage of chromosomes involved (Table 2). The chromosomal associations are mostly associated with univalents and trivalents. Quadrivalents are rather rare. The average association per PMC is $10.56_I + 6.5_{II} + 5.56_{III} + 0.94_{IV}$. Some of the univalents faintly stained failed to orient on the equator and are located peripherally. They displayed a tendency towards precocious disjunction during M I (7.2%), A I (8.9%), M II (0.9%) and A II (6.2%).

Karyomorphology and chromosomal associations confirm allotetraploid nature of this naturally occurring tetraploid hybrid. High frequency of structural hybridity reflects on genomic instability.

TABLE I : Details of Karyotype analysis of *Gloriosa masterpiece (2n=44)*

Chromosome number	Chromosome length in μm			Relative chromosome length	Arm ratio r=L/S	Centromeric index $\frac{100S}{C}$	Centromeric notation
	Long arm L	Short arm S	Total C				
1	8.80	7.30	16.10	12.29	1.21	45.34	nm
2	6.20	5.60	11.80	9.00	1.11	47.46	nm
3	5.80	2.80	8.60	6.56	1.86	32.56	nsm (–)
4	4.30	3.40	7.70	5.88	1.26	44.16	nm
5	4.70	2.40	7.10	5.42	1.96	33.80	nsm (–)
6	4.90	1.90	6.80	5.20	2.58	27.94	nsm (–)
7	3.90	2.60	6.50	4.97	1.50	40.00	nm
8	3.40	2.60	6.00	4.58	1.31	43.33	nm
9	3.60	2.30	5.90	4.50	1.57	38.98	nm
10	3.00	2.30 (0.5+1.80)	5.30	4.01	1.30	43.40	nm
11	3.60	1.70	5.30	4.01	2.12	32.08	nsm (–)
12	3.60	1.50	5.10	3.89	2.40	29.41	nsm (–)
13	2.60	1.90	4.50	3.44	1.37	42.22	nm
14	2.60	1.90	4.50	3.44	1.37	42.22	nm
15	2.30	1.90	4.20	3.21	1.21	45.24	nm
16	3.00	1.10	4.10	3.13	2.73	26.83	nsm (–)
17	2.40	1.70	4.10	3.13	1.41	41.46	nm
18	2.30	1.50	3.80	2.90	1.53	39.47	nm
19	2.30	1.50	3.80	2.90	1.53	39.47	nm
20	2.30	1.50	3.80	2.90	1.53	39.47	nm
21	2.10	1.30	3.40	2.60	1.62	38.24	nm
22	1.50	1.10	2.60	1.98	1.36	42.31	nm

Absolute chromosome length=131.00 μm
Average chromosome length = 5.95 μm
Ratio of the shortest to the longest chromosomes of the complements=0.16
Size range=2.60 to 16.10 μm



Figs. 1–11 : Somatic chromosomes (x 1000) and meiotic peculiarities of *Gloriosa masterpiec*. 1. Somatic chromosomes, 2n = 44. 2. Photoidiograms, 2n = 44. 3. Diakinesis with univalents and multivalents. 4. Poleward precocious movement of univalents and multivalents. 5-6. Late Anaphase-I showing bridge and laggards. 7. Prophase-II showing laggards. 8-9. Diads with nonsynchronous divisions with laggards. 10-11. Telophase-II with bridges and laggards.

TABLE : 2 Chromosome associations at diakinesis in *Gloriosa masterpiece*

Type of configuration	Average/PMC	%age of chromosomes involved
Quadrivalents	0.94	8.55
Trivalents	5.56	37.91
Bivalents	6.50	29.54
Univalents	10.56	24.00

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IMPORTANT

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A COMPARISON OF GENE-ENZYME VARIATION AMONG THREE *DROSOPHILA* SPECIES OF MONTIUM SUBGROUP

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SUMMARY

Allozymic variation of eight gene-enzyme systems was analysed through starch gel electrophoresis in 3 *Drosophila* species (*D. jambulina, D. punjabiensis* and *D. kikkawai*) of the montium species subgroup. Data reveal that each of the 5 enzyme systems (ACPH, AO, ADH, ODH and *a*-GPDH) is controlled by an autosomal locus while EST, APH and MDH have revealed multiple zones of activity. The 3 types of electrophoretic patterns include complex Acph isozymes coded by duplicate alleles, conformational isozymes of ADH and *a*-GPDH and standard allozymic variation for the remaining 5 gene-systems. The overall indices of genetic variation as well as determinations of percentages of common allozymic bands indicate similarity in the 3 species of montium species subgroup. Data on the species specific genetic structure at 12 loci in these 3 *Drosophila* species have been discussed.

Key Words : Gel electrophoresis, allozymes, *Drosophila*, genetic polymorphism.

INTRODUCTION

Gel electrophoretic analysis of gene-enzyme systems constitutes a powerful tool in the study of genetic differentiation among species populations (Ayala et al. 1974, Wills 1981). The gel electrophoretic technique separates proteins on the basis of their net electric charge and the resulting enzyme banding patterns transformed into allelic and/or non-allelic genetic variation on the basis of specific genetic crosses (Lewontin 1974, Nei 1975). Such data on enzyme genotypes are used to describe the genetic structure of natural populations (Ferguson 1980, Wills 1981). The technique has been widely applied to natural populations of cosmopolitan as well has other *Drosophila* species of temperate region but there is lack of such information on the drosophilids occurring in the oriental region. The *Drosophila melanogaster* species group comprises 115 species under 13 species subgroups which include the largest montium species subgroup (50%), 4 major subgroups (25%) and 8 minor subgroups (25%) (Bock 1980). A single study has been made so far to examine the extent of genic variation in laboratory strains of 6 species of montium subgroup (Triantaphyllidis et al. 1978). Thus, the information on most of the species of the montium subgroup is still lacking. The present paper reports the comparative petterns of electrophoretic variability for 8 gene - enzyme systems in 3 species of the montium subgroup.

MATERIALS AND METHODS

Isofemale lines of *D. jambulina*, *D. punjabiensis and D. kikkawai* were characterised after Parshad & Paika (1964), Bock & Wheeler (1972) and Bock (1980). About 12–15 homogenates of species specific single individuals were loaded in each horizontal starch gel slab (15×10×1 cm) and run electrophoretically at 250 V and 30 mA at 4°C for 4 h and the gel slices were stained for different gene-enzyme systems (Smith 1976, Harris & Hopkinson 1976). The gene–enzyme systems include octanol dehydrogenase (ODH, E.C. 1.1.1.73), esterases (EST, E. C. 3.1.1.1), acid phosphatases (ACPH, E. C. 3.1.3.2), α-glycerophosphate dehydrogenase (α-GPDH, E. C. 1.1.1.8.), aldehyde oxidase (AO, E. C. 1.2.3.1.), malate dehydrogenase (MDH, E.C. 1.1.1.37) alcohol dehydrogenase (ADH, E. C. 1.1.1.1) and alkaline phosphatase (APH, E. C. 3.1.3.1). The genetic basis of enzyme banding patterns was interpreted from the segregation ratios of electrophoretic phenotypes of the parents and progeny (F_1/F_2/ Backcross) of species specific genetic crosses. The genetic interpretation of electrophoretic data and calculation of genetic indices were followed from other sources (Ferguson 1980, Zar 1984). The data on per cent similarity of allozymic bands among the 3 species of the montium subgroup was calculated after following Nair et al. (1971).

RESULTS AND DISCUSSION

Genetic basis of electrophoretic phenotypes

The comparative starch gel electrophoretic phenotypes of eight gene enzyme systems in *D. jambulina*, *D. punjabiensis* and *D. kikkawai* have been represented in in Figs. 1 and 2. The monomorphic zones include α-GPDH, ADH, APH–1 and–2 and MDH–2; and the electrophoretic mobilities of all such zones are identical in the 3 species. Out of species specific esterase zones of activity, some of the zones are represented by segregating single–band variants and two-banded patterns while other zones are monomorphic. The segregation behaviour of polymorphic esterase zones in parents and progeny of genetic crosses are in agreement with monogenic inheritance patterns. The occurrance of two-banded patterns in heterozygotes has indicated that esterases are monomeric. The polymorphic zones of AO, ACPH, MDH–1, APH–3 and ODH are represented by segregating single band variants and triple-banded patterns. Genetic crosses between individuals having triple-banded and single-banded patterns (for ACPH, ODH, AO, MDH–1 and APH–3) produced about equal proportions of progeny with electrophoretic phenotypes like the parents and the segregation ratios (1:1) have been found to be in accordance with monogenic mendelian inheritance patterns. The banding patterns did not vary with the sex and thus the enzyme phenotypes are coded by distinct autosomal loci. The single-band variants and triple-band patterns homozygous and heterozyous genotypes respectively and such enzymes are dimeric in nature. The occurrence of non-segregating

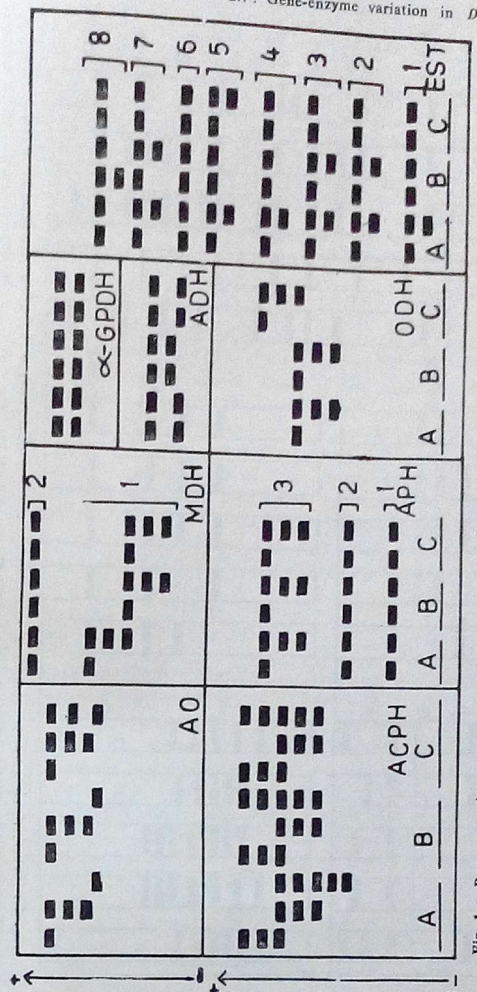

Fig. 1 : Representation of starch slab gel electrophoretic patterns for 8 gene-enzyme systems in single individual homogenates of *D. jambulina* (A), *D. punjabiensis* (B) and *D. kikkawai* (C). Multiple zones of activity occur for APH, MDH and EST while other enzymes are represented dy a single zone of activity. Single-band variants and triple-band patterns for AO, MDH-1 APH-3 and ODH represent homozygous and heterozygous genotypes. Three-banded and five-banded complex ACPH patterns are coded by a duplicate locus Two-banded ADH and α - GPDH patterns are coded by monomorphic loci. Arrow indicates the direction of current flow.

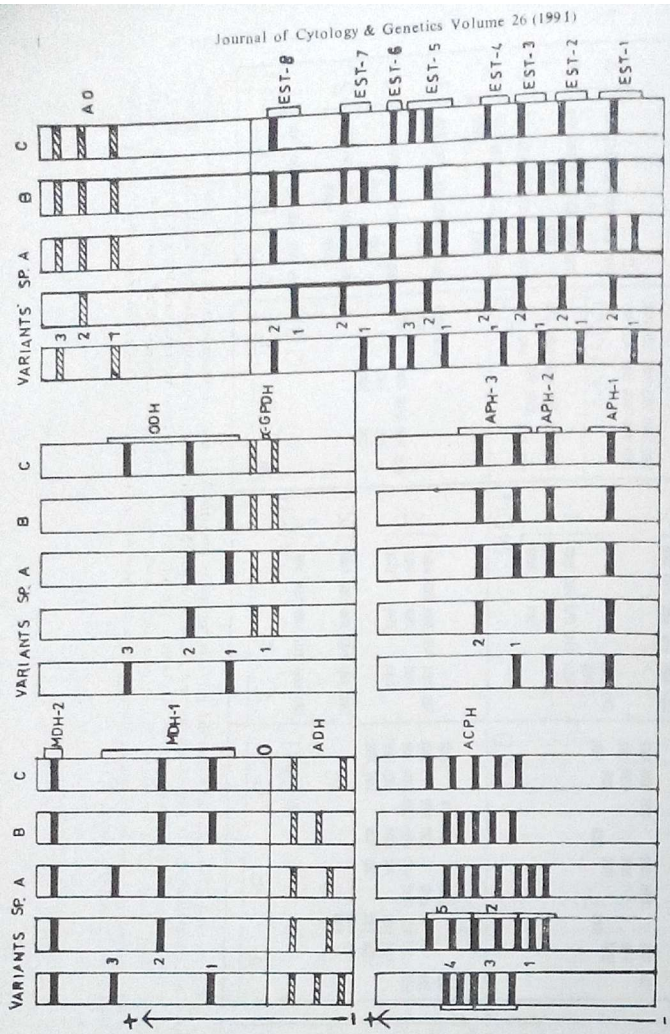

Fig. 2 : Schematic comparative representation of allelic isozymes (allozymes) of 8 gene-enzyme systems in three *Drosophila* species of the montium species subgroup. Each gene-enzyme system has been shown in 5 bar-type diagrams. Allelic variants (electromorphs) for each enzyme are shown in first two bars while species specific allozymes of *D. jambulina, D. punjabiensis* and *kikkawai* are shown in bars 3,4 and 5 respectively. The bands have been drawn according to their electrophoretic mobilities. The banding patterns of cathodal ADH and anodal α-GPDH have been superimposed. O refers to the origin for sample homogenates.

two–banded patterns of ADH as well as of α-GPDH in homozygous strains represented electromorphs or allozymes, and thus could be under the independent control of a single locus. The present observations on ADH and α–GPDH concur with other reportes that in NAD requiring dehydrogenases, more than one electromorphs (conformational isozymes) may arise due to posttranslational differential binding of coenzyme NAD (Niesel et al. 1982). The observed patterns of genic variation at various polymorphic loci in the 3 species of *montium* species sub group concur with the functional constraint hypothesis that the glucose-metabolising enzymes are least variant as compared to nonglucose-metabolising enzymes (Gillespie & Kojima 1968, Johnson 1974).

The single polymorphic ACPH zone revealed either three-banded or five-banded ACPH patterns and the mobility patterns are species specific. The progeny of isofemale lines depicting particular triple-banded patterns did not reveal segregation of the ACPH bands in any of these 3 species. Genetic crosses involving 2 distinct types of triple-banded ACPH phenotypes resulted in five-banded ACPH patterns in the F_1 individuals. Genetic crosses among five-banded individuals resulted in offsprings having 2 distinct types of triple-banded patterns and five-banded patterns in accordance with mendelian segregation ratio of 1:2:1. Thus the genetic data indicated that the observed complex ACPH patterns in each of the 3 species is controlled by a duplicate locus and three-banded and five-banded phenotypes represent homozygous and heterozygous ones respectively.

Population genetic structure

The data on allelic frequencies, observed and expected heterozygosity, Wright's inbreeding coefficients and G–values for log-likelihood X^2 test for fit to Hardy-Weinberg expectations at various polymorphic loci in *D. jambulina, D. punjabiensis* and *D. kikkawai* have been represented in Tables 1 & 2. The patterns of allelic frequencies are almost identical at three loci (ADH, α-GPDH and AO) while the three species seem to be genetically different at other loci (MDH-1, APH-3, ACPH and EST loci) due to differential distribution of allelic frequency patterns (Tables 1 & 2). The range of heterozygosities observed at various polymorphic loci correlate well with the number of alleles and allelic frequencies in three species. Significant deviation from Hardy-Weinberg expectations have been observed at ACPH, AO, MDH-1, APH-3 EST-1, 4, 5, 7 in *D. jambulina* ; at ACPH, AO, EST-3 in *D. punjabiensis* and at AO and APH-3 loci in *D. kikkawai*. The high values of Wright's coefficient at EST-1 and –4 loci in *D. jambulina* and ACPH & EST-3 and EST-8 in *D. punjabiensis* and at ACPH and APH-3 loci in *D. kikkawai* indicate deficiency (+f) or excess (—ve f values) of heterozygotes at such loci.

TABLE 1 : Data on distribution of genotypes, allelic frequencies, heterozygosities (obs./exp.), Wright's fixation index (f) and effective number of alleles (n_e) and G-values for log-likelihood x^2 test for fit to Hardy Weinberg expectations at five loci in three species of montium species group (A=*D. jambulina*, B=*D. punjabiensis*, C=D. *kikkawai*).

Locus	Species	observed & expected genotypes						Sample size (N)	Allele freq.			Het.			
		FF	MM	SS	FM	FS	MS		F	M	S	obs./exp.	f	n_e	G-values
ACPH	A	8	80	20	12	0	48	168	.08	.66	.26	.36/.49	.26	1.96	42.08*
		1.07	73.18	11.36	17.74	6.98	57.66								
	B	88	22	—	32	—	—	142	.73	.27	—	.23/.39	.41	1.65	23.95*
		55.98	10.35		55.98										
	C	15	0	—	24	—	—	66	.41	.59	—	.36/.48	.25	1.90	3.14 n.s.
		11.09	0.53		31.93										
AO	A	21	28	14	42	7	0	112	.40	.44	.16	.44/.62	.29	2.62	56.03*
		17.92	21.68	2.87	39.42	14.34	15.77								
	B	10	20	15	50	0	0	95	.37	.47	.16	.53/.62	.14	2.60	88.85*
		13	20.99	2.43	33.04	11.26	14.29								
	C	25	25	0	45	15	0	110	.50	.43	.07	.54/.56	.04	2.27	21.06*
		27.5	20.34	0.54	47.30	7.70	6.62								
ODH	A	—	44	8	—	—	40	92	—	.70	.30	.43/.42	—.02	1.72	0.09 n.s.
			45.08	8.28			38.64								
	B	—	40	10	—	—	55	105	—	.64	.36	.52/.46	—.13	1.85	2.14 n.s.
			43.01	13.61			48.38								
	C	36	12	—	48	—	—	96	.625	.375	—	.50/.47	—.06	1.88	0.03 n.s.
		37.5	13.5		45										
		FF		SS		FS			F		S				
MDH-1	A	72/77.9		0/6.2		56/43.9		128	.78		.22	.43/.38	—.26	1.53	15.9*
	B	24/21.67		42/39.7		54/58.6		120	.42		.58	.45/.49	.80	1.96	0.75 n.s.
	C	30/30.01		18/18.6		48/47.3		96	.56		44	.50/.49	—.02	1.97	0.03 n.s.
APH-3	A	51/56.06		9/13.97		66/55.97		126	.67		.33	.52/.44	—.17	1.78	4.20*
	B	16/12.32		34/30.14		31/38.59		81	.39		.61	.38/.48	.19	1.91	3.85 n.s.
	C	3/10.98		25/32.96		46/38.05		82	.37		.63	.56/.47	—.19	1.88	4.15*

Expected genotypes are given below or against obs. genotypes ; * Significant at 5% level ; n.s. = non-significant.

TABLE 2 : Data on the distribution of genotypes, allelic frequencies, heterozygosities (obs./exp.), Wright's fixation index (f) and effective number of alleles (n_e) and G—values for log—likelihood X^2 test for fit to Hardy-Weinberg expectations at esterase loci in three species of montium species sub group (A = *D jambulina*, B = *D punjabiensis* C = *D. kikkawai*).

Locus	Species	Observed/expected genotypes			Sample size (N)	Allele freq.		Het. obs./exp.	f	n_e	G-value
		FF	SS	FS		F	S				
EST-1	A	39/28.81	42/31.21	39/59.98	120.	.49	.51	.32/.50	.36	2.0	14.99*
EST-2	A	45/41.39	30/25.56	57/65.05	132	.56	.44	.43/.51	.15	1.97	2.08 n.s.
	B	6/7.53	48/49.76	42/38.71	96	.28	.72	.43/.40	—.07	1.67	0.67 n.s.
EST-3	A	30/29.84	41/41.11	69/70.05	141	.46	.54	.49/.50	.02	2.0	0.04 n.s.
	B	15/7.13	72/64.12	27/42.75	114	.25	.75	.24/.37	.35	1.6	14.19*
EST-4	A	42/26.24	52/36.16	30/61.60	124	.46	.54	.24/.50	.52	2.0	39.13*
EST-5	A	26/18.83	46/38.99	40/54.18	112	.41	.59	.36/.48	.25	1.92	7.71*
	C	11/9.26	40/38.15	34/37.59	85	.33	.67	.40/.44	.09	1.78	0.75 n.s.
EST-7	A	36/30	36/30	48/60	120	.50	.50	.40/.50	.20	2.0	4.85*
	B	9/10.06	36/37.90	42/39.04	87	.34	.66	.48/.45	—.06	1.82	0.43 n.s.
ESS-8	B	30/31.26	12/12.77	42/39.97	84	.61	.39	.50/.48	—.04	1.91	0.20 n.s.

D. jambulina is monomorphic for EST-6 and EST-8 loci ; *D. punjabiensis* is monomorphic for EST—1, EST-4, EST-5 and EST-6 loci, while *D. kikkawai* is monomorphic at all the esterase loci except EST-5. *Significant at 5% level ; n.s. = non-significant.

The earlier electrophoretic analysis of 5 species (*D. auraria, D. biauraria, D. triauraria, D. serrata and D. Kikkawai*) of montium subgroup revealed species inter-relationships based on genic variation patterns for 5 enzyme systems (EST-6, EST-C, ACPH, α-GPDH and LAP). Thus, Triantaphpllidis et al. (1978) reported a maximum of 66.6% genetic similarity between *D. serrata* and *D kikkawai* while a greater degree of genetic differentiation was observed between *D. auraria, D. biauraria* and *D. triauraria*. In the present studies also, the data on interrelationships between 3 species of montium subgroup *(D. jambulina, D. punjabinesis* and *D. kikkawai)* based on present similarity of allozymes among species pairs are given in Table 3. The data revealed maximum genetic/allozymic similarity (86.67%) between *D. jambulina and D. punjabiensis* which constitute the known sibling species pair. However, *D. kikkawai* is also closely related to the

TABLE 3 : Data on species inter-relationship on the basis of percent genetic similarity of the allozyme bands between three species of montium species sub-group.

Species	*D. jambulina*	*D. punjabiensis*	*D. kikkawai*
D. jambulina	X	86.67	75.86
D. punjabiensis		X	78.57
D. kikkawai			X

other 2 species of montium group. The present studies point out the need to survey genetic polymorphism in many other species of the montium subgroup which occur in India and thereby to establish their overall phylogenetic relationships.

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CYTOLOGY AND IN VITRO MORPHOGENESIS IN *CODIAEUM VARIEGATUM* CV. WARENII

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SUMMARY

Codiaeum variegatum cv Warenii exhibits a high degree of chromosomal variations with 2n=24, 48, 56, 60, 68, 72, 84, 88, 96, 102, 104, 120 and the most common number being 2n=80. Further, meiosis in it is not very regular. The formation of univalents, multivalents, laggards, non-synchrony in division, disrupted polar orientation and split spindles are some of the meiotic configurations. The possible reasons for chromosomal numerical variations are unequal disjunction, split spindles and clumping of chromosomes. In vitro studies using explants like stem, leaf, petiole, decoated seeds and inflorescence have been carried out. The cytologcal instability and chromosomal abnormalities reveal the heterozygous nature of the plant.

Key Words : *Codiaeum variegatum* cv Warenii, chromosomal instability, morphogenesis.

INTRODUCTION

Codiaeums popularly known as crotons have attracted the attention of horticulturists all over the world as foliage plants because of their unusual colour combination and infinite patterns of leaf variegation. The occurrence of variations in a single species of *Codiaem variegatum* is attributed mainly to their capacity for hybridization and response to vagetative propagation (Sharma & Bal 1958). At present, there are about 600 cultivars of *Codiaeum variegatum* under cultivation offering much scope for cytological and tissue culture studies. About 100 of them have been investigated cytologically by Sharma & Bal (1958), Pancho & Hilario (1963), Gill et al. (1973) Chikkannaiah & Gayatri (1977), Chennaveeraiah & Wagley (1985) and Gayatri & Shanta (1989) revealing striking chromosomal and morphological variations. Further, tissue culture studies have been made in 9 cultivars by Chikkannaiah & Gayatri (1974), Gayatri (1975) and Gayatri & Shanta (1987). In the present investigation, *C veriegatum* cv Warenii is selected for cytological and in vitro morphogenetic studies.

MATERIALS AND METHODS

Plants of *Codiaeum veriegatum* cv Warenii were obtained from Lalbagh Horticultural Society, Bangalore and were maintained in pots. It is a rare ornamental plant having a tall and robust stem topped by a crown of long, linear, pendant, spirally twisted leaves, red in centre with yellow bloches and margins coppery green (Fig. 1). For mitotic studies, young root tips 4-5 mm long were cut and pretreated with 0.002 M 8-hydroxyquinoline for 5 h at 4°C and transferred to acetic

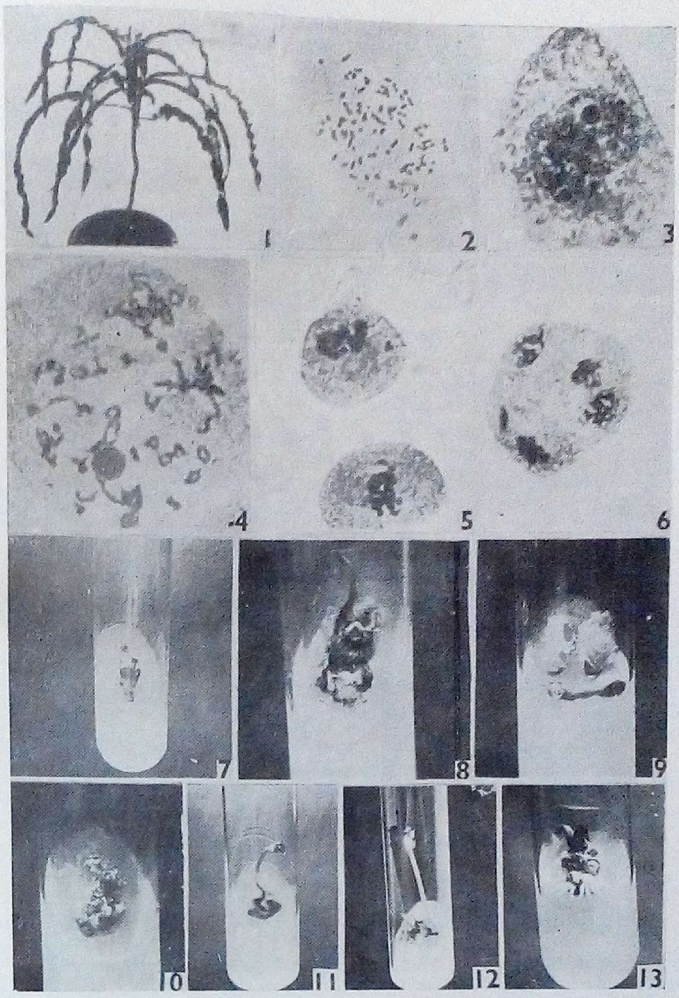
acid-ethonol (1 : 3) for 30–40 min. Slides were prepared by squash method using 2% aceto-orcein. For meiotic studies, anthers were fixed in Carnoy's fluid for 24 h and squashed in 2% acetocarmine.

Parts of stem, leaf, petiole, inflorescence and decoated seeds from field-grown plants were taken for in vitro investigations. The explants were surface sterilized in saturated chlorine water for 15 min followed by mercuric chloride (0.1%) for 3 min. The explants were cultured on Modified White's (Rangaswamy 1961) (MWBM), Murashige & Skoog's (1962) (MS) and Bourgin & Nitsch 'H' (1967) basal media (NHBM). 2% sucrose was added to the medium and it was solidified with agar (0.7%); the pH was adjusted at 5.6 to 5.8. Different adjuvants like cocount milk (CM), casein hydrolysate(CH), benzyl aminopurine (BAP), N-(2-Chloro-4-pyridyl), N-Phyenylurea (4-PU), indole-3-acetic acid (IAA), 2,4-dichloro-phenoxy acetic acid (2,4-D) and Kinetin (Kin) were used as supplements in the media.

RESULTS AND DISCUSSION

The chromosomes are generally small varying in number considerably within the plant and even within the root tip. The most common number is 2n=80. The lowest number is 2n=24 and the highest is 2n=120. Other chromosome numbers encountered are 2n=48, 56, 60, 68, 72, 84, 88, 96, 102 and 104. There is a mixture of both small, dot-like to slightly larger chromosomes varying in size from 0.5 to 2.1μm (Fig.2).

PMCs show some abnormal features along with normal stages. Occasionally, 2-10 nucleoli are found at leptotene (Fig.3). During diplotene and early diakinesis a network like strutcure is formed due to sticky nature making it difficult to count the chromosomes. In addition to bivalents, univalents trivalents, and multivalents are commonly observed at diakinesis and some of them are attached to the nucleolus (Fig.4). The orientation at metaphase I is not always normal. The bivalents and multivalents have a tendency to arrange themselves in groups (Fig. 5). Occasionally, chromatin bridges with one or more laggards are seen at anaphase I and II. The laggards and chromatin bridges are expressive of the hybrid nature of the plant. The most common feature is the occurrence of split spindles and non-synchrony in divisions leading to disrupted orientations (Fig. 6). Despite these irregularities, some normal tetrads are also formed.



Figs. 1–13: 1. *Codiaeum variegatum* cv Warenii. Potted plant. 2. Somatic metaphase plate showing 2n = 96. 3. PMC with 4 nucleoli. 4. Diakinesis showing univalents, trivalents and qudrivalents. 5. PMCs at metaphase I showing groups of chromosomes. 6. PMC showing disrupted orientation. 7. Stem segment with apical bud. 8. White mass of callus at the basal region after 3 weeks in culture. 9. 2-week-old callus at the basal region after 3 weeks in culture. 10. Endosperm callus. 11. Radicle showing characteristic curvature with callus at its apex and base of cotyledonary leaves. 12. Rhizogenesis in hypocotyl just below the callused radicular apex. 13. Rhizogenesis from basal region of stem apex.

Culture of shoot apex showed both callusing and development of shoot. Only callusing was observed on MWBM+CM (10%) (Fig. 7). When MWBM was supplemented with CM (10%) and CH (500 ppm) or BAP (2 ppm) and 4 PU (10 ppm) callusing as well as shoot development was noticed (Fig. 8) and after 6 weeks, roots developed from the callus. Stem explant with node and internode showed a few shoot bud formations in the axillary region on MWBM with CM (10%), BAP (2 ppm) and 4-PU (10 ppm). However, these explants failed to form shoot buds on MS medium but only callus formation was observed. Leaf and petiole explants cultured on MS supplemented with BAP (2 ppm), 4-PU (10 ppm) and IAA (1 ppm) showed yellowish-green callus (Fig. 9). Decoated seeds when cultured on MWBM supplemented with CM (10%), CH (500 ppm) and 2, 4-D (1 and 2 ppm) showed proliferation of endosperm (Fig. 10), negatively geotropic germination (Fig. 11) and formation of roots just below the radicular apex (Fig. 12) apart from normal germination of decoated seeds. Direct organogenesis was observed in the from of rhizogenesis in stem (Fig. 13) and leaf explants. The inflorescences responded on NHBM with adjuvants like CM (10%) and Kin (1 ppm) forming friable as well as compact callus within 3-4 weeks. After 16 weeks of culture, roots were formed from the callus.

The cytological studies in the present investigation reveal striking variation in somatic chromosome number which, however, is not in multiples of any of the basic chromosome number reported for the species so far. The most common number is 2n=80 which confirms the chromosome number reported for cv. Warenii by Sharma & Bal (1958). The random variation in chromosome number may be formed due to spindle abnormalities (Darlington & Thomas 1937). The disjunction of chromosomes to the poles is uneven when split spindles are formed. Also, the groups of chromosomes formed due to secondary association may have a chance to fuse or two or more groups may get enclosed within a common wall resulting in higher chromosome number as has been reported earlier (Chennaveeraiah & Wagley 1985).

The present study revealed that the explants have the capacity to regenerate on a simple medium as reported in other cultivars (Gayatri & Shanta 1987) thus providing a scope to raise a large number of plants through micropropagation.

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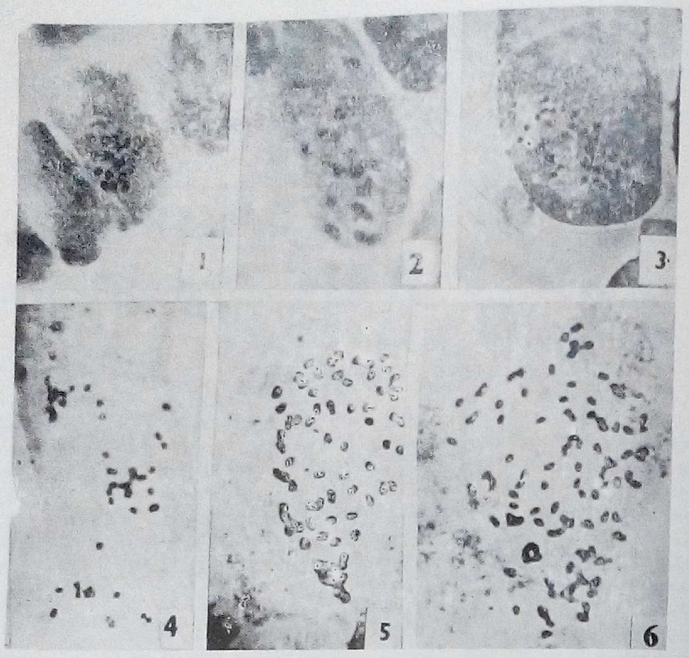

Figs. 1-6 (all x 1500) : Root and shoot tip cells of *Jasminum* at mitosis. 1 & 2, root tip cells of *J. sambac*. 1, 2n = 26 chromosomes. 2, an aneuploid cell showing 24 chromosomes. 3 & 4, shoot tip cells of *J. angustifolium*. 3, 2n = 52 chromosomes. 4, an aneuploid cell with 48 chromosomes. 5 & 6, *J. azoricum* (hexaploid). 5, root tip cell with 2n = 78. 6, a hyperploid cell with more than 78 chromosomes.

DISCUSSION

Present data on chromosome number reveal that in *Jasminum* the variability in somatic number is not in multiples of any basic number. Such mixoploid conditions are encountered occasionally in *Triticum* (Li and Tu 1947), *Caladium bicolor* and *Zephyranthes mesochloa* (Sharma 1956) and in some garden crotons (Chennaveeraiah & Wagley 1985).

In *Jasminum*, the origin of nuclei with altered chromosome numbers certainly involves various abnormal cytological mechanisms. One possibility is chromosome duplication involving the entire chromosome set or partially which is otherwise

called partial endoreduplication. Such a mechanism may easily give rise to a large number of aneuploid and polyploid cells as recorded by Sharma & Ghosh (1954). However, in *Jasminum* cytomictic channels between adojoining cells with complete or partial chromosome complements migrating through them (cytomixis) ultimately give rise to a mixoploid tissue of cells with altered chromosome numbers. However, the minor role played by unequal segregation and lagging chromosomes in connection with somatic chromosome instability in *Jasminum* cannot be ruled out.

According to Sumitra Sen (1973) polysomaty must have originated out of gene–environment interaction at different levels of tissue growth. Based on the present study the authors are of opinion that in sexually reproducing plants, normal mitosis is ensured by putting genes together in their genetic system which enable them to resist various adverse environmental conditions while in vegetatively reproducing plants where such recombinations are not possibie the genes turn to be plastic and fluctuating in accordance with varying conditions of environment. This in turn gives rise to various cytological abnormalities such as cytomixis predominatly and ultimately to mixoploidy and chimeral variations.

The phenomenon of chromosome instability has a definite role to play in the origin of new genotypes. In a mixoploid tissue, if a few cells with altered chromosome numbers undergo repeated mitotic divisions, homogeneous groups of heteroploid tissue may consequently result from which a new shoot bud can be generated in the from a chimera. Such chimeral variations have been reported by George (1967) in *Stachytarpheta indica*. From such chimera new strains with altered genotypes can be generated. In cases where new genotypes have selective value their survival in nature is quite expected.

ACKNOWLEDGEMENTS

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CYTOLOGY OF THIRTEEN SPECIES OE *LEUCAS* R. Br. (LABIATAE) WITH A NOTE ON CYTOTAXONOMY OF THE GENUS

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SUMMARY

Cytology of 13 species and a variety of *Leucas* was studied. The chromosome numbers reported are 2n=28 in *L. biflora*, 2n=28 in *L. biflora* var. *procumbens*, 2n=28 in *L. marrubioides*, 2n=22 in *L. prostrata*, 2n=28 in *L. chinensis*, n=11 in *L. diffusa*, 2n=22 in *L. eriostoma*, and *L. vestita*, n=11 in *L. hellanthemifolia*, *L. indica*, *L. lancefolia*, *L. plukenetii*, *L. zeylanica* and n=14 in *L. martinicensis*. It is shown that x=11 and 14 are predominant basic numbers in the genus. It is also shown that sect. *Astrodon* of the genus is based on x=11, sect. *Hemistoma*, *Loxostoma* and *Ortholeucas* are based on x=14 and sect. *Plagiostoma* is characterized by both x=11 and 14. It is suggested that x=14 is the original basic number of *Leucas* and that occasional dysploid increase to x=15 or 16 has occurred in sect. *Ortholeucas* while occasional reduction to x=11 is seen in sect. *Plagiostoma*.

Key Words : *Leucas*, cytology, cytotaxonomy.

INTRODUCTION

Leucas is a tropical genus with about 60 species, distributed in Asiatic and African regions (Cramer 1981). Chromosome numbers of 27 species are known (Gill 1970, Goldblatt 1981, 1984, 1988). Yet no attempt has been made in the past to examine the taxonomy of the genus in light of cytological data. Cytology of 13 species of *Leucas* is reported here with a note on the cytotaxonomy of the genus.

MATERIALS AND METHODS

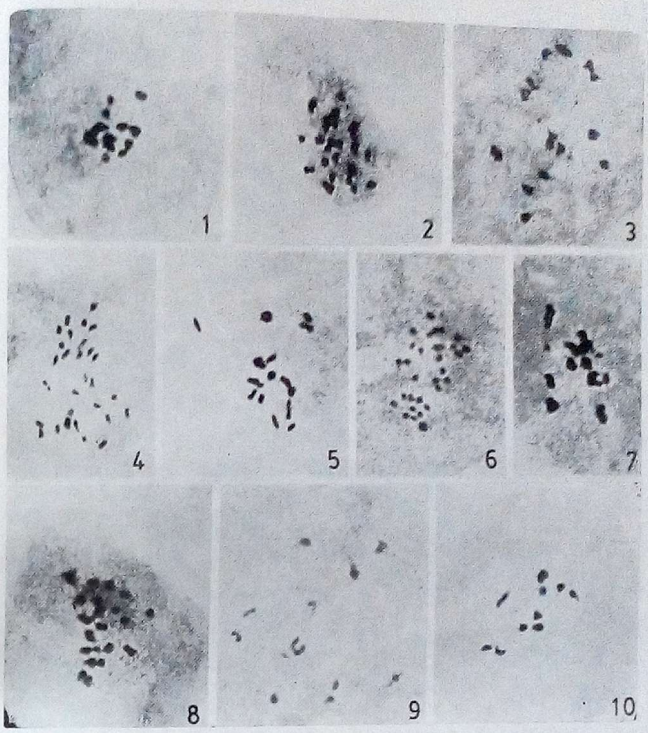
The source localities of the various species reported here are given in Table 1. Root tips were treated with 8- hydroxiquinoline at approximately 4°C for 3 h prior to fixation. Young flower buds and root tips were fixed in ethanol-acetic acid-chloroform (3 : 1 : 1) and were squashed in 1% acetocarmine. Voucher specimens are preserved in KUBOT.

OBSERVATIONS

The chromosome numbers observed during the present study are listed in Table 1 and illustrated in Figs. 1–19. Meiosis was not observed in *L. eriostoma* and *L. vestita*. All the other species showed normal meiotic division and very low pollen sterility ranging from 1.6 to 9%. However, 22.3% pollen sterility was observed in *L. marrubioides*. Mitotic chromosomes were observed in *L. eriostoma*,

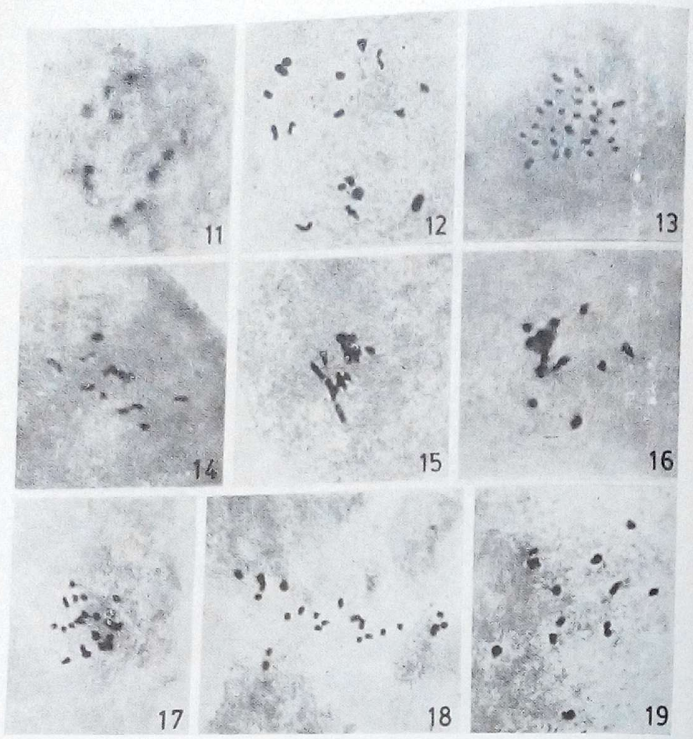
TABLE 1 : Chromosome numbers in 13 species of *Leucas*

Taxon	Present study: Locality	Chromosome number: n	2n	Previous Reports
L. biflora R. Br.	Munnar	14	28	
L. biflora R. BR. var *procumbens* Desf.	Trivandrum	14	28	
L. marrubioides Desf.	Wynaad	14	28	
L. prostrata Gamble	Ootacamund	11	22	
L. chinensis R. Br.	Kodaikanal	14	28	n=15 (Saggoo & Bir 1982 Saggoo 1983).
L. Diffusa Benth.	Munnar	11	–	2n=22 (Ayyangar & Vembu 1980, Krishnan 1980, Vembu & Sampathkumar 1980)
L. eriostoma Hook.	Wynaad	–	22	2n=22 (Krishnappa & Basavaraj 1982)
L. helianthemifolia Desf.	Ootacamund	11	–	n1=1 (Sagoo & Bir 1982, Saggoo 1983)
L. indica R. Br. (*L. linifolia* Spreng.)	Maruthamalai	11	–	2n=22 (Chopde 1965) n=11+0–1B (Saggoo 1983) n=11 (Saggoo & Bir 1986)
L. lancefolia Desf.	Ootacamund	11	–	n=11 (Saggoo & Bir 1982, Saggoo 1983)
L. martinicensis R. Br.	Coimbatore	14	–	2n=28 (Morton 1962, Krishnappa & Basavaraj 1982) n=14 (Gill 1978, Saggoo 1983)
L. plukenetii Spreng. =*L. aspera* Link	Trivandrum	11	–	2n=22 (Jha & Sinha, 1960 Ayyangar & Vembu 1980, Krishnan 1980) n=14 (Mehra & Gill 1968, Vij & Kashyap 1975 1976)
L. vestita Benth.	Ponmudi	–	22	n=11 (Saggoo 1983, Saggoo & Bir 1986) n=11 (Saggoo & Bir 1982. Saggoo 1983)
L zeylanica R. Br.	Ootacamund	11	–	n=11 (Saggoo & Bir 1982, Saggoo 1983) 2n=22 (Krishnappa & Basavaraj 1982)



Figs. 1-10 : Chromosome numbers of *Leucas* species (all X 920). 1. *L. biflora* metaphase I (n=14), 2. *L. biflora* metaphase (2n=28), 3. *L. biflora* var. *procumbens* diakinesis (n=14), 4. *biflora* var. *procumbens* metaphase (2n=28), 5. *L. chinensis* metaphase I (n=14) 6. *L. chinensis* metaphase (2n=28), 7. *L. diffusa* diakinesis (n=11), 8. *L. eriostoma* metaphase (2n=22), 9. *L. helianthemifolia* metaphase I (n=11), 10. *L. indica* metaphase I (n=11).

L. vestita, *L. biflora*, *L. biflora*, var. *procumbens*, *L. chinensis*, *L. marrubioides* and *L. prostrata*. In general, the chromosomes of *Leucas* are small ranging from 0.9



Figs. 11-19 : Chromo ome numbers of *Leucas* species (all X 920). 11. *L. lan·efolia* metaphase I (n=11), 12. *L. marrubioides* metaphase I (n=14), 13. *L. marrubioides* metaphase (2n=14), 14. *L. martinicensis* diakinesis (n=14), 15. *L. plukenetii* metaphase I (n=11), 16 *L. prostrata* diakinesis (n=11), 17. *L. prostrata* metaphase 2n=22), 18. *L. vestita* metaphase. (2n=22), 19. *L. zeylanica* metaphase I (n=11).

to 1.8 μm in length. Six chromosomes in *L. biflora* var. *procumbens*, 4 each in *L. biflora* and *L. chinensis*, 2 in *L. marrubioides* and most of the chromosomes in *L. prostrata* showed almost median primary constrictions. The rest of the chromosomes in all the presently investigated species are almost rod-shaped with no visible constrictions, which may indicate that these are acro- aer telocentrics.

DISCUSSION

The chromosome numbers in *L. biflora*, *L. biflora* var. *procumbens*, *L. marrubioides* and *L. prostrata* are reported for the first time in this work. The chromosome number 2n = 28 in *L. chinensis* is a new count for the species in which n = 1 has been reported earlier (Saggoo & Bir 1982, Saggoo 1983).

Morton (1962) and Gill (1970) suggested x = 7 as the basic number for the genus. However, none of the species so far cytologically known has a chromosome number n = 7. Vij & Kashyap (1976) suggested two basic numbers x = 11 and 14 for the genus, while Saggoo and Bir (1986) have indicated the genus to be multibasic with x = 11, 14, 15, and 16. Nine of the 14 taxa reported here are based on x = 11 and four on x = 14. It is also seen that 13 of the total of 30 species of the genus so far cytologically studied have the gametic chromosome number n= 11 and 10 species have n =14. This would suggest that *Leucas* is predominantly based on x = 11 and 14. The gametic chromosome number n = 15 is reported in *L. procumbens* (Saggoo & Bir 1982, Saggoo 1983), *L. lanata* (Bhatt et al. 1975) and *L. chinensis* (Saggoo & Bir 1982, Saggoo 1983). But n = 14 is also reported in at least *L. lanata* (Krishnappa & Basavaraj 1982) and *L. chinensis* (present work). Hence, it is quite probable that n = 15 and n = 16, reported only in *L. angularis* (Saggoo & Bir 1982, Sagoo 1983), are derived from n = 14 by stepwise increase.

Bentham (1876) divided the genus into 6 sections namely *Hemistoma*, *Ortholeucas*, *Plagiostoma*, *Astrodon*, *Loxostoma* and *Physoleucas* based on calyx characters. The calyx is membraneous, mouth oblique, split above and produced below in sect. *Hemistoma*. *Plagiostoma* has tubular calyx with very oblique mouth. In the sections *Ortholeucas* and *Astrodon* the calyx is straight and the mouth nearly equal, the throat being naked in the former and villous in the latter. The calyx in sect. *Loxostoma* is tubular, mouth oblique and produced below. The calyx is inflated and subglobose and the mouth contracted in sect. *Physoleucas*.

Though chromosome numbers in about half the number of species of the genus are known, no attempt has been made so far to employ this data in elucidating the taxonomic relationships of the different sections of the genus. The sole species of section *Physoleucas*, *L. arabica* is confined to Arabia and its chromosome number is not known. The 30, cytologically known species of *Leucas* could be placed under the remaining five sections of the genus as in Table 2.

It is seen from Table 2 that sect. *Astrodon* is based on x = 11. Most of the species of sect. *Ortholeucas* are based on x = 14, though in some, dysploid numbers such as x = 15 and 16 are also known. Species under the sect. *Hemistoma* and sect. *Loxostoma* have x = 14, while species of sect. *Plagiostoma* are based

TABLE 2 : Chromosome numbers* and basic chromosome numbers in different sections of *Leucas*

Section	Species	bromo-some Cnumber (2n)	Basic chromo some number
Hemistoma	*L. membrancea* Mort.	28	14
	L. urticaeflia R. Br.	28	14
Loxostoma	*L. deflexa* Hook	28	14
	L. oligocephala Hook. sub sp. *oligocephala*	28	14
Ortholeucas	*L. mollissima* Wall.	28	14
	L. procumbens Desf.	30	14
	L. biflora R. Br	28	14
	L. angularis Benth.	32	16
	L. lanata Benth.	28,30	14,15
	L. marrubioides Desf.	28	14
	L. chinensis R. Br	28,30	14,15
	L. montana Spreng.	28	14
Plagiostoma	*L. nutans* Spreng.	28	14
	L. martinicensis R. Br.	28	14
	L. capitata Desf.	22,28	11,14
	L. cephalotes Spreng.	22,28	11,14
	L. plukenetii Spreng.	22	11,14
	L. clarkei Hook.	22	11
	L. indica R. Br.	22	11
	L. zeylanica Br.	22	11
	L. lavandulaefolia Sm.	22	11
	L. diffusa Benth.	22	11
Astrodon	*L. vestita* Benth.	22	11
	L. ciliata Benth.	22	11
	L. hirata Spreng.	22	11
	L. prostrata Desf.	22	11
	L. suffruticosa Benth.	22	11
	L. helianthemifolia Desf.	22	11
	L. lancefolia Desf.	22	11
	L. eriostoma Hook.	22	11

* Compiled from previous as well as present work.

on x = 11 or 14. Quite interestingly both 2n = 22 and 2n = 28 are reported in some species such as *L. capitata* (Mehra & Gill 1968, Saggoo 1983), *L. cephalotes* (Gill 1970, Vij & Kashyap 1975, 1976, Vembu & Sampathkumar 1980, Krishnappa & Basavaraj 1922) and *L. plukenetii* (Jha & Sinha 1960, Mehra & Gill 1968, Vij & Kashyap 1975, 1976, Ayyangar & Vembu 1980, Saggoo 1993, Saggoo & Bir 1986 and Present study) belonging to the sect. *Plagiostoma*. This indicates the very close phylogenetic affinity between x = 11 and x = 14 *in Leucas*.

Leucas belongs to the subfamily Lamioideae (Erdtman 1945) which is predominantly based on x = 7. Therefore, it is more likely that x = 14 is the original basic number of the genus and x = 11 is the derived condition. While retaining the original situation x = 14 in the sect. *Hemistoma* and *Loxostoma*, *Leucas* represents two distinct lines of cytological evolution, namely dysploid increase in number from x = 14 as revealed by some species of sect. *Ortholeucas* and a dysploid reduction of chromosome number to x = 11 as exhibited by sect. *Astrodon* and some species of sect. *Plagiostoma*. An especially noteworthy aspect of the cytological evolution of the genus is the absence of dysploid numbers such as x = 13 and 12, connecting x = 11 to the original basic number x = 14, in any of the cytologically known species. This cytological gap together with the fact that the sect. *Astrodon* is entirely based on x = 11 strongly suggest the necessity for further detailed examination of the systematics of this section as against the rest of the genus.

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FRUIT DIVERSITY IN *TERMINALIA ARJUNA* BEDD. AND *T. TOMENTOSA* W.&

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Genetic divergence, as measured by D^2 statistics, was studied in 22 populations of *Terminalia arjuna* Bedd. and *T. tomentosa* W. & A. exhibiting mixed morphological characters and their 2 control morphotypes for 4 fruit characters. All of them were grouped into 10 clusters. Clusters I, IV and VI to IX had 2 populations each, while II and V had 4 populations each and III and X had 3 and 1 populations respectively. Based on such studies, fruit weight, fruit length, breadth of wing and germination percentage were found to contribute 32.97, 32.60, 16.30 and 18.11 per cent respectively towards divergence. The intercluster D^2 values ranged from 44.61 to 2367.06 suggesting very wide diversity between the populations. The clustering revealed lack of correlations between geographical distribution and genetic divergence. The canonical analysis has also confirmed the fruit diversity as measured by D^2 statistics.

Key Words : *Terminalia arjuna*, *T. tomentosa*, divergence, D^2 statistics, Canonical analysis.

INTRODUCTION

Variability, being the genetic base of the crops, is the basic requirement for genetic improvement of any crop. Potent variability in indigenous cultivars is the result of prolonged natural and artificial selection which is heritable and hence important. Therefore, in order to create the new reservoirs of genetic variability, the use of crossing between the most genetically diversed parents has become essential as it helps in recombination of genes from diverse sources. The D^2 statistics permits precise comparison among all possible pairs of populations before effecting actual crosses in modelling the varieties in a desired genetic architecture. Thus, the genetic diversity in breeding for high yielding varieties is obviously important (Murty & Arunachalam 1966, Jain et al. 1981). In order to assess the degree of diversity (Mahlanobis 1936) D^2 statistics based on multivariate analysis has been adopted by many workers for different crops viz., rice (Singh et al. 1987, Vaidyanath & Reddy 1985), wheat (Srivastav et al. 1987), sugarbeet (Kapur et al. 1987) and mulberry silkworm (Jolly et al. 1988). No such information is available on multipurpose forest tree species (MPTs) like *Terminalia arjuna* and *T. tomentosa* which not only serve as food plants of tasar silkworm *Antheraea mylitta* D. but are also exploited in many other industries like timber, tannin, leather and medicine (Srivastav 1991). Earlier, Srivastav et al. (1990) studied genetic diversity in half sib seedling of *T. arjuna* and their hybrid populations which excluded fruit diversity in 22 hybrid populations and two typical populations of *T. arjuna* and *T. tomentosa*.

MATERIALS AND METHODS

22 plus trees (superior genotypes) of *T. arjuna* and *T. tomentosa* exhibiting mixed morphological characters were selected and marked in forests of Madhya Pradesh and Orissa. B_1-B_6 (Bijlkhaman), S_1-S_3 (Samradihi), O_1-O_2 (CTR&TI, Office) were selected in Sundergarh and N_5-N_6 (Nowrangpur) were selected in Umerkote in Orissa. In Madhya Pradesh, the plus trees selected were D (Dhamtari), Ds_1-Ds_4 (Dhamtari Sorgaom) and N_1-N_4 (Jagdalpur- Raipur Road). Besides, C_3 (*T. tomentosa*) and C_1 (*T. arjuna*) were selected at Piska Nagri farm, Ranchi (Bihar) to serve as typical controls for both the species (Table 3). The fruits containing undetachable single seeds from these 24 trees were measured for length of fruits, breadth of wings, weight of fruits and their germination percentage following standard nursery technique (Tewari et al. 1978). For recording above parameters three replications, each containing 100 fruits, were used in each treatment (morphotype/genotype). Canonical analysis and Mahlanobis's (1936) generalised distance were used for assessing the genetic divergence between populations. The transformation of co-related variables to uncorelated variables and the grouping by Tochers approach was made as described by Rao (1952). Canonical analysis was done as described by Anderson (1959).

OBSERVATIONS

Analysis of variance revealed significant differences among 24 populations for all the four fruit characters studied, indicating the existence of potent genetic variability among the populations. Maximum variation was observed for fruit weight followed by length of fruits, breadth of wings of fruits and germination percentage in descending order (Table 1). After computing the D^2 values the 24 populations were grouped into ten clusters (Table 2). This indicates existence of a large amount of diversity in various hybrid populations of both taxa. The fruits of various hybrid genotypes within the individual clusters were having a smaller D^2 values among themselves than those belonging to any other cluster. The grouping based on the Mahalanobis D^2 statistics does not conform to the geographical origin of various populations (Table 3).

Cluster I included 2 populations (B_5, N_6) from Sundergarh and Umerkote areas of Orissa. Cluster II comprised of 4 populations (B_3, N_1 N_3, Ds_4) from Sundergarh (Orissa), Raipur - Jagdalpur Road and Dhamtari Sorgaom (M.P.). Cluster III had 3 populations (B_6, D, C_1) from 3 different states Orissa, M.P. and Bihar. Likewise, clusters IV, VI, VII, VIII and IX had 2 populationseach, while cluster V was comprised of 4 populations (B_2, O_2 ,S_1, C_3) from Sudergarh (Orissa) and Ranchi (Bihar). While 3 Clusters (I, VI, VIII) had 2 populations each from the same states, the other three clusters (IV, VII, IX) comprised of two populations each belonging to different states. The culster X had only 1 population (O_1) from Sundergarh (Orissa). Thus, a close examination of geographical

TABLE 1 : ANOVA showing F values and d independent contribution of fruit characters towards divergence in *Terminalia arjuna* and *T. tomentosa*

Characters	Mean sum of Squares	F Values	Ranked I	Contribution (%)
1. Weight of fruits	4.06	539.11*	91	32.97
2. Length of fruits	1.53	150.31*	90	32.67
3. Breadth of wings of fruits	0.40	41.80*	45	16.30
4. Germination percentage	979.12	15.95*	50	18.11

P ≥ 0.05 *significant at 5% level.

TABLE 2 : Average Inter- and Intra- cluster distances for fruit characters in *Terminalia arjuna* and *T. tomentosa*

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
I	6.14	1662.01	271.31	170.54	913.22	107.08	187.37	207.47	688.10	646.91
II		27.79	641.28	867.35	163.78	1440.12	901.67	2367.06	299.40	341.25
III			21.09	70.95	236.71	365.36	124.83	751.82	198.28	169.56
IV				17.93	391.34	139.57	44.61	471.42	238.38	165.97
V					38.85	1016.81	576.89	1809.67	198.19	194.69
VI						26.85	82.25	132.77	475.90	465.62
VII							37.58	376.74	208.24	195.65
VIII								39.98	1043.97	1031.23
IX									43.10	60.34
X										0.00

TABLE 3 : Source of origin and clustering of 24 superior genotypes/ plus trees through D^2 analysis for fruit characters

Cluster No:	Plus trees (superior genotypes) included	Source
I	B_5	Sundergarh (orissa)
	N_6	Umerkote (orissa)
II	B_3	Sundergarh
	N_1	Raipur-Jagdalpur Road (M.P.)
	N_3	-do-
	DS_4	Dhamtari Sorgaon (M.P.)
III	B_6	Sundergarh
	D	Dhamtari (M.p)
	C_1	CTR & TI, Farm, Ranchi (Bihar)
IV	B_4	Sundergarh
	N_4	Raipur-Jagdalpur Road
V	B_2	Sundergarh
	O_2	-do-
	S_3	-do-
	C_3	CTR & TI,-Farm, Ranchi
VI	S_2	Sundergarh (Orissa)
	S_1	-do-
VII	B_1	-do-
	DS_1	Dhamtari-Sargaon
VIII	N_2	Raipur-Jagdalpur Road
	DS_2	Dhamtari Sorgaon
IX	N_5	Umerkote
	DS_3	Dhamtari Sorgaon
X	O_1	Sundergarh

origin of various populations included in different clusters revealed that the populations from the same region entered into different clusters also (Table 3).

The inter-and intracluster distance (D^2) among ten clusters presented in Table 2 revealed that the lowest intracluster distance was observed for cluster I (D^2 -6.14)

and maximum (D^2 -43.10) For cluster IX. Clusters V, VII and VIII also had high D^2 values (38. 85, 37. 58, 39. 98 respectively) which indicates that these clusters had more diversity among their constituent populations (Table 2).

The maximum intercluster D^2 values were observed between the clusters II and VIII (D^2 -2367.06), followed by V and VIII (D^2 -1809.67), I and II (D^2- 1662.02), II and VI (D^2 - 1440 12), VIII and IX D_2-1043.97, and V and VI (D^2 -1016.81) which indicates enormous distance between above corresponding pairs of clusters. The minimum intercluster D^2 values were found in clusters IV and VII (D^2 -44.61) followed by IX and X (D^2 -60.34), III and IV (D^2 -70.95) and VI and VII (D^2- 82.25) indicating that these clusters were less diversed genetically corresponding to one another in each cluster pairs (Table 2).

The character means of 10 clusters with respect to 3 fruit characters are given in Table 4. While cluster II had lowest mean fruit weight (1.63), cluster

TABLE 4 : Cluster Means of fruits characters in 24 populations of *Terminalia* species through D^2 analysis

Clusters	Weight of fruits	Length of fruits	Width of wings of fruits	Germination %
I	4.71	5.61	0.96	78.50
II	1.63	3.36	0.86	69.33
III	3.45	4.99	1.32	72.44
IV	3.86	5.00	1.11	38.33
V	2.40	4.30	1.19	71.16
VI	4.45	4.76	1.30	66.83
VII	3.85	4.57	1.40	62.33
VIII	5.28	5.05	1.01	70.05
IX	2.87	3.74	0.60	72.00
X	2.95	4.20	1.27	21.00

VIII had highest mean fruit weight (5.28). The mean fruit length ranged from 3.36 in cluster II to 5.61 in cluster I. While the lowest mean width of wings of fruits (0.6) was observed in cluster IX, the highest mean width of fruit wings (1.4) was found in cluster VII. Likewise, lowest mean germination percentage (21.0) was found in cluster X as compared to highest mean germination percentage (78.5) observed in cluster I.

Among the four fruit characters, fruit weight and fruit length contributed maximum (32.97 and 32.60 per cent respectively) to divergence whereas, breadth of wings (16.30%) and germination percentage (18.11%) had less contribution in divergence. Out of 276 D^2 values, fruit weight, fruit length, breadth of wings and germination percentage had 91, 90, 45 and 50 times repetibility respectively (Table 1).

DISCUSSION

The evaluation of the parents through variability studies involves parental analysis for yield components, adaptability and genetic divergence by adopting the multivariate analysis proposed by Mahlanobis (1936) commonly known as D^2 statistics as it is more effective in the assessment of the genetic divergence. The material used in present study originated from Madhya Pradesh, Orissa and Bihar states of India.

Barring C_1 *(T.arjuna)* and C_8 *(T.tomentosa)* all other 22 populations exhibited mixed morphological characters. The present analysis has indicated considerable amount of genetic diversity in fruit characters of these populations. The clustering pattern of the populations did not follow the geographic distribution as they come from diverse sources (M. P , Bihar, Orissa) and had been grouped into II to V, VII and IX clusters. Further, populations B_1 - B_6, S_1 - S and O_1 - O_2 collected from same geographical origin i.e. Sundergarh (Orissa) fell in different clusters. Similar reports of non-correspondence of the genetic divergence and geographic diversity were made by Murty & Arunachalam (1966), Upadhyay & Murty (1970), Katiyar & Singh (1979) and Jain et al. (1981). On the other hand Ram & Panwar (1970) suggested the Vavilovian way of thought that the genetic diversity is broadly attributable to geograpical diversity. However, this does not hold true for a crop like *Triticale* which is very recent in origin and has not passed throuhg natural forces of slection (Kamboj & Mani (1983). On the contrary, under present investigation, the natural selection under different environmental conditions in *Terminalia* species through thousands of years has resulted in greater divergence than the geographical distance. Further, it may be conceived from the distribution of the different hybrid populations that the fixed spontaneous selection criteria and the similarly in parentages may be the cause of genetic similarity to a large extent (Das & Borthakur 1973).

The choice of the character for the studies is quite important as pointed out by Sokal & Daly (1961) who stated while studying insect behaviour that the original aim of the investigation could not be achieved since the choice of experimental variables had been unfortunate. As a matter of fact, while selecting genetically divergent parents only component characters of yield should be taken into

account because of their economic importance under crop improvement programme. Under present investigation, fruit weight and fruit length are equally important contributers to genetic diversity as they accounted for 32.97 and 32.60 per cent of the total divergence in comparison to breadth of wings and germination percentage which contributed 16.30 and 18.11 per cent only.

The second criteria for selection of genotypes/varieties as parents for hybridization D^2 analysis is the intercluster distances. Existence of high intercluster distances indicates genetically divergent genotypes as included in the clusters I, II, V, VI, VIII and IX. Hence, it would be logical to incorporate such genotypes/varieties in future breed- futurer breeding programmes.

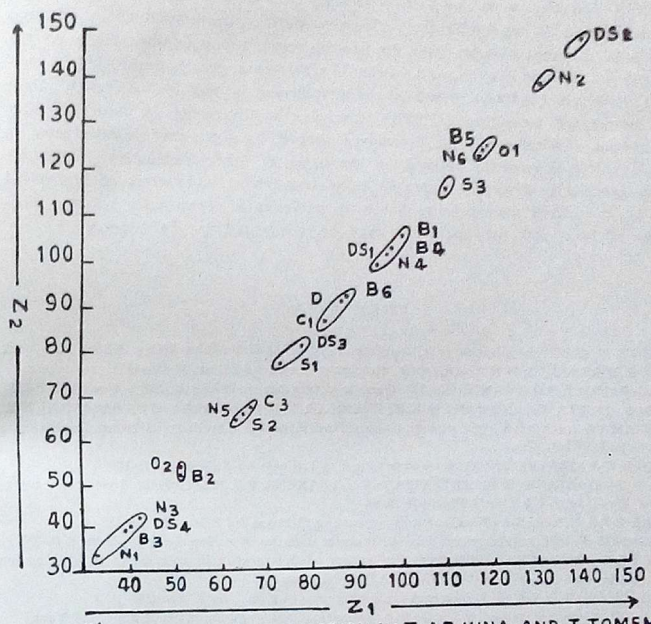

Fig: 1: CLUSTER FORMATION IN T. ARJUNA AND T. TOMENTOSA FOR FRUIT CHARACTERS THROUGH CANONICAL ANALYSIS.

The cluster constellations obtained by D^2 analysis has been confirmed by canonical analysis (Fig. 1) following the procedure suggested by Rao (1952) The canonical vectors corresponding to the two largest roots (λ_1 and λ_2) supplied the two best orthogonal vectors Z_1 and Z_2 whose values were same. Around 99.99 (Z_1 =42.58%, Z_2 =57.41%) per cent of the total variability was accounted by both the vectors. Thus, canonical analysis also confirmed the existence of substantial genetic divergence with respect to fruit characters.

When the present investigation is taken in conjunction with the one which is made with respect to seedling diversity (Srivastav et al. 1990) one is tempted to conclude that clustering made earlier on the basis of seedling diversity is different from that of the fruit diversity as on the basis of seedling diversity a total of eight clusters are made instead of ten under fruit diversity. Further, the constituent populations falling under different clusters also do not correspond to groupings made on the basis of fruit vis-a-vis seedling diversity. Furthermore, while seedling height contributed maximum (38.04%) towards the divergence in seedling diversity, fruit weight contributed maximum (32.97%) towards the divergence in fruit diversity in *Terminalia*. Therefore, foliar divergence should be given more importance as compared to the divergence derived on the basis of other characters and while selecting genetically divergent parents, only component characters of leaf yield should be considered for evolving heterotic varieties of *Terminalia* so that productivity of tasar silk per unit area may be enchanced in the country.

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ANNOUNCEMENT

The Fourth All India Conference on Cytology and Genetics is proposed to be held on 5th to 7th November, 1992 at BANGALORE under the aegis of the KARNATAKA STATE SERICULTURE DEVELOPMENT INSTITUTE, (KSSDI), Thalaghattapura. There will be paper reading sessions for 3 days and one day Symposium on "Silkworm and Mulberry Genetics and Breeding". The discussion will be focussed on the following topics : (1) Animal cytology and genetics, (2) Plant cytology and genetics, (3) Molecular genetics, (4) Microbial genetics, (5) Human genetics, (6) Biotechnology (tissue culture relating to cytogenetics), (7) Biosystematics and (8) Mutagenesis. Contact Prof. M. S. Chennaveeraiah, Hon. Secretary (SCG1), 9, Byrasandra Main Road, First Block East, Jayanagar, Bangalore 560 011.

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IN VITRO MORPHOGENETIC AND CYTOLOGICAL STUDIES IN *LABLAB PURPUREUS* (L.) SWEET

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(Received 20 November 1991, accepted 14 December 1991)

SUMMARY

Different vegetative and reproductive explants of *Lablab purpureus* (L.) Sweet var. HA-3 were cultured on MS medium supplemented with various auxins and cytokinins. In addition to callusing direct and indirect rhizogenesis were observed from hypocotyl, cotyledon and cotyledonary nodal cultures. Quantitative analysis of the growth of hypocotyl callus in presence of auxins and cytokinins was made. Histological studies revealed both root and shoot meristemoids scattered in the callus tissue. Regeneration of shoots and whole plants with multiple shoots were obtained from shoot tip and cotyledonary nodal cultures. Cytological analysis of both normal and regenerated plants via shoot tip cultures was made. Karyotypic and meiotic studies showed high genetic stability of the regenerants which can be exploited further to get homogeneous genetic stocks of the variety.

Key Words : *Lablab purpureus* (L.) var. HA-3, morphogenesis, meristemoids, cytology.

INTRODUCTION

The improvement of legumes in terms of their yield and disease resistance is essential for their continued exploitation as sources of human nutrition and other products. The lack of genetic variability is a major factor for the slow progress made in the improvement of these crops. The inherently low genetic variability in legumes caused by long periods of cultivation and in turn perpetuated by a high degree of self pollination, have imposed limitations on using conventional plant breeding approaches. The use of unconventional methods for increasing genetic variability have, therefore, assumed much significance. The value of plant tissue cluture technique in this respect has been fully recognised. In addition, uniform clones of elite varieties can be obtained by applying tissue culture technique. *Lablab purpureus* (L.) Sweet var. HA-3, commonly known as hyacinth bean, is a new variety obtained from a cross between 'Hebbale Avare-1' and 'Co-8'. Since it is a photo-insensitive variety, the cultivation of the crop has become feasible in all the seasons of the year. The present investigation deals with the responses of different explants of this variety to tissue culture techniques and chromosome number in plantlets produced under in vitro conditions.

METERIAL AND METHODS

Aseptic seedlings were raised on MS basal medium from the surface sterilised seeds which are obtained from the University of Agricultural Sciences, Bangalore.

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Aseptic seedlings were raised on MS basal medium from the surface sterilised seeds which are obtained from the University of Agricultural Sciences, Bangalore.

The vegetative explants such as root, hypocotyl, cotyledons, cotyledonary node, stem, leaf and shoot tip were excised from the aseptic seedlings whereas anthers and gynoecium were obtained from field grown plants. Surface sterilisation was done with freshly prepared chlorine water and 0.1% mercuric chloride. Thereafter, they were thoroughly washed with sterile distilled water. The explants are then inoculated on MS medium containing 2% sucrose and 0.8% agar. Different growth regulators were supplemented at various concentrations. The pH of the medium was adjusted to about 5.8 before autoclaving at 103.41 kpa. The cultures were maintained at 25 ± 2°C under 16 : 8 h light : dark cycle. For histological studies, the callus was fixed in FAA and dehydrated through the ethanol-butanol-series. Customary paraffin technique was followed and sections were cut at 10-12 μm thickness and stained with Heidenhain's haematoxylin and counterstained with erythrosine. To study somatic chromosomes, the root tips were pretreated in 8-hydroxyquinoline (0.008 M) for 4 h at 10-12°C and followed aceto-orcein technique for squash preparations. For meiotic studies, buds of proper stages were fixed in acetic-alcohol (1:3) and smears were prepared using 2% acetocarmine.

RESULTS AND DISCUSSION

Callus cultures

Profuse friable callusing from all the explants was observed in MS + 2, 4-D (2mg/l)+ CM (10%). However, CM with IAA/IBA/NAA was less effective in producing callus. The synergistic effect of CM + 2, 4-D on profuse callusing is well established in various plant systems (Gill et al. 1986). Nodular callus was obtained from hypocotyl and cotyledon when MS was supplemented with BAP.

Growth studies

Various auxins such as 2,4-D, IAA, IBA and NAA were selected at the concentrations of 0.1, 0.5, 1, 2, 3 and 4 mg/l for analysis of growth in callus cultures (Table 1). The initial average fresh and dry weights of the explant at the start of the experiment were 40 mg and 5 mg respectively. In the presence of all auxins there was an increase in callus growth after 15 days of culture. There was an initial lag phase of about one week in all the combinations. Similar observations were made in *Crotalaria juncea* (Tejavathi & Sujatha 1990). The maximum and minimum fresh and dry weights were obtained on a medium supplemented with NAA (1 mg/l) and 2,4–D (0.5 mg/l). However, the increase in fresh weight in presence of NAA is due to the profuse indirect rhizogenesis at lower concentrations (1 mg/l). Whereas, the higher concentrations of IAA/IBA induced rooting thereby increasing the fresh weight. While lower concentrations of 2,4–D (0.5 mg/l) was favourable for callus growth but without any rhizogenesis. In *Dolichos lablab* var. *lignosus* (Sounder Raj et al. 1991), 2, 4–D (1 and 2 mg/l) was alone effective in inducing callus from different explants, whereas

TABLE 1 : Effect of auxins and cytokinins on growth of callus from hypocotyl explants in *Lablab purpureus*

MS Medium + Growth regulators (mg/l)	Average gain in fresh weight (mg)	Average gain in dry weight (mg)
NAA 0.1	530	35
0.5	570	40
1	640	45
2	526	35
3	510	35
4	400	27
2,4–D 0.1	100	10
0.5	150	16
1	145	15
2	110	11
3	76	8
4	40	5
IAA 0.1	110	11
0.5	120	12
1	125	12
2	150	15
3	160	16
4	160	16
IBA 0.1	80	7
0.5	80	7
1	100	10
2	135	13
3	200	20
4	210	20
BAP 0.1	120	10
0.5	150	15
1	185	19
2	200	20
3	205	20
4	210	20
Kin 0.1	90	8
0·5	90	8
1	100	10
2	120	12
3	110	11
4	110	11

the presence of IAA/IBA/NAA in the medium showed to be less effective in both callusing and rhizogenesis. Similarly, response of hypocotyl explants to cytokinins was studied by the addition of BAP and Kin to the basal medium at various concentrations ranging from 0.1–4 mg/l (Table 1). The maximum fresh and dry weights were observed in the medium containing BAP at 2 and 4 mg/l. The callus was green, compact and nodulated. However, in *C. juncea* (Tejavathi & Sujatha 1990) Kin was better in inducing compact callus.

Histological studies of callus cultures

Histological studies are necessary to define the steps in the developmental sequences which could be suitable for evaluating the effects of various physical and chemical agents on the sequences leading to differentiation. The initial cytodifferentiation which is commonly seen in the callus cultures is the differentiation of tracheids. Spiral tracheary elements are seen in the callus cultures of hypocotyl and cotyledon on MS+BAP (1 mg/l) + Kin (1 mg/l). Whereas in *Glycine*, *Daucus* and *Syringa* (Roni Aloni 1980) high level of auxins was required for differentiation of xylem and phloem elements. Sections of nodulated callus revealed the presence of a large number of meristemoids randomly scattered among the callus cells. The meristemoidal cells are small, isodiametric with densely stained nuclei and cytoplasm. Two types of meristemoids were observed, root meristemoids which are smaller and lightly stained (Fig. 1) and shoot meristemoids which are larger and darkly stained (Fig. 2). Whereas, root meristemoids had given rise to roots in presence of auxins, the differentiation of shoots from shoot meristemoids has not been obtained.

Rhizogenesis

Both direct and indirect rooting were observed in the cultures of hypocotyl whereas in cotyledon and cotyledonary nodal cultures only indirect rhizogenesis was observed. High auxin to cytokinin ratio is necessary to induce rhizogenesis (George & Sherrington 1984). However, Flick et al. (1983) reported that in legumes the frequency of root initiation is quite high despite the concentrations of auxins and cytokinins as observed in the present investigation. MS supplemented with NAA (1 mg/l) and BAP/Kin (4 mg/l) was found to be an ideal combination for profuse rooting as was reported earlier in *Cajanus cajan* (Kumar et al. 1983) and *Dolichos lablab* var *lignosus* (Sounder Raj et al. 1991).

Figs. 1–10 : 1. Section of hypocotyl callus showing root meristemoid (arrow). 2. Section of shoot tip callus showing shoot meristemoid (arrow) 3. Regeneration of whole plant from shoot tip culture on MS+IAA (1 mg/l). 4. Induction of multiple shoots from shoot tip culture on MS+BAP (0.1 mg/l). 5. Suppressed shoot tip growth with profuse callusing on MS+NAA (0.5mg/l). 6. Regeneration of single axillary shoot from cotyledonary node on MS+IAA (0.5 mg/l). 7. Regeneration of shoots from both axillary meristems of cotyledonary node on MS+IBA (1 mg/l), 8. Regenerated whole plants from shoot tip cultures transferred to pots. 9. Somatic plate of regenerated plant showing 22 chromosomes. (X 1000). 10. PMC at metaphase I shwing II bivalents. (X 450).

Shoot tip cultures

When shoot apices were cultured on MS without any growth regulators, the growth of the shoot apex was observed. Similar observations were made in other legumes (Kartha et al. 1981. Sounder Raj et al. 1989, 1991). When the medium was supplemented with NAA at lower concentrations (0.1 mg/1), complete plantlets were obtained. However, the higher concentrations of NAA inh bited the growth. Whereas, IAA/IBA favours the growth of the shoot apex in both lower and higher concentrations (Fig. 3). When cytokinins like BAP and Kin added alone to MS, shoot growth was observed depending on the concentrations. Lower concentrations of BAP (0.1 mg/1) induced shoot growth along with multiple shoots with negligible callus at the base (Fig. 4), whereas higher concentrations lead to stunted growth with substantial callus at the base as was reported in *Dolichos lablab* var. *lignosus* (Sounder Raj et al. 1991). However, the higher concentrations of Kin induced shoot growth along with multiple shoots with roots. Addition of cytokin n to a medium containing auxin at different levels stimulate shoot regeneration in all selected legumes (Kartha et al. 1974, Sastri et al. 1982). When Kin concentration was increased to 5 mg/ l keeping NAA concentration at 0.5 mg/1, the explants showed excessive callusing at the basal part and the growth of the shoot apex ceased (Fig. 5). MS + IAA (1 mg/1) + BAP/Kin (1 mg/1) was also effective in supporting the shoot growth. Thus exogeneous supply of auxin and cytokinin were essential for better growth of the shoot ap ces as reported in a few other legumes (Gamborg et al. 1974). The complete plantlets were separated and transferred to filter paper bridges in test tubes containing sterile distilled water. Finally, they were transferred to pots containing sterilised vermiculate before planting them in the soil (Fig. 8).

Cotyledonary nodal cultures

On the basal medium, a single axillary shoot growth was observed. Addition of NAA and 2, 4–D failed to initiate the axillary shoot growth, whereas IAA and IBA were found to be best suited for the induction of axillary shoots. The presence of IAA initiated the growth of any one of the axillary buds (Fig. 6) whereas IBA was successful in inducing the growth in both the axillary buds along with multiple shoots (Fig. 7). Whereas in *Dolichos lablab* var. *lignosus* (Sounder Raj et al. 1991) IAA suppressed the growth of the axillary buds in nodal cultures. As in shoot tip cultures higher concentrations of BAP alone or with NAA suppressed the axillary buds. Even the presence of IAA with BAP/Kin was effective in regenerating axillary shoots unlike in *D. lablab* var. *lignosus* (Sounder Raj et al. 1991) where IAA with BAP/Kin induced callusing. However, multiple shoots were formed from both the axillary buds in presence of Kin (2 mg/1).

Cytological studies

Somatic plates of normal seedlings obtained from germinated seeds as well as regenerated plantlets raised from shoot tip cultures showed 22 chromosomes in each which conform to the earlier reports (Goldblatt 1981) (Fig.9). Thus, the genetic stability of the shoot tip culture is established as reported earlier in various other plant systems (Cheng & Smith 1975). The meiosis is normal in plants raised in vitro with 11 bivalents (Fig. 10). Normal fruits with viable seeds were obtained.

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