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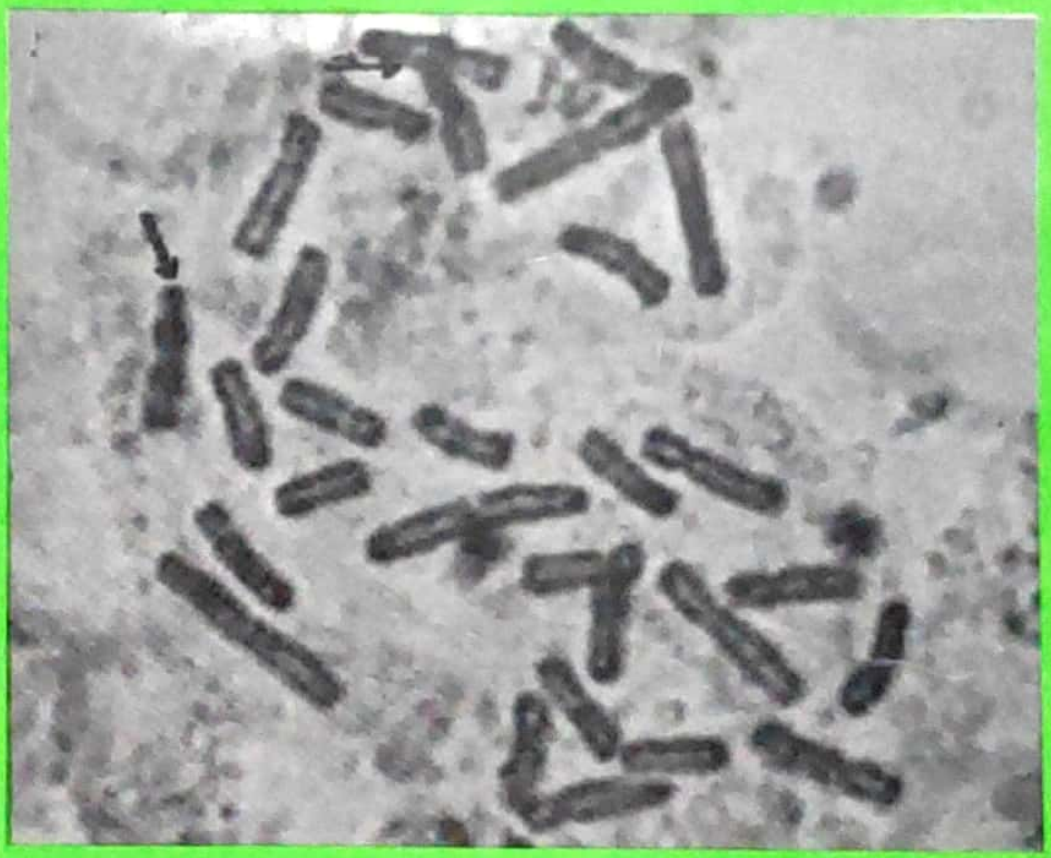

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KNOB CONSTELLATIONS IN NORTHEASTERN HIMALAYAN MAIZE

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SUMMARY

Northeastern Himalayan maize is known for its uniqueness, racial diversity and antiquity. The cytological characterization of 41 maize collections from different areas of this region has been done on the basis of pachytene knob analysis and certain knob constellations have been identified. One new knob at 2LT position has also been identified.

Key Words : Maize, knob, Himalayan, lineages.

INTRODUCTION

A lot of genetic diversity exists in the northeastern Himalayan (NEH) maize due to variation in ecology, microniches, altitudes and complete isolation due to conservative socio-cultural practices of the aborigines cultivating these strains for centuries (Stonor & Anderson 1949, Singh 1977). Primitiveness and uniqueness of these landraces are well confirmed (Sachan & Sarkar 1982, Pandey et al. 1986, Kumar & Sachan 1992,1994). Cytological characterization of 41 landraces of this region on the basis of their knob composition has been attempted in the present investigation.

MATERIALS AND METHODS

A total of 41 NEH maize germplasm collections, maintained at Division of Genetics, Indian Agricultural Research Institute, New Delhi, were screened for their knob composition. The maize germplasms included in present investigation consisted of 16 from Sikkim (S-4,S-21, S-24, S-25,S-27, S-38, S-39, S-43, S-44 (SP), S-47, S-50, S-51, S-53, S-54, S-55 and S-59) 11 from Meghalaya (3 S.P. strains of M-1, M-15 and M-25; M-6, M-8, M-12, M-17, M-240, M-249, M-250, M-252) 6 from Tripura (T-6, T-8, T-12, T-14, T-24 and T-26(SP) 4 from Nagaland (N-21, N-24, N-26 and N-29), 2 from Assam (AS-60 and AS-312) and one each from Arunachal Pradesh and West Bengal (A-1 and K-2)

Young emerging tassels were fixed in ethanol-acetic acid (3:1) fixative and stored at 4±1°C. Pachytene analysis of pollen mother cells (PMCs) from desired sized anthers were done by standard acetocarmine squash technique.

The position of knobs in different chromosomes was identified following the standard pachytene karyotype of maize (Neuffer et al. 1968). Characterization of these maize germplasms was done on the basis of their knob composition and certain knob complexes frequently present in the region were identified (Table 1). Finally these germplasms were categorised in different tentative groups based on the similarity in their knob compositions.

RESULTS AND DISCUSSION

Twentyone knob positions in different combinations were identified in these maize germplasms, with their number ranging from 4 to 7, A-1 possessing the highest number of knobs. Of these 21 knob positions, a large new knob forming position at 2LT was also identified in M-240 from Meghalaya. 6S knob was invariably present in all germplasms associated with nucleolus organising region (NOR) while other common knob forming positions with high frequency in these germplasms (Table 1) were 9ST (78.04%), 8 La (75.60%), 4 L(60.97%) and 2 La(56.20%), 7 L(29.26%) and 3L(21.95%). Knobs at 1La, 5L and 6Lc positions were observed in 9.75%

TABLE 1: Characterization of northeastern Himalayan strains based on knob composition.

Collections	Total Knob No.	Knob Positions																										Groups
		1S		2L		2S	2L		3ST	3L	4S	4L	5L	6S	6L		7ST	7L	8L		9ST	9L	10La		K10			
		Sa	ST	La	Lb		La	Lb	LT						La	Lb	Lc		La	Lb		La	Lb					
M-25(S.P.)	4	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	+	-	-	-	-		
T-26(S.P.)	4	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-		
M-6	4	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-		
S-4	4	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-		
T-6	5	-	-	-	-	-	+	-	-	-	-	+	-	+	-	+	-	-	-	-	-	+	-	-	-	-	I	
S-55	4	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-		
M-250	4	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-		
M-249	5	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	II	
M-252	5	-	-	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-		
N-29	5	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	+	-	-	-	-		
T-14	5	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	-	+	-	-	-	-	III	
S-25	5	-	-	-	-	-	+	-	-	-	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-		
S-59	5	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+	-	-	-	-	IV	
S-39	5	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	+	+	-	-	-		
S-38	5	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+	-	-	-	-	+	+	-	-	-	V	
S-54	5	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	+	-	-	-	+	-	-	-	-		
S-50	6	-	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	+	-	+	-	+	-	-	-	-		
S-53	5	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	+	-	+	-	-	-	-		
M-8	6	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	+	-	+	-	-	-	-	VI	
K-2	5	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	-	-	+	-	+	-	-	-	-		
AS-60	6	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	-	-		
N-26	6	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	VII	
N-24	6	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-		
S-51	6	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	+	+	+	-	-	-	-		
N-21	6	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	+	+	+	-	-	-	-	VIII	
M-240	6	+	-	-	-	-	+	-	*	-	-	-	-	+	-	-	-	-	-	+	+	+	-	-	-	-		
T-8	6	-	-	-	-	-	+	-	-	-	-	+	-	+	-	+	-	-	-	+	-	+	-	-	-	-		
T-12	6	-	-	-	-	-	+	-	-	-	-	+	-	+	-	+	-	-	-	+	-	+	-	-	-	-	IX	
T-24	6	-	-	-	-	-	+	-	-	-	-	+	-	+	-	+	-	-	-	+	-	+	-	-	-	-		
M-1(S.P.)	6	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	+	-	-	-	-		
M-15(S.P.)	6	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	-	-	X	
S-24	6	-	-	-	-	-	↘	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	-	-		
S-27	6	+	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	-	-	XI	
S-43	6	-	-	+	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+	-	+	-	-	-	-		
M-12	6	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	+	+	-	-	-	-	-	-		
M-17	6	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	+	+	-	+	-	-	-	-	XII	
AS-312	6	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-		
S-47	6	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+	+	-	+	-	-	+	-	-	-	-		
S-21	6	+	-	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	XIII	
S-44(S.P.)	6	-	-	-	-	-	+	-	-	+	-	-	+	+	-	-	-	-	-	+	-	+	-	-	-	-		
A-1	7	-	-	+	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	+	-	+	-	-	-	-		

* New knob position

germplasms whereas 1Sa and 8Lb knob positions were present in 7.3% of the germplasms. Distribution of knobs in different germplasms as well as geographical regions varied widely and did not follow any specific pattern.

Rare knob positions observed in these germplasms were, terminal knobs on short arms of chromosomes 1, 4, and 7 (1ST, 4ST and 7ST) present in one collection each from Nagland and Sikkim respectively and interstitial knobs on long arms of chromosomes 6, 9 and 10 (6La, 9La, 10La) in strains from Meghalaya, Sikkim and Nagaland (Table 1). Out of these, 2 terminal knobs at 1ST and 7ST positions were observed in N-29 collection from Nagland. Similarly, a larger new knob at 2LT position in addition to normal knob position of 2La was identified in M-240 collection from Meghalya. The 6La and 10La knobs were observed in M-8 (Meghalaya) and N-24 (Nagland) respectively. While 9La knob was observed in two collections of Sikkim, S-38 and S-39.

These germplasms were tentatively characterised in 13 groups I to XIII depending upon similarities in their knob positions. Among these 41 germplasms, 5 Sikkim Primitives (SP) collections, namely M-1, M-15, M-25 (all from Meghalaya), S-44 (Sikkim) and T-26 (Tripura) were also included. Out of these, M 25 and M-26, although both having 4 knobs each, differed in their knob composition. But M-1 and M-15, both having 6 knobs each, resembled each other in their knob composition except the knobs at 7L and 8La, perhaps due to their adaptive value in different microniches, hence, they were placed in a single group-X. Similarly, S-44, differed with other S.P. strains having 3ST knob position. Strangely, this 3ST knob was restricted to Sikkim only, being present in 3 collections, namely, S-21, S-44(SP) and S-50 all of which possessed six knobs each, although placed in different groups due to difference in their total knob composition. Similarly, 2 collections from Assam, AS-60 and AS-312 though possessed 6 knobs each, differed in their knob composition, hence, placed in different groups.

Knob at 5L position was present in only 2 collections, K-2(W.B.) and AS-312 (Assam), but these collections differed widely in their knob composition as well as geographical distribution.

These NEH maize germplasms belonging to different areas and altitudes show a wide variation in their total knob number as well as knob composition. Knob frequency is negatively correlated with latitude and altitude (Pande et al. 1988) hence, variation in the knob frequency in maize strains of NEH region is quite understandable. Knob heterochromatin plays an active (though indirect) role in the adaption of maize to its environment (Chughtai & Steffensen 1987, Chughtai et al. 1993). Knobs have extensively been utilized for characterizing the races of maize (Longely & Katq 1965, McClintock et al. 1981).

Tentative grouping of different NEH maize germplasms in the present report indicates that some of the germplasms have common source of introduction in the region in prehistoric times (Sarkar et al. 1974, Kumar & Sachan, 1991). After initial introductions, they got diversified and adapted to local environments by either acquiring some new knobs through introgression/hybridization between adjacent populations or through fixation/or loss of some knobs. Since knobs possess adaptive value, the regional differences in knob composition found at present are the reflections of past changes that occurred in ancestral population complex, and the variation found within any given region would represent adjustments that occurred in a more recent past or are occurring

at the present time because of variations in the local environment(Longley & Kato 1965, McClintock 1978). It has been further suggested that migration of small populations could lead to loss or fixation of one or more knobs by accident or in adaptation to local environment (Longley & Kato 1965); these contentions appear to hold true in case of NEH maize also, where it is grown in small isolated microniches by various ethnic groups under stringent selection. Presence of a new terminal knob at 2 LT position, hitherto unknown in American races, along with commonly found knob at 2La position in M-240 suggests its adaptive value (Kumar & Sachan 1994). Since this 2LT knob was present in the same plant along with normal 2La knob, its origin can be explained as genome's response to climatic conditions of that area. This knob has been reported in some maize strains of Kashmir (Jotshi 1982) also.

Similarities in the knob composition, with minor variation, of the maize strains belonging to different region as in case of group IV, VI, VIII etc. suggests their common lineages (Table1). At the same time, presence of different knob constellations in the same geographical area, say Sikkim or Meghalaya, can be explained on the basis of different introductions of ancentral germplasm, which due to conservative socio-cultural practices of the tribals, continued to be cultivated in small isolated pockets for centuries leading to fixation of these knob complexes.

Hence, it can be concluded that the most frequent knob complex in this area comprises of knobs at 6S, 9ST, 8La, 4L and 2La positions followed by less frequent knobs at 6Lb, 7L and 3L positions. These knobs were present in ancestral introductions in the region (Kumar, 1990). Conservative nature of knob constellations in different geographical regions has been emphasized by many workers (Longley & Kato 1965, McClintock 1981, Jotshi & Patel 1984, Pande et al. 1988, Kumar 1990). When the knob composition of these germplasms is compared with that of ancient American primitive races, they show similarities with races like Confite Morocho of Peru (mean knob number 3.1 with a range of 1-7), due to presence of common frequent knob positions in these NEH maize germplasms and Confite Morocho at 9ST, 4L, 2La, 6Lb positions along with less frequent knobs at 8La, 8Lb, 5L and Pl positions. The presence of knobs on the long arm of chromosome 6 in a majority of these collections places them in the lineages of Confite Morocho and not Palomero Toluqueno, another American primitive race from Peru which has been earlier proposed as the ancestral germplasm introduced in this region. Presence of some additional knobs at lower frequency in these maize germplasms in NEH can be explained as a result of hybridization with recent introductions in the region.

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CHARACTERIZATION OF INDUCED MORPHOLOGICAL MUTANTS IN BARLEY

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SUMMARY

Four morphological mutants in barley viz., dwarf, semidwarf, early flowering semidwarf (semidwarf with earliness) and early maturing types were characterized and assessed for their breeding value. The shoot system was altered in the mutants and considerable reduction in plant height was noticed in all. Shortening of lower internodes of culm along with reduced internode number was mainly responsible for reduced plant height of mutants. Early maturing mutants matured 9-10 days earlier than control. Grain yield was higher in semidwarf and early maturing mutants. Semidwarf mutant with its vigorous growth, short stature, enhanced tillering, increased yield and higher seed protein content is highly desirable plant type with immense breeding value. Among others, early maturing mutant is more suitable for late sown conditions while dwarf mutant can be utilized in recombination breeding.

Key Words : Barley, induced mutants, early maturity, short stature, protein content.

INTRODUCTION

Mutants are useful for studying plant development. They can serve as genetic tracers to establish fate maps of organs. Characterization of mutants is necessary as it provides useful information on the stage of expression of a particular mutation, the response of mutant in a particular environment, and the characters associated with it and also help in proper understanding of the genetic architecture of the concerned crop. Though there were several reports on the induction and isolation of a large number of mutants for various characters in barley, detailed studies were lacking on many of them. This paper deals with the morphological characterization of four induced mutants in barley namely, dwarf, semidwarf, early maturing and early flowering semidwarf (semidwarf with earliness) types and their practical utility in barley breeding.

MATERIALS AND METHODS

Four morphological mutants for plant height (dwarf and semidwarf) and maturity period (early maturing and semidwarf with earliness) were isolated from gamma ray treated M_2 populations of barley cv. K-169 in 1991. The M_4 seeds of these mutants along with their control were used in the present investigation. The growth and development of the mutants along with their control were closely observed at various growth stages and the data on a number of quantitative characters were recorded from time to time. Seed protein content was estimated by the conventional Kjeldahl method with slight modifications following rapid chromic acid procedure of Sharma & Sud (1978).

RESULTS AND DISCUSSION

Morphological characterization of 4 induced mutants namely, dwarf, semidwarf, early maturing and semidwarf with earliness was made along with their control (cv. K-169) and the data on various quantitative characters are presented in Table 1.

TABLE 1 : Data on morphological and agronomical characteristics in control and induced mutants of cv. K-169 of barley.

Character	Control	Dwarf	Semidwarf	Early flowering semidwarf	Early matuing
Plant height (cm)	120.55[a]±1.45[b]	55.73**±0.93	76.64**±1.56	76.05**±1.99	105.30**±1.16
Culm length (cm)	95.35±1.22	38.95**±0.89	56.33**±1.51	59.05**±1.91	86.50*±1.25
Peduncle length (cm)	38.05±0.64	17.76**±0.50	24.70**±0.78	23.74**±0.75	33.40**±0.67
Spike length (cm)	25.20±0.50	16.76**±0.26	20.30**±0.27	17.00**±0.32	19.95**±0.23
Boot leaf area (cm^2)	26.10±1.62	17.44**±0.83	31.55±1.57	14.75**±1.23	17.59**±1.44
Days to 50% heading	98.00±0.19	103.00**±0.16	102.00**±0.16	88.00**±0.16	86.00**±0.19
Days to maturity	131.00±0.16	132.00±0.19	135.00**±0.19	122.00**±0.16	121.00**±0.16
Productive tiller No.	12.55±0.89	10.26±0.75	12.64±0.87	11.00±1.13	13.80±0.80
Spikelets per spike	71.14±3.33	73.60±1.60	75.60±2.40	59.00**±1.77	70.60±1.35
Seed set(%)	93.48±0.71	93.95±0.86	94.72±0.40	93.91±0.56	93.46±0.38
100 grain wt. (g)	4.67±0.03	4.40**±0.03	4.61±0.16	4.34**±0.10	4.73±0.10
Biological yield/plant (g)	79.28±6.85	48.20*±2.96	92.80±6.29	66.40±5.43	76.20±4.82
Grain yield/plant (g)	32.00±4.33	20.40*±2.13	35.90±2.01	30.10±2.98	33.10±2.17
Harvest index (%)	39.58±2.82	41.77±2.59	39.61±2.17	45.09±1.46	43.44±0.98
Seed type	Bold	Medium	Medium	Medium	Medium
Seed length (cm)	1.32±0.04	0.96*±0.03	1.23±0.03	1.01±0.02	1.22±0.04
Seed width (cm)	0.37±0.01	0.35±0.01	0.33±0.01	0.33±0.01	0.37±0.01
Seed protein content (%)	8.75±0.50	11.08**±0.29	15.46**±0.29	11.67**±0.29	6.42**±0.29

a=mean; b=S.E.value; *Significant at 5%; **Significant at 1% level.

The Growth of plants in the early vegetative phase was slow both in control and mutants but later on it was faster in control but not in mutants. All these mutants studied here belong to the category of reduced plant height mutations, the most frequently arising types through mutagen treatments (Gottschalk & Wolff 1983). They do not represent a uniform group neither morphologically nor anatomically or genetically. Significant reduction in plant height was observed in all the mutants because of length reductions in culm (including peduncle) and spike. The reduction in culm length of the mutants was due to reduction in number as well as the length of the internodes. Shortening of internodes may be a result of decrease in cell number, cell length or both (Weber & Gottschalk 1973). In wheat, short internodes (Nilson et al. 1957) and coleoptiles (Allan et al. 1968) were found to associate with reduced cell number, whereas in barley, the better lodging resistance of 'erectoides' mutants has been associated with structural properties of the stem, that are functions of cell size (Blonstein & Gale 1984). In the mutants studied here, the length reduction in the lower internodes of the culm is comparatively higher than in the upper ones. The reduced culm length, in general, is associated with an improved straw stiffness resulting in an increased lodging resistance.

In all these mutants, spike length was significantly reduced in comparison to that of parent variety. The early maturing mutants (early maturing as well as early flowering semidwarf types) came to flowering quite early and also matured earlier (by about 10 days) than control. Flag leaf, which has a pivotal role (alongwith peduncle) in grain filling thereby contributing in the yield of barley, was found to be significantly reduced in size in all the mutants with the exception of semidwarf mutant. Grain yield was considerably high in semidwarf mutant and also in early maturing mutant to some extent over their control. However, the grain yield in dwarf mutant was significantly low (Table 1).

The dwarf mutant, showing more than 50% reduction in plant height as compared to its parent variety, is very low yielding because of reduction in tiller number, spike length flag leaf size, seed size and weight. The seed of this mutant, however, has significantly higher protein content over the parent variety. Further, harvest index is also higher in the mutant mainly due to more reduction in biological yield than in grain yield. This mutant, although may not be useful for direct commercial cultivation because of its poor yield, can be utilized in cross breeding experiments particularly for transferring some of its agronomically desirable traits like short-stature, better harvest index and high protein content.

Semidwarf mutant of K-169 with more than one third reduction in plant height has shown vigorous growth with larger foliage, enhanced tillering and increased dry matter production and grain yield. The mutant seed has a remarkably higher protein content to the extent of 15.46% as compared to 8.75% of control. In modern varieties, the increase in yield is mainly due to increase in harvest index and only to a lesser extent to increase in biological yield (Kertesz 1984). However, in this semidwarf mutant the increase in yield was mainly due to increase in biological yield without any significant increase in harvest index. This mutant with its better plant type, increased yield and higher seed protein content can be utilized for direct commercial cultivation as a new improved mutant variety after successful yield trials.

Early flowering semidwarf mutant with significant reduction both in plant height and maturity period has shown a slight reduction in yield due to reduced number of grains per spike.

However, the seed contained almost 3% more protein content over that of control. This mutant with its highly desirable characters like semidwarf stature, early maturity and high seed protein content can be utilized in cross breeding experiments for transferring these agronomically desirable traits into other high yielding barley genotypes. Further, it can also be considered for direct commercial cultivation in place of its parent cultivar (K-169). especially for late sown conditions.

The early maturing mutant besides showing 10 days earliness in flowering and maturity is also high yielding over parent variety with increased tiller number and grain weight. The harvest index is also significantly high. This mutant with increased yield and early maturity is highly recommended in place of K-169, particularly for late sown conditions. However, the seed of this mutant contained significantly lower protein content (6.42%).

Seed protein content, in general, exhibited negative correlation with grain yield as seen in dwarf, early flowering semidwarf and early maturing mutants. However, in semidwarf mutant a positive correlation between protein content and grain yield was recorded, which is of great practical value in barley breeding.

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FREQUENCY AND SPECTRUM OF MUTATIONS INDUCED BY GAMMA IRRADIATION IN RICE (*ORYZA SATIVA* L.)

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SUMMARY

Rice plants were exposed to 3 doses, 2.5, 5 and 10 KR, of acute gamma rays at 8 developmental stages. In M_2, both chlorophyll and morphological mutants occurred with maximum frequencies after irradiation at the seedling stage followed by tillering and panicle initiation stages. In the M_3 generation of plants irradiated at meiotic and gametic stages, mutants occurred in significantly higher frequencies closely followed by those at the anthesis stage. Also, the spectra of chlorophyll mutants observed at various developmental stages showed significant differences. In the M_3, the widest spectrum, viz., 15 types of morphological mutants, was found after irradiation at the anthesis stage. Another significant feature of the M_3 mutant spectra was the recovery of certain rare and promising mutants in relatively higher frequencies after irradiation at anthesis stage. These results amply suggest that flowering period is the ideal phase for irradiation and recovery beneficial mutants.

Key Words : Rice, developmental stages, anthesis, gamma irradiation, beneficial mutants.

INTRODUCTION

Irradiation of growing plants at different vegetative and reproductive phases might increase induced mutation frequency with altered mutant spectra. Changes in the radiosensitivity were reported by irradiation of plants at different ontogenetic stages in wheat (Matsumura & Fuji 1963), oat (Shestakov et al. 1955), maize (Sparrow & Singleton 1953), tulip (Meshitsuka et al. 1963), tobacco (Devreux & Scarascia Mugnoza 1964) and barley (Hermelin 1970). In rice, limited information is available on the mutagenic effects of chronic gamma irradiation at 4 developmental stages (Kawai & Inoshita 1965). In the present study, rice plants were exposed to acute gamma rays at different developmental stages with the objective of identifying stage(s) which might give maximum frequency and wider spectrum of mutations.

MATERIALS AND METHODS

Rice plants (cv. sona) were exposed to acute gamma rays at 8 developmental stages, viz., seedling, tillering, panicle initiation, meiotic, gametic, anthesis, milk and dough stages. At each stage, five to ten pots,, each with 4 plants, were exposed at a distance of 100 cm from the gamma source around a circular disc. Irradiations were done at the dose rate of 1150 R/h in the 'Gamma Shine' (1500 curies) with a ^{60}Co unit at the Department of Genetics, Osmania University. Plants at different stages were exposed to 2.5, 5 and 10 KR of gamma rays. M_2 progenies were raised from about 100 - 200 selfed panicles collected from M_1 plants irradiated at each stage. In M_2 and M_3 generations, data on the frequency and spectrum of chlorophyll and morphological (viable) mutants were recorded from seedlings and adult plants, respectively, and averaged over 3 doses of gamma rays. The data were based on 3,500 - 10,000 M_2 plants and 25,000 - 40,000 M_3 plants. Differences in the mutant frequencies were tested by standard normal deviate (Z) test and variations in the spectra of chlorophyll mutants were detected using χ^2 test. Mutagenic efficiency and effectiveness were estimated by the method given by Konzak et al. (1964).

RESULTS AND DISCUSSION

Irradiation at the seedling stage induced a marked growth retardation, and after 10 KR treatment about 30% of the seedlings did not survive; at other stages of irradiation no growth retardation or decrease in survival were noticed. Maximum amount of seed sterility (41.6%) resulted from irradiation at meiosis followed by that at gametic stage (33.7%), while at other stages it ranged from 13 to 25%. The sterility may mainly be caused by chromosomal aberrations and, to a lesser extent, by gene mutations. Chromosomal aberrations would be induced most frequently by irradiation at meiotic and gametic stages at which cell division is most active.

In the M_2, higher frequencies of chimeric plants were observed in those irradiated at anthesis stage (0.08 - 0.51%) as compared to those irradiated at milk (0.03 to 0.31%) and dough (0 - 0.17%) stages. This indicates the greater radio-sensitivity of early proembryos than later stages of embryogeny. It also reveals that the primordia for chloroplasts are already present in the cells of early proembryos. The maximum frequency of both chlorophyll and morphological mutants, in M_2, were obtained from irradiation at the seedling stage followed by those at tillering and panicle initiation stages (Table 1). However, after irradiation at meiotic and gametic stages, the mutant frequencies were negligibly low, while irradiation at later stages produced no mutants. The significantly higher frequency of chlorophyll mutants induced in early developmental stages are attributable to the identical genetic constitution of male and female gametes for the mutant genes. On the other hand, in later ontogenetic stages, mutations are induced independently in male and female gametes and hence, the incidence of homozygous mutants is rare in the M_2 generation.

In the M_3 generation, mutants were found after irradiation at various developmental stages; they occurred in significantly higher frequencies in the plants irradiated at meiotic and gametic stages closely followed by those at anthesis stage. This is, presumably, because of both enhanced induction and fixation of mutational events at these stages (Kawai & Inoshita 1965).

In M_2 the spectra of chlorophyll mutants due to irradiation at the seedling (χ^2=14.94) and tillering (χ^2=20.52) stages differed significantly from that of panicle initiation stage. In M_3, the widest spectrum, viz., 8 types of chlorophyll mutants, was observed in plants irradiated at anthesis stage followed by those at meiotic (7 types) and gametic (6 types) stages. Out of 28 possible comparisons made among 8 stages for the spectra of mutants, 16 showed significant differences when tested by χ^2 heterogeneity. These spectral differences may be attributed to the differential diplontic and haplontic selections operating after gamma irradiation.

In the M_2 8 types of viable mutants were observed out of which 6 types were found after irradiation at seedling stage and 7 each at tillering and panicle initiation stages. However, a majority of these mutants were characterized by high seed sterility (15 - 20%) and other undesirable attributes. In M_3, a total of 17 types of morphological mutants were observed. The widest spectrum, viz., 15 types of mutants, was found at the anthesis stage followed by that at gametic (11 types) and meiotic (10 types) stages (Table 2). In the remaining stages, the spectra of mutants were relatively narrow. A significant feature of the M_3 morphological mutant spectra was the recovery of certain rare and promising mutants, viz., exserted panicle, short-slender grain, medium-slender grain, glutinous endosperm, tapering grain, early maturing and dwarfs, in relatively higher frequencies at

TABLE 1 : Frequency of chlorophyll and morphological (viable) mutants induced by gamma rays in M_2 and M_3 generations.

Stage of Irradiation	M2 generation		M3 generation			
	Frequency of chlorophyll mutants % ± S.D.	Frequency of morphological mutants % ± S.D.	Frequency of chlorophyll mutants % ± S.D.	Frequency of morphological mutants % ± S.D.	Mutagenic efficiency	Mutagenic effectiveness
Control	-	-	-	-	-	-
Seedling	1.02 ± 0.10	0.67 ± 0.05	0.69 ± 0.06	0.27 ± 0.03	0.04	0.12
Tillering	0.88 ± 0.07	0.47 ± 0.05	*0.38 ± 0.04	0.15 ± 0.02	0.03	0.07
Panicle initiation	***0.44 ± 0.05	0.42 ± 0.04	0.49 ± 0.05	0.18 ± 0.03	0.02	0.08
Meiotic	***0.10 ± 0.02	-	***2.46 ± 0.13	***1.05 ± 0.08	0.06	0.42
Gametic	***0.04 ± 0.01	-	**1.84 ± 0.10	**0.72 ± 0.07	0.05	0.32
Anthesis	-	-	**1.60 ± 0.09	**0.73 ± 0.07	0.06	0.28
Milk	-	-	**0.58 ± 0.06	**0.36 ± 0.04	0.03	0.10
Dough	-	-	*0.37 ± 0.05	0.19 ± 0.03	0.03	0.06

Differences from the mutant frequency induced at seedling stages :

* = significant at 5% P level; ** = significant at 1% P level; *** = significant at 0.01% P level.

TABLE 2 : Spectra of morphological (viable) mutants induced in the M_2 generation.

Stage of Irradiation	No. of viable mutants	No. of plant type mutants	No. of maturity mutants	No. of panicle type mutants	No. of grain type mutants	Total types of mutants
Seedling	106	3	2	-	-	5
Tillering	94	4	2	-	-	6
Panicle inititation	112	3	2	-	1	6
Meiotic	545	5	2	1	2	10
Gametic	445	5	2	-	4	11
Anthesis	360	6	2	1	6	15
Milk	191	4	2	-	4	10
Dough	75	3	2	-	2	7

the anthesis stage. Whereas, most of the early-maturing and grain type mutants recovered after irradiation at meiotic and gametic stages were beset with high seed sterility (25-40%) and other developmental abnormalities. On the other hand, mutants obtained after irradiation at anthesis were characterized by normal seed fertility (>90%) and were free from any developmental abnormality. Mutagenic efficiency and effectiveness were relatively higher in the meiotic, gametic and anthesis stages when compared to all other stages.

In the present investigation, gamma irradiation at anthesis gave a fairly high frequency and widest spectrum of viable mutants with normal seed fertility, suggesting that flowering period is the ideal phase for irradiation and recovery of beneficial mutations.

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KARYOTYPE ANALYSIS OF THREE SPECIES OF *ZEPHYRANTHES* (AMARYLLIDACEAE)

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SUMMARY

Karyotypes from 3 species of *Zephyranthes*, viz., *Z. grandiflora* *Z. candida*, and *Z. flava* have been studied. The haploid chromosome number recorded in *Z. grandiflora* is n=12. Except *Z. gradiflora* (2n=24) other two species exhibit great variations in chromosome number ranging from polyploid to aneuploid, *Z. candida* (2n=19, 32, 38, 40) and *Z. flava* (2n=28, 42, 48). Besides, there is prevalence of nearly asymmetric karyotype representing advanced nature of the species. Presence of great numerical variation in chromosomes might play an important role in speciation.

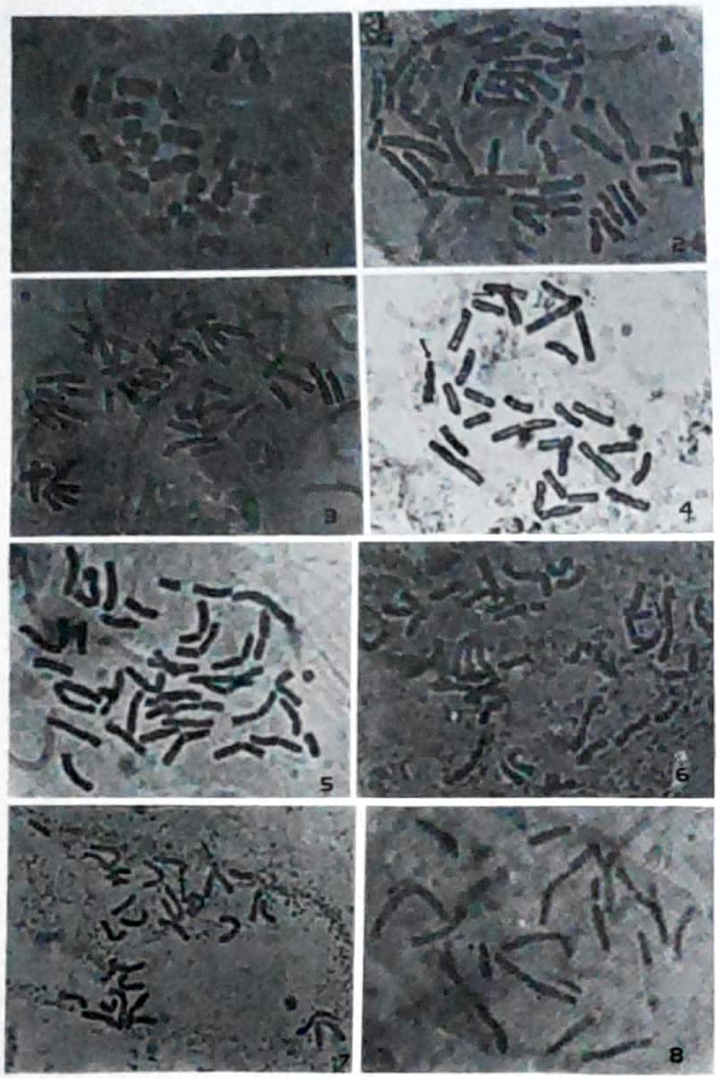
Key Words: Karytope. polyploid, aneuploid, asymmetric, *Zephyranthes*.

INTRODUCTION

The genus *Zephyranthes* is represented by about 60 species (Lawrence 1967). It is native to tropical and subtropical America (Dimitri 1978). Three species are found in the northeastern region of India viz., *Z. grandiflora* Lind1, *Z. candida* Haines and *Z. flava* Haines. *Z. grandiflora* and *Z. candida* are propagated by asexual means while *Z. flava* is propagated by both asexually and through seeds. They are herbaceous, perennial plants, and distinguishable from each other on the basis of structure of leaves, flower colour etc. *Z. grandiflora* has pink, *Z. candida* has white and *Z. flava* has yellow flowers. These species have great relevance in both horticulture and pharmaceutical sectors due to their dual importance; ornamental and medicinal. It has been reported that the decoction of the leaves of *Z. candida* is used as hypoglycaemic in diabetes and it yields many important alkaloids. Farmanova & Oledzka (1978) reported that *Z. grandiflora* (= *Z. carinata*) has tumour inhibitory property. Many important alkaloids have been extracted from *Z. flava* (Ghosal et al. 1987).

The study of karyotype and its various component characteristics are very important for deciphering the karyoevolutionary trends in given taxa. Karyotypic features like relative chromosome size and the position of centromere are very important which have allowed reasonable assessment of chromosomal affinities based on the concept of symmetry vs asymmetry. However, the general form of karyotype with respect to centromeric position and relative arm ratio provides useful information to deduce an approximate degree of similarity of the karyotype as shown by T.F. % (Lavania & Srivastava 1992).

Many reports exist in literature on the studies of chromosomes of Amaryllidaceae. Chromosome mosaicism in the shoot species of some members of Amaryllidaceae identicated that these somatic alterations play a significant role in the origin of species through vegetative means (Roy et al. 1983). Several tribes studied in Amaryllidales were charactrized by long chromosomes. It has



Figs. 1-8 : Mitotic metaphase chromosomes of *Zephyranthes*. 1. *Z. grandiflora*, 2-4. *Z. flava*, 5-8. *Z. candida*. Setellited chromosomes are indicated by arrows.

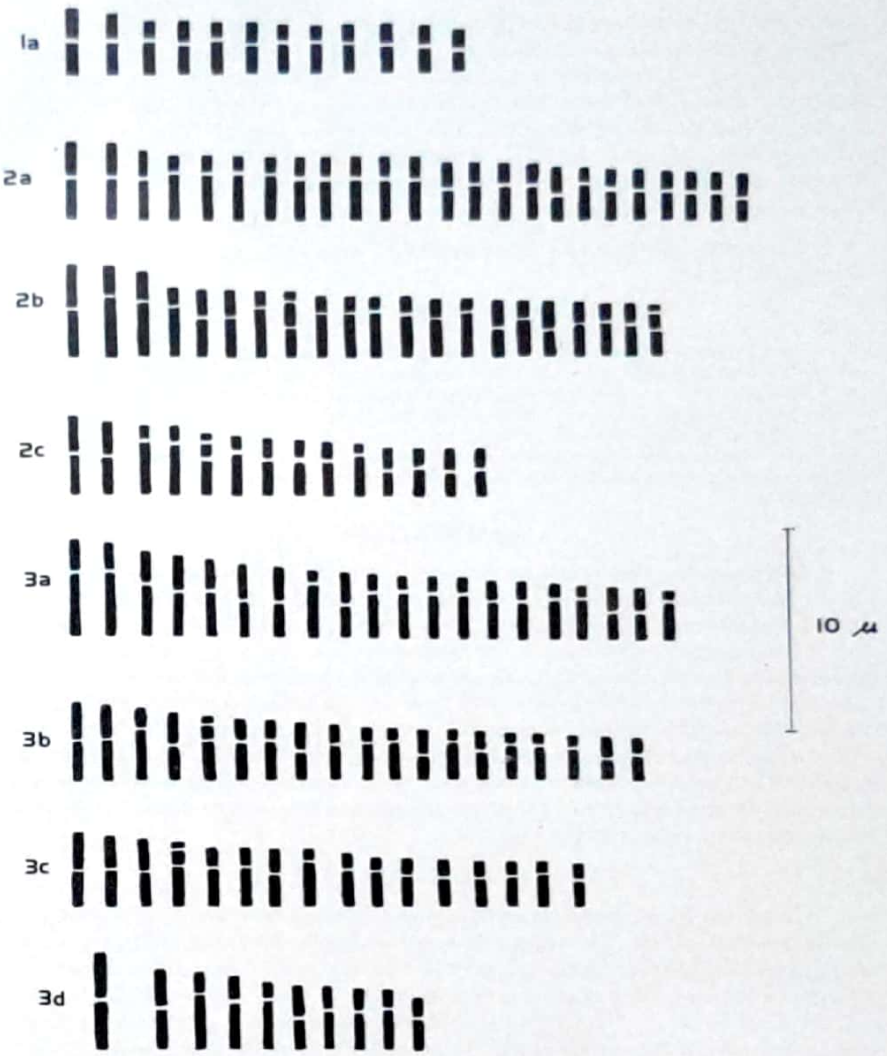


Fig. 9 : Idiograms of mitotic metaphase chromosomes of *Zepharanthes*. 1a. *Z. grandiflora*, 2a-c. *Z. flava*, 3a-d. *Z. candida*.

been reported that the chromosomes of 75 taxa of *Zephyranthes* were from 3x to 6x and aneuploid chromosome numbers are also prevalent (Roy et al. 1983). They have observed that homomorphic karyotype was up to tetraploid level and heteromorphic karyotypes were at the higher levels. Many authors have also reported that polyploidy and aneuploidy are widespread in the genus *Zephyranthes* (Darlington & Wylie 1955, Ornduff 1968, 1969, Moore 1973, 1977, Naranjo 1969, 1974, Fedorov 1974, Greizerstein et al. 1987). Although reports are available regarding the mitotic chromosomes, no systematic and comparative studies of these 3 species studied here have been reported so far.

The present study deals with the comparative kayotypic analysis of *Z. candida*, *Z. flava* and *Z. grandiflora*.

MATERIALS AND METHODS

The bulbs of the plants of each species of *Zephranthes* were collected locally from Dibrugarh and planted under natural conditions in pots and petridishes as well. Preparations for karyotype analysis were made using the method of Sharma & Sharma (1980). Root tips of 0.5 to 1 cm long were excised from the plants at 10.30 a.m. and pretreated with 0.5% colchicine for 3 h at 11° C. After washing with tap water they were fixed in 1:3 cold acetic acid - ethanol mixture for 24 h. Then they were hydrolysed in 1:9 1 N HCl-2% acetocarmine mixture at 60°C for 10 min and squashed in 2% acetocarmine. Selected best plates were then photographed. From the photomicrographs chromosome lengths were measured using a curveter and idiograms of each species were made using the method of Battaglia (1955). Total form percentage (T.F. %) were calculated using the formula given by Huziwara(1962).

OBSERVATIONS

In *Zephyranthes*, the best time for collection of root tips was found around 10.30 a.m. The chromosome number of 2 species viz., *Z. flava* and *Z. candida* was found to be variable representing 2n = 28, 42 and 48 in the former and 2n = 19, 32, 38 and 40 in the latter. However, in *Z. grandiflora* 2n = 24 was found to be constant. All the species taken for study were characterized by large chromosomes. Chromosomes of all the species were mostly metacentric and submetacentric while secondarily constricted ones were either with subterminal or submedian primary constrictitions. Satellited chromosomes were not observed in *Z. grandiflora*. Karyotypes in all the three species were observed to be nearly asymmetrical types with average T.F. value of 39.53% in *Z. grandiflora* and 37.53% in *Z. candida*. Based on the analysis of chromosome morphology, the karyotypes of all the varieties are given in Table 1. The photomicrographs and idiograms of somatic karyotypes for all the three species are shown in the Figs. 1-9.

DISCUSSION

The karyotype analysis of 3 species of genus *Zephyranthes* shows considerable variation in chromosome number. Only *Z. grandiflora* shows normal diploid chromosome number of 24. The other 2 species i.e., *Z. candida* and *Z. flava* show variation in numbers from polyploid to aneuploid. The different somatic numbers recorded in these species are 2n = 19, 32, 38 and 40 for *Z. candida* and 2n = 28, 42 and 48 for *Z. flava*. Some of the chromosome numbers observed in the present study are different from the previous records. The previous records observed by some authors are *Z. grandiflora* 2n = 24, 36 (Kapoor & Tandon 1963 b), 2n = 24, 48 (Raina & Khoshoo 1972 a) ; *Z. candida* 2n = 19, 20 (Tandon & Sachdeva 1963). 2n = 38, 40, 41 (Raina & Khoshoo 1971b, 1972a, Khoshoo 1979). 2n = 50 (Khoshoo & Raina 1976) and *Z. flava*, 2n = 42, 48 (Singh & Roy 1973a).

TABLE 1 : Details of karyotypes of *Zephyranthes*.

Sl. No.	Species	2n*	Types of chromosomes			Secondary Constriction		Average Arm ratio	Length of chromosomes in microns			Average T.F. %
			Median	Sub median	Sub terminal	Sm	St		Absolute length	Mean length	Range in length	
1.	*Z.grandiflora*	24	3	7	2	-	-	0.60	17.2	0.71	0.5-1.1	39.53
2.	*Z.flava*	48	5	14	5	-	-	0.67	44.0	0.91	0.6-1.4	37.91
3.	*Z.flava*	42	3	9	7	1	1	0.53	45.0	1.07	0.5-1.7	37.91
4.	*Z.flava*	28	4	5	4	1	-	0.60	27.2	0.97	0.7-1.5	37.91
5.	*Z.candida*	40	5	7	8	-	-	0.56	44.8	1.12	0.5-1.8	37.53
6.	*Z.candida*	38	5	8	4	-	1	0.64	30.8	0.81	0.5-1.4	35.53
7.	*Z.candida*	32	3	11	1	-	1	0.63	27.0	0.84	0.5-1.3	37.53
8.	*Z.candida*	18+1	1	6	3	-	-	0.58	23.2	1.22	0.7-1.8	37.53

Four types of chromosomes are observed, metacentric submetacentric, subtelocentric and setellited chromosomes. But in *Z. grandiflora*, satellited chromosome is not observed. In case of *Z. candida* and *Z. flava* also, satellited chromosome is not observed. In *Z.candida*, satellited chromosome are observed in plants with 2n = 32 and 38. Similarly, in *Z. flava*, satellited chromosome are observed only in plant with 2n = 28 and 42. In *Z. candida* (2n = 19, 40) and *Z. flava* (2n = 48) satellited chromosomes are not observed. This difference in the same species is possibly due to coinciding of secondary constriction in size with the nucleolus and if the latter is very small, the constriction may be invisible or many disappear later (Sharma 1976). Among the 3 species, the size of the chromosomes of *Z. flava* are relatively large and also the highest chromosome number is observed in this species.

In the present study, the variations in the number of chromosome are observed in the cells of the same tissue collected from the same bulb. The variations in chromosome number within the cells of the same tissue in the family Amaryllidaceae has been extensively studied. For example, in *Z. mesochloa*, the normal somatic chromosome number is 2n = 48, but variant cells within the same tissue contain 2n = 24, 42, 54, 60, 66 and 72 (Sharma 1976). The variation of chromosome numbers within the cells of same tissue may be due to non-disjunction, somatic reduction and possibly partial endomitosis. In the somatic tissues, non-disjunction involves unequal distribution of chromosomes in the daughter nuclei (Sharma 1976). The variation may be due to repeated cell division of the bulb and sometimes cytokinesis does not occur during the formation of callus for vegetative reproduction. Due to the abnormality in meiotic division sex cells with a wide range of numbers are involved in hybridization in nature, a cycle of aneuploid variability arise which are preserved through a agamospermy and vegetative multiplication (Raina 1980). Among the species studied here chromosome number variation is observed n *Z. candida*. In this species, the number ranges from polyploid to aneuploid.

The highest chromosome number among these three species is 2n=48 as observed in *Z. flava*. Based on chromosome numbers we can not ascertain their evolutionary trends because in some plants primitive ones have lower chromosome number and advanced ones have higher chromosome number and they are derived from lower chromosome number during the course of evolution. But in some plants, for example in *Crepis*, the evolution may involve in descending series. In this genus, the most primitive species have higher chromosome number and the advanced species have lower chromosome number (Sharma 1976).

The karyotype of all the three species are nearly asymmetrical and show their advanced nature. There is slight variation between the karyotypes of these three species. The presence of somatic cells with variation in karyotypes i.e., polysomaty is not a rare feature in vegetatively reproducing plants and such variations in chromosome number and structure within the karyotypes of cells of a single individual or of different individuals play an important role in speciation (Sharma 1976).

In the progressive evolution from a symmetrical karyotope to asymmetrical ones, 2 reasons can be given, (a) submedian and subterminal centromeric chromosomes are derived from median by reduction in length of one arm of the chromosome and (b) reduction in size of some chromosomes in

progressively unequal sizes (Sharma 1976). Those chromosomes which have equal and more or less similar size and length show symmetric karyotype (Sharma 1976, Stebbins 1950). Another point is that due to the shift of position of centromere from metacentric to submetacentric or acrocentric or accumulation of differences in relative size between chromosomes of the same complement, the gradual evolution from symmetrical to asymmetrical karyotype take place (Sharma 1976, Stebbins 1971). In the present study, among the three species, the highest T.F. value is observed in *Z. grandiflora*, 39.53% and the lowest T.F. value in *Z. candida*, 37.53%.

So, among the 3 species, the karyotype of *Z. candida* is more asymmetrical and shows the advanced nature of the species and *Z. grandiflora* shows primitive nature. From the above mentioned reasons about variations in chromosome numbers within the cells of the same tissue, structural differences and their T.F. values, it may be concluded that *Z. candida* is the most advanced species and *Z. grandiflora* is the most primitive among 3 species.

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MEIOTIC STUDIES IN A RADIATION INDUCED MUTANT IN *PHLOX DRUMMONDII*

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SUMMARY

Meiosis was studied in a mutant isolated in a population raised from gamma ray irradiated seeds of *Phlox drummondii* (2n=14). The mutant was characterised by flower colour variation and highly abnormal meiotic behaviour like occurrence of less than 7 bivalents (10.63%), cytomictic cells (15.72%), tetraploid condition (7.6%), stickiness (28.43%), laggards (11.2%), bridges (13%), unequal distribution of chromosomes (7.4%) and micronuclei (5.6%). The pollen grains were also characterised by minipollen and cytomixis.

Key Words: *Phlox drummondii*, radiation, mutant, cytomixis.

INTRODUCTION

Various cytogenetical methods like induction of polyploidy and breeding, induced mutation etc. can be used to achieve genetic improvement of economically important plants. *Phlox drummondii* (Polemoniaceae) is one of the most common annual, herbaceous ornamentals which produces beautiful flowers of various colours. Under the genetic improvement programme of this plant, colchiploids of various colours and a few mutants have been produced (Verma & Raina 1982, 1991, Verma et al. 1993, Reddy & Verma 1994). The present investigation was aimed at isolating useful mutants using gamma ray treatment. An useful mutant was isolated from a large population of plants raised from treated seeds. In the present communication, meiosis of a mutant has been described.

MATERIAL AND METHODS

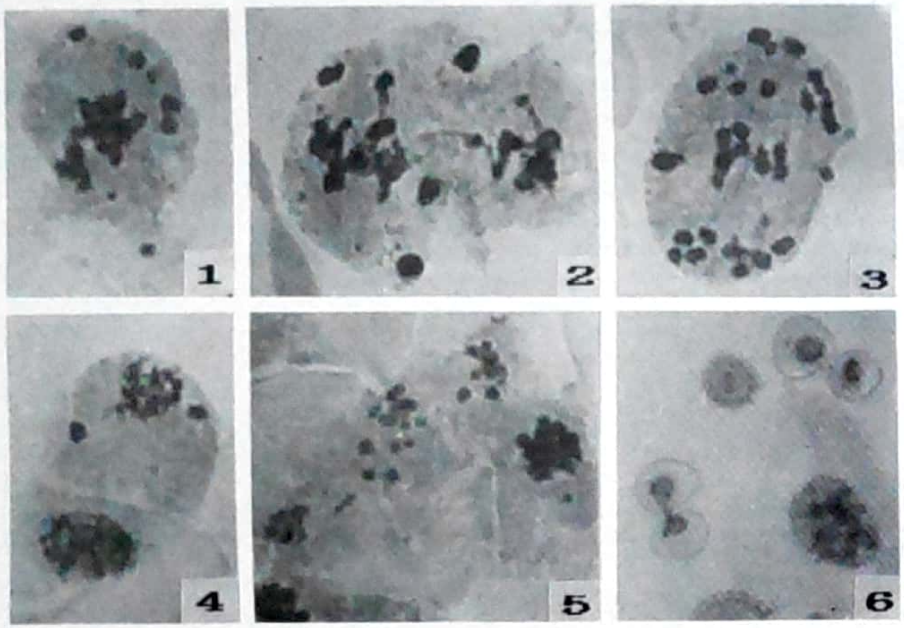
Seeds of *Phlox drummondii* were collected from the lines maintained by us. Seeds were irradiated by 5, 8, 10, 12 and 15 KR doses of gamma rays at the N.B.R.I., Lucknow. For meiotic studies, young flower buds were fixed in acetic acid : absolute alcohol (1:3) mixture for 24 h. Anthers were squashed in 1% acetocarmine. Photomicrographs were taken from temporary slides. Pollen stainability was determined using iron-acetocarmine.

OBSERVATIONS

In the population raised from 5 KR treated seeds of diploid (red flower), one plant was morphologically different from the others. From the very beginning, the growth of the plant was slower as compared to the others and the leaves had some cream-coloured stripes. The flower petals had white stripes on red and the proportion of 2 colours varied in different flowers. The petals were uneven on margins as compared to smooth margins in control.

The PMCs in untreated materials showed 7 bivalents at diakinesis and metaphase I. The number of chiasmata per cell ranged from 12-20, mean number being 16.8, out of which 9.7 were terminalized giving terminalization coefficient of 0.57. Anaphase I had equal distribution (7:7) of

chromosomes. The pollen stainability was 93.9% and the seeds per plant were about 300. However, in plant treated with gamma rays all cells analyzed at diakinesis and metaphase I were abnormal. In none of the PMCs 7 bivalents were observed. The bivalents ranged from 1-5 in each cell (10.63%) remaining were univalents or the associations were not countable. Cytomixis was observed in 15.72% of cells (Fig.5). At early anaphase I, unequal distribution like 5 : 6 : 3 and 8 : 6 : 8 were observed in 7.4% cells (Fig.3). Bridges (13%) and fragments, lagging (11.2%), and other abnormalities like stickiness (Figs.1,2) were also observed. Telophase I and II were highly irregular. Micronuclei (5.6%) ranging from one to many or unequal distribution of chromosomes were observed (Fig.4). Many post-meiotic abnormalities like different sizes of pollen grains, high percentage of sterility and cytomixis (Fig.6) between pollen grains were observed.



Figs.1-6 : Meiosis in the mutant *Phlox drummondii*. 1,2. Mutant M I ; note stickiness. 3. Anaphase I showing unequal distribution. 4. Telophase I ; note unequal distribution of chromatin at poles and micronuclei. 5. Cytomixis between PMCs. 6. Pollen grains; note cytomixis and pollen grains of different sizes.

DISCUSSION

The mutant of *Phlox drummondii* was characterised by the presence of high frequency of univalents at diakinesis and metaphase I. This indicated that the univalents might have been

produced by partial asynapsis. When the chiasmata are strictly terminal, there is a probability of some of them being slipped off precociously and convert the bivalents to pairs of univalents which move to the poles. In the present material, the association between chromosomes might have been maintained by some sort of stickiness till they separate at anaphase I. Abnormal associations at diakinesis and metaphase I led to highly abnormal anaphase I. Not even a single cell was observed to have 7:7 distribution of chromosomes. In some cases, a few univalents were left at the equatorial region. Other abnormalities like bridge formation, chromosome fragmentation at anaphase I, as observed in *Scilla* (Rees 1952), *Sorghum* (Magoon et al. 1961) were observed in the present material.

In 15.72% of cells and in some pollen grains cytomixis and transmigration was observed. The origin, development and function of cytomixis during microsporogenesis have been reported and discussed by various workers in several families including normal plants, hybrids, mutants, triploids and apomicts (Verma et al. 1986). Cytomixis resulted into the formation of micronuclei and minipollen grains. Due to stickiness also there may be unequal distribution during microsporogenesis resulting in the non-functional germ cells. This explains to some extent the sterility of mutant plant observed in the present investigation.

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EFFECT OF GAMMA RADIATION ON MEIOTIC CHROMOSOMES OF *BOMBYX MORI* L.

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SUMMARY

The multivoltine Pure Mysore strain of *Bombyx mori* larvae (V instar) were exposed to ^{60}Co gamma rays (500 r, 1000 r and 1500 r) to study the effects on meiotic chromosomes. Karyotypes were prepared from the larval chromosomes of the same as well as of the succeeding generations to study the carry over effects. Gamma rays induced chromosomal aberrations (such as fragmentation, translocation, stickiness, ring formation, clumping, etc.) during spermatogenesis and oogenesis at first and succeeding generations.

Key Words: *Bombyx mori*, gamma rays, meiotic chromosomes

INTRODUCTION

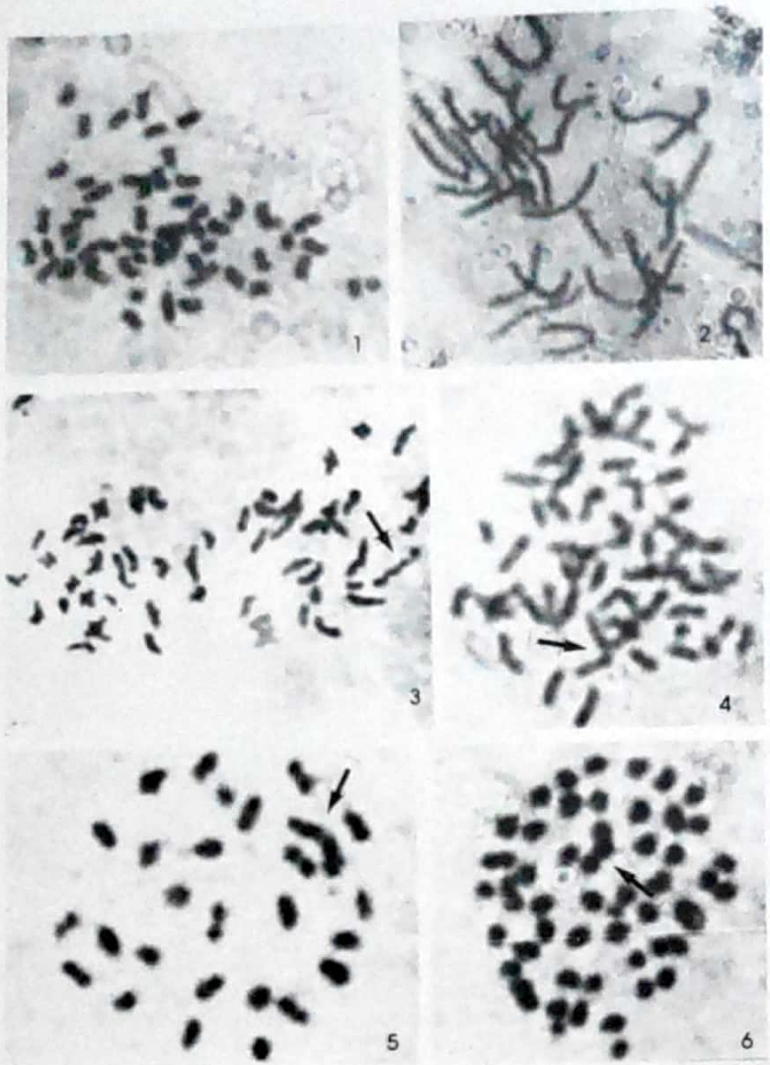
The impact of ionizing radiations like X- and gamma rays in inducing chromosomal aberration has been reported in many holokinetic species including *Bombyx mori* (La Chance et al. 1967, 1970, North & Holt 1968a, b, Cooper 1970, Murakami & Imai 1974, Nayak & Padhy 1979, Tempelar 1979, Carpenter 1991). Among Bombycidae, genetical and cytological analysis of meiosis in male and female of *B.mori* have yielded valuable information on the mechanism of meiosis. Inspite of the importance of silkworms as genetic tools, the effect of gamma radiation during the meiosis has not been studied in *B.mori*. This paper presents a report on the incidence of visible chromosomal aberrations induced by gamma rays.

MATERIAL AND METHODS

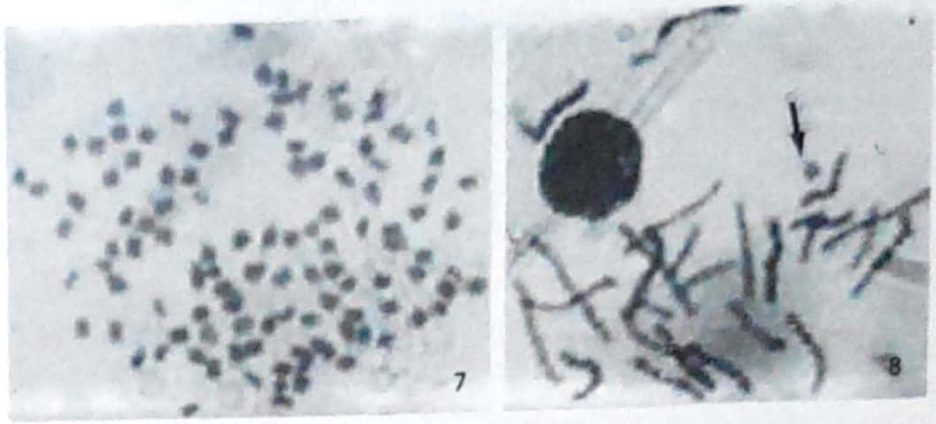
In the present study, the multivoltine *Bombyx mori* L. (Pure Mysore strain) was used. Newly moulted V instars were irradiated with gamma rays from ^{60}Co source (Theratron 78-C-AECL, Canada). The radiation doses of 500 r, 1000 r and 1500 r at the rate of 150 r/min were given at room temperature (24±2°C). The larvae were allowed to spin the cocoons. The rearing methods followed were that of Krishnaswami (1978). The larvae from III to V instars picked up from the same and next generations were dissected and the gonads were taken for the chromosomal preparation. The meiotic chromosomes from testis and ovary were prepared following the methods of Premila Chanu et al. (1988).

OBSERVATIONS

The diploid chromosome number of *B. mori* was 56 and the nature of chromosome was holokinetic (Fig. 1). Gamma radiation-induced aberrations in the silkworm chromosomes were frequently observed in both spermatogenesis and oogenesis. The aberrations were of gross as well as individualistic in type. The gross type aberrations were represented by stickiness, clumping, etc., whereas individualistic types were represented by point mutation such as fragmentation, reciprocal translocation, ring chromosomes etc. (Figs. 2-8). The occurrence of tetraploidy was also noticed in larvae irradiated with 1500 r (Fig. 7).



Figs.1-6 : Photomicrographs of meiotic chromosomes from larvae of *B.mori*. 1. Male . Metaphase II (unirradiated). 2. Female. Pachytene (unirradiated). 3. Male. Diakinesis with an abnormal chromosome (500 r). 4. Female . Metaphase II with sticky branched chromosome (500 r). 5. Male - Metaphase I with heteromorphic bivalent (1000 r). 6. Female . Metaphase I with heteromorphic bivalent (1000 r).



Figs.7-8 : 7. Male. Tetraploid complement (1500 r). 8. Female. Pachytene with minute fragment (1500 r). Arrow indicates the abnormal chromosome.

DISCUSSION

The chromosomal aberrations induced by gamma rays were of gross and of point mutation type. Murakami & Imai (1974) also observed similar point mutations in *B. mori* when treated with X-rays (1000 r). Like X-rays, gamma rays could be used for involving chromosomal aberrations. Tazima (1961) has opined that the mutagenic efficiency in the silkworm is equivalent between X-rays and gamma rays. Nayak & Padhy (1979) reported the chromatid and isochromatid breaks in *Philosamia ricini* when irradiated with gamma rays. The sub-chromatid type aberrations were not reported so far in silkworms.

Since the silkworm is an insect of economic importance, induction of sterilization or lethality is not a good criterion unlike other insects which are pests to agricultural crops, animals and human beings. In the present investigation, the doses of 500 r, 1000 r and 1500 r have not caused any effect of sterilization or lethality in *B. mori*. Further, these low doses of radiation could be used in sericulture industry to induce beneficial mutations. However, more detailed studies are needed to know the type of chromosomal translocation/genes involved in a particular chromosome to understand the beneficial mutations.

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AUTOTETRAPLOIDS OF *SESAMUM INDICUM* AND *S. ALATUM* : CYTOGENETICS AND CROSSABILITY

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SUMMARY

Interspecific hybridization in the genus *Sesamum* (Pedaliaceae) is constrained by the presence of a high degree of cross incompatibility. The species with identical chromosome number of 2n=26 viz., *S. indicum* and *S. alatum* also showed a very poor crossability due to some strong isolation barriers. Fertile autotetraploids of *S. indicum*, the cultivated sesame and *S. alatum*, a wild species reported to be resistant to phyllody disease, were induced through colchicine treatment in order to assess the cross compatibility after genome doubling. Cytological studies showed much similarities in terms of chromosome configuration in meiosis. A maximum of 6 quadrivalents was observed at metaphase I of the tetraploids of both the species followed by a normal anaphase I separation leading to high pollen fertility. The meiotic chromosome behaviour of autotetraploids of *S. alatum* is reported for the first time while the cytogenetical behavior of autotetraploids of *S. indicum* clarifies the earlier contradicting reports on the quadrivalent formation at metaphase I. There was not much improvement in the cross compatibility as these autotetraploids exhibited similar compatibility relationships as their primary species with other species of *Sesamum*

Key Words : *Sesamum*, autotetraploids, quadrivalents, interspecific hybridization.

INTRODUCTION

Interspecific hybridization is being performed in sesame (Pedaliaceae), a crop known for its high quality seed oil, to transfer the desirable agronomic traits like resistance to biotic and abiotic stresses from the wild relatives. *S. alatum*, a wild species with 2n = 26 reported to be resistant to phyllody disease. Attempts to transfer this trait to *S. indicum* (2n=26) were not successful due to the cross incompatibility between them and the genomic relationship could not be studied. The purpose of this experiment was to induce chromosome doubling in these two incompatible species and study the crossability relationship between them after genome doubling. Autotetraploids of different cultivars of sesame have been developed using colchicine (Richharia & Persai 1940, Mazzani 1954 & Srivastava 1956) and described for various morphological characters and cytological behaviour. But such studies on cytogenetical behaviour of autotetraploids of *S. indicum* have been at variance. Autotetraploids of *S. alatum* have been recently identified (Prabakaran et al. 1992) but the chromosome behaviour in meiosis was not described. Though the improved and seed fertile autotetraploid forms of the *S. indicum* will have very little scope for direct utilization as cultivars, the studies on experimental autotetraploids are likely to elucidate problems of fundamental nature connected with

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the type and optimal level of ploidy in the genus. Further, the utility of these autotetraploids in species crosses to bring out the genomic relationship between species of *Sesamum* with higher chromosome number is unlimited. Hence, attempts were made to induce autotetraploids of *S. alatum* Thonn, and *S. indicum* L. and study the cytogenetical behaviour in meiosis and their crossability relations with other species of *Sesamum*.

MATERIALS AND METHODS

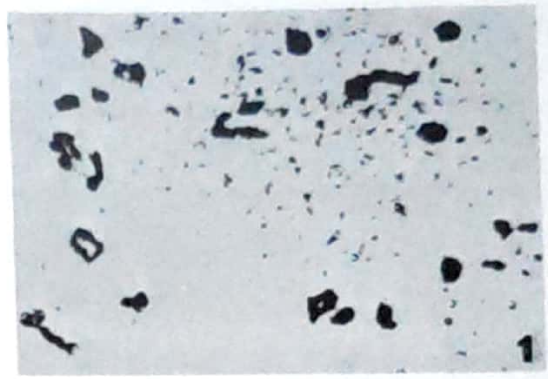
Seeds of *S. indicum* var. CO 1 were presoaked in water for 12 h and treated with 0.05% (w/v) aqueous solution of colchicine for 3 h. After treatment, the seeds were washed several times with distilled water and sown in pots. Owing to poor germination of seeds of *S. alatum*, young seedlings at 2-leaved stage were applied with 0.01% of colchicine on the shoot apices (by wrapping a small piece of cotton wool), thrice a day for 5 or 6 d continuously. The treated plants were observed for morphological deviations at different stages of plant growth. For cytological investigations, young flower buds of appropriate size were fixed in Carnoy's fluid at 11.15 a.m. and kept at 4°C overnight before transferring it to 70% alcohol for storage. The smears were prepared using 1% acetocarmine. The induced fertile autotetraploids obtained in the present study were crossed both in direct and reciprocal directions with all the other species of *Sesamum* by following the standard hand emasculation and pollination method.

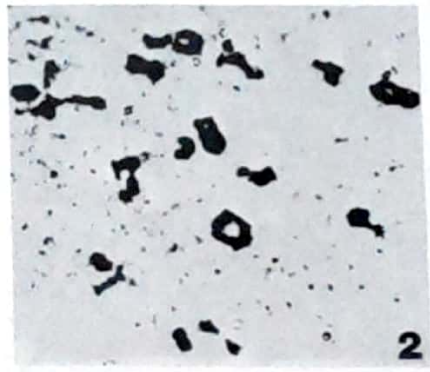
OBSERVATIONS

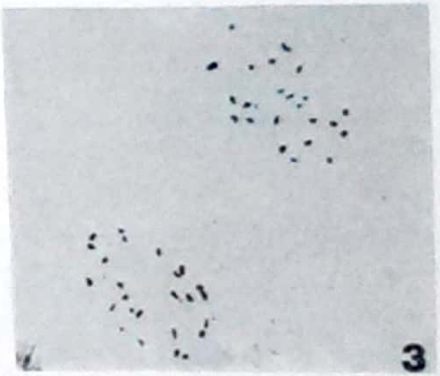
The autotetraploids of both the species were recognisable based on their morphological distinctness from the respective diploids. The growth rate in tetraploids was very slow as compared to their corresponding diploids. The tetraploids were identified based on thick stem with larger, broader, thick, leathery and dark green leaves. Stomatal observations revealed increased stomatal size with decreased frequency. Flowering was delayed and the flower size of tetraploids was almost double that of their respective diploids. The tetraploids exhibited short but broader capsules. The total number of seeds per capsule got reduced while the seed size increased.

The chromosome number of the induced autotetraploids of *S. indicum* and *S. alatum* was confirmed to be 2n=52. Meiotic studies revealed different chromosome associations at diakinesis and metaphase I with varying number of quadrivalents, bivalents and univalents (Table 1). The maximum number of quadrivalents observed was 6 but the most frequent number was 5 or 6 in the autotetraploids of both the species. At metaphase I, the quadrivalents were of different shapes from chain- to ring-types (Figs. 1,2). The chromosome association in each PMC varied from $6_{IV} + 14_{II}$ to $5_{IV} + 15_{II} + 2_{I}$ with a mean of $5.55_{IV} + 14.85_{II} + 0.1_{I}$ in the autotetraploid of *S. indicum* (Table 1). The autotetraploid of *S. alatum* showed an average association of $5.52_{IV} + 14.88_{II} + 0.18_{I}$ in each PMC with a range of $6_{IV} + 14_{II}$ to $5_{IV} + 15_{II} + 2_{I}$ (Table1). However, the anaphase I separation was regular (26:26) in all the PMCs observed (Fig. 3) and normal tetrads were formed in the autotetraploids of both *S. indicum* and *S. alatum* leading to high pollen fertility of 69.23 - 75.33 % and 67.25 - 78.37%, respectively.

The selfed seeds of the autotetraploids of both the species were sown and studied subsequently in C_1, C_2 and C_3 generations. All the progeny plants were confirmed to be tetraploids. When the induced fertile autotetraploids were crossed with different species of *Sesamum*, they readily crossed with their respective diploids (Table 2). However, the autotetraploid of *S. alatum* was not crossable with any other species in the genus *Sesamum* including the induced autotetraploids of *S. indicum* while the autotetraploid of *S. indicum* readily crossed with *S. malabaricum* (2n=26) and *S. laciniatum* and *S. prostratum* (2n=32). Attempts to cross *S. occidentale* or *S. radiatum* (2n=64) with the induced autotetraploids of *S. indicum* and *S. alatum* (2n=52) failed to yield any seed set.







Figs.1-3: Meiotic associations in *S. alatum* and *S. indicum*. 1. Metaphase I in autotetraploids of *S. alatum* showing $6_{IV} + 13_{II} + 2_{I}$. 2. Metaphase I in autotetraploids of *S. indicum* showing $5_{IV} + 15_{II} + 2_{I}$. 3. Anaphase I in autotetraploids of *S. indicum* showing equal disjunction. (1,2 x 1500, 3 x 1000).

DISCUSSION

Experiments on induction of autotetraploids of sesame (*S. indicum*) are in progress since 1940 when Richharia & Persai (1940) reported the first autotetraploid in sesame. The autotetraploids were characterized based on their morphological features and cytological behaviour (Kobayashi & Shimamura 1952, Srivastava 1956). The first autotetraploid of *S. alatum* was identified by us based on their distinctness in phenotypic expression (Prabakaran et al. 1992). In the present investigation, the induced autotetraploids of both *S. indicum* var. CO 1 and *S. alatum* exhibited larger, thicker and dark leaves, delayed flowering, larger flowers, larger seed size and lesser number of seeds per capsule which is in agreement with the earlier reports. The striking

TABLE 1: Chromosome association at metaphase I in the induced autotetraploids of *S. indicum* and *S. alatum*.

S. indicum					*S. alatum*				
Chromosome association				No. of PMCs	Chromosome association				No. of PMCs
IV	III	II	I	analysed	IV	III	II	I	analysed
5	-	15	2	4	5	-	16	-	53
5	-	16	-	48	5	-	15	2	6
6		13	2	2	5	-	14	4	2
6	-	14	-	61	6	-	13	2	1
					6	-	14	-	64

TABLE 2: Crossability relationship of the fertile autotetraploids of *S. indicum* and *S. alatum* with different species of *Sesamum*.

Parental species		*S.indicum* (2n=26)	*S.indicum* (2n=52)	*S.alatum* (2n=26)	*S.alatum* (2n=52)	*S.laciniatum* (2n=32)	*S.prostratum* (2n=32)	*S.radiatum* (2n=64)	*S.occidentale* (2n=64)
S.indicum (2n=52)	D	H	H	X	X	H	H	X	X
	R	H	H	X	X	H	H	X	X
S.alatum (2n=52)	D	X	X	H	H	X	X	X	X
	R	X	X	H	H	X	X	X	X

D - Direct Cross; R - Reciprocal cross; H - Viable seed set; X - No seed set

difference in the dimension of capsule was its reduced length and increased breadth in the autotetraploids.

Though higher chromosomal configurations like quadrivalents were observed at diakinesis and metaphase I, due to normal separation of chromosomes in anaphase I and II, regular tetrads were observed. The tetraploids of *S. indicum* and *S. alatum* produced fertile pollen to a maximum of 75.33% and 78.37%, respectively which is as high as that reported earlier on fertile autotetraploids of some cultivars of *S. indicum* (Kobayashi & Shimamura 1952, Srivastava 1956). However, reports on the chromosome association in meiosis have been at variance. Richharia & Persai (1940) observed 0-5 quadrivalents while Kobayashi & Shimamura (1952) reported 13 quadrivalents at metaphase I. In the present investigation, 5-6 quadrivalents were observed at metaphase I followed by normal anaphase separation in the autotetraploids of both *S. indicum* and *S. alatum*. The variable number of quadrivalents reported in the induced autotetraploids of different genotypes of

S. indicum and *S. alatum* clearly explains the genotypic control of quadrivalent formation. Morrison & Rajhathy (1960) speculated that in all autotetraploids approximately two-third of chromosomes form quadrivalents. But the present study does not support the above conclusion. In the autotetraploids of *S. indicum* and *S. alatum*, less than one-half of the chromosomes associate as quadrivalents, as observed by Mehta et al. (1963) in *Trifolium*. Theoretically, the autotetraploids are expected to form 13 quadrivalents because of the repetition of homologous pairs by doubling. But the reduced number of 5 or 6 quadrivalents observed in the present study may be due to the smaller size of chromosomes of sesame, reduced chiasma frequency due to reduced rate of pairing in the autotetraploids thereby resulting in some reduction in pollen fertility. Reduction in multivalent formation in the subsequent generation leading to improvement in seed setting has been observed in many plant species. However, cytological studies on autotetraploids of *S. indicum* and *S. alatum* revealed no reduction in the occurrence of odd chromosome configuration like quadrivalents in the subsequent generation and hence, there was no rapid natural diploidization and increased fertility in the later generations.

The cytological studies on autotetraploids of the 2 species utilized in the present investigation showed much similarity in terms of chromosome configuration at metaphase I, anaphase I separation and pollen fertility (Table 1). A chance hybrid of *S. alatum* x *S. indicum* reported for the first time by Prabakaran et al. (1992) showed a normal course of meiosis by forming 13 bivalents at metaphase I. This is a clear indication of intergenomic homology between the 2 parental species. The interspecific hybrid also showed high fertility and therefore, it was premised that the differentiation of the 2 species might be due to a small structural changes and gene mutations (Prabakaran et al. 1992). The identical chromosome behaviour observed in the autotetraploids of *S. indicum* and *S. alatum* evidently supports the earlier assumption of more genome homology between these 2 species.

Direct use of autotetraploids is limited due to their low seed set and poor yield despite their larger seed size. But their utility in wide crosses where *S. indicum* and *S. alatum* have poor crossability, is very high. Interestingly, *S. indicatum* (2n=58), an amphidiploid of the cross *S. indicum* (2n=26) x *S. prostratum* (2n=32) showed a high degree of crossability with *Ceratotheca sesamoides* (2n=32), a related genus of *Sesamum*, while the primary species *S. indicum* and *S. prostratum* were unable to produce viable seeds when crossed with *C. sesamoides* (Joshi 1961). However, this cannot be generalized as the increased ploidy status did not show any improvement in crossability. The induced autotetraploids of *S. indicum* and *S. alatum* exhibited a similar crossability relationship with all the other species belonging to the 3 chromosome groups of *Sesamum* as reported in their diploid primary species (Prabakaran et al. 1995). The 2 incompatible species of the same chromosomal group (2n=26) viz., *S. indicum* and *S. alatum*, were imcompatible at tetraploid level too indicating a strong isolation mechanism which was unaffected by the doubled genome size. Similarly, *S. occidentale* and *S. radiatum* having 2n=64 also showed a high degree of incompatibility when forced pollination was done with the fertile autotetraploids. Hence, the transfer of desirable genes through interspecific hybridization is continued to be constrained by a high degree of cross incompatibility between the species of *Sesamum* which could not be overcome by the attempted ploidy manipulation. However, the new interspecific hybrids between the autotetraploid of *S. indicum* and other compatible species will be of immense use in future cytogenetical studies.

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COMPUTER AIDED IMAGE ANALYSIS OF PEARL MILLET CHROMOSOMES AFTER Q-BANDING

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SUMMARY

The somatic chromosomes of pearl millet (*Pennisetum glaucum* (L.) R.Br.) were stained with quinacrine and the Q-banding pattern was analysed using an image processing system for the first time. The image of 14 chromosomes was processed through different filters and a high contrast chromosome image with a clear background was produced. Various quantitative data viz., lengths of short arm and long arm and the size and position of bands were used to construct the Q-banded karyotype of pearl millet. Histograms and frequency analysis were attempted for each of the 14 somatic chromosomes. The presence of Q-bands in the telomeric and centromeric regions and the absence of intercalary bands were evident from the above parameters. Polymorphism in Q-banding patterns in the homologues of chromosome 1,3,4 and 6 of pearl millet reported earlier by us was confirmed.

Key Words : Q-bands, pearl millet, image processing, histogram, frequency analysis.

INTRODUCTION

The first computer aided system for human chromosome analyses was developed in 1964 by Ledley based on the increasing demand for obtaining high quality data and also for sparing the time and efforts of the researchers in the cytological, microscopical and photographical procedures. McGurk & Rivlin (1983) used a 'BASIC' computer programme for chromosome measurements and analysis. Green et al. (1984) introduced the computer assisted analysis of karyotype and utilized them for generation of idiograms and pairing homologues. Image analysis methods have only been recently introduced in plant cytology and chromosome studies (Fukui 1985, 1986, 1988). Fukui et al. (1988) applied CHIAS (Chromosomes Image Analysing System) in the identification of rice chromosomes and the mean length, relative length and arm ratios were analysed for karyotyping. The density distribution along the chromosomal axis can be obtained easily using an image analysing system. It could provide new information on the distribution pattern of condensed regions and also the degree of condensation. The analysis of banded chromosomes by defining the density distribution along the chromatids or the midrib of the chromosomes was not attempted earlier. However, such analyses will be very effective in determining the accurate position of the banded regions on the chromosomes. Hence, an attempt was made to analyse the Q-banding pattern of pearl millet chromosomes using an image analysing system and to confirm the banding pattern reported earlier.

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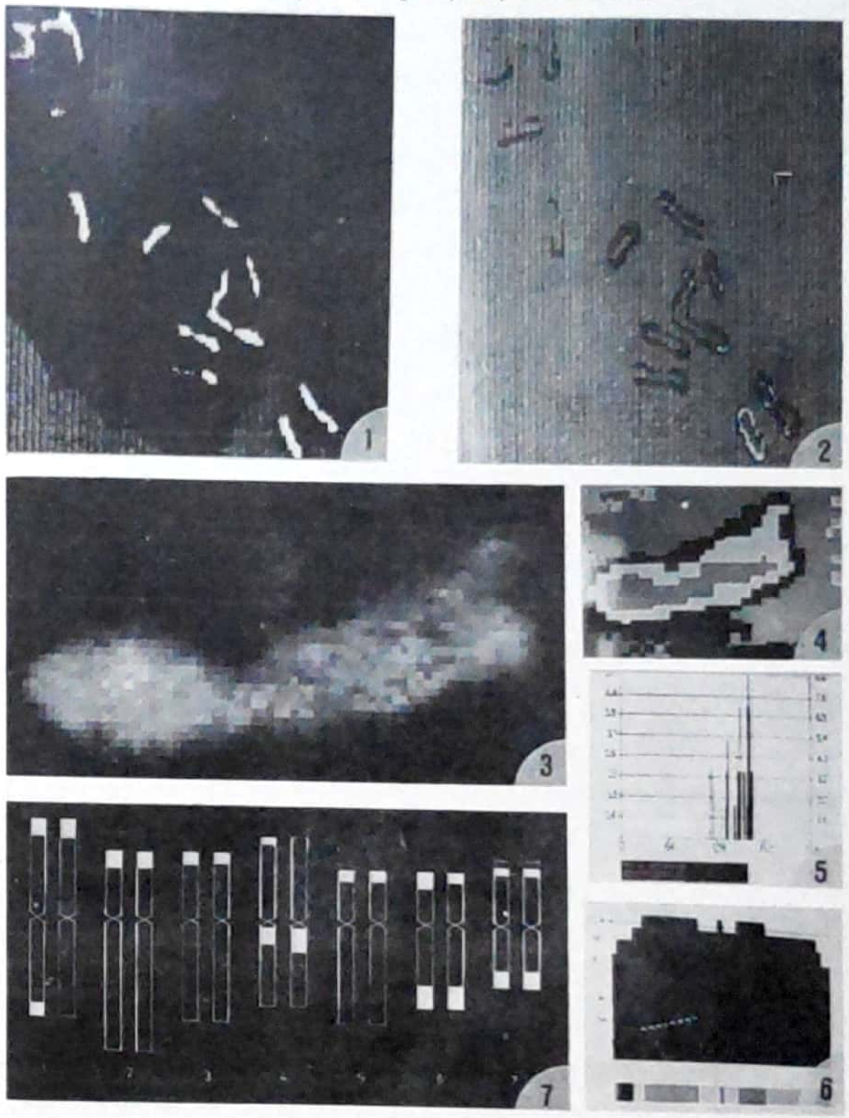
MATERIALS AND METHODS

Chromosome spreads were prepared using root tips of pearl millet, air dried and stained with quinacrine by following the detailed procedure outlined by Prabakaran et al. (1991). Photomicrographs of the Q-banded chromosomes were taken under Zeiss ultraphot microscope using 400 ASA 35 mm colour film. The image of photomicrograph or the negative film was taken into the system using a video camera with zoom lens and stored in a MHS image processing unit originally designed for biomedical analyses. The stored images of 10 well spread cells were processed using different types of filters. Histograms and frequency graphs which are the measures of the intensity of fluorescence along the length of the chromosomes were obtained for each of the 14 somatic chromosomes of the complement. A TV monitor was used to visualize the above characteristics and the banded regions.

RESULTS AND DISCUSSION

Contrast of the image was usually low at high magnifications but the same could be enhanced once the image was processed through different filters of the system viz., high pass, low pass and sobal filters. The unwanted particles in the background were eliminated and the image of 14 chromosomes with a clear background was obtained (Figs. 1,2,3). Midrib lines were drawn on every chromosome manually to mark the short arm, long arm and the size and position of bands. The various quantitative data obtained were used to construct the Q-banded karyotype of pearl millet. The quinacrine-stained chromosomes of pearl millet showed Q-bands in telomeric and centromeric regions and intercalary bands were absent. This was clearly evident from the histograms and the frequency distribution graphs obtained for each chromosome after image processing (Figs. 4,5,6). Further, by viewing the magnified image of the chromosomes in a TV monitor through different filters, it was possible to identify the banded regions more clearly. The measurement of the size of every band was made easy by getting a print out of the processed image of the chromosomes. The Q-banded karyotype of pearl millet is given in Fig. 7 which is identical to that reported by Prabakaran et al. (1991). When the chromosomes were paired and compared based on the similarities in frequency distribution and histograms, the homologues of the chromosomes 1,3,4 and 6 showed slightly dissimilar pattern of frequency distribution and histograms, thus exhibiting polymorphism. This is in conformity with the earlier observations of Prabakaran et al. (1991). The homologues of chromosome 1,3,4 and 6 differed by having bands of unequal size or different band positions thus exhibiting polymorphism. Fukui et al. (1988) used CHIAS to identify rice chromosomes based on mean length, relative length and arm ratio and constructed the karyotype. But the analysis of banded chromosomes by defining the density distribution along the chromatids or midrib of the chromosomes has not been reported earlier. Image analysis seems to be very effective in determining the accurate position and the size of the banded regions on the chromosomes.

Though image analysis has recently been used in plant cytology, its applications in this field are many. Apart from saving a great deal of time by automatization of detection of chromosome aberrations, it has become possible to obtain various kinds of quantitative data viz., number of chromosomes, lengths and arm ratios with much precision in a shorter time. Further, to construct karyotypes of banded chromosomes it will be much useful to locate the bands and to measure the band size accurately. Even very faint and small bands can be located and the polymorphism in the band size accurately. Even very faint and small bands can be located and the polymorphism in the banding pattern can be brought out. It appears to be a very powerful tool and continuously generates the data that cannot be obtained by the conventional methods. Thus, computer aided image analysis will be of immense use in future cytological and chromosome investigations to obtain quick and precise results and to circumvent the conventional methods.



Figs.1-7: Images of the quinacrine stained somatic metaphase chromosomes of pearl millet. 1. Before processing the image. 2. After the elimination of the background. 3. A single chromosome. 4. Image of a single chromosome through sobal filter. 5. Histogram. 6. Frequency graph. 7. Q-banded karyotype. (1,2 x 1500, 3 x 15000, 4 x 6000)

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DEVELOPMENTAL EXPRESSION AND VARIABILITY OF MALATE DEHYDROGENASE IN *ANOPHELES STEPHENSI* (DIPTERA : CULICIDAE)

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SUMMARY

Two separate gene loci seem to govern the developmental expression of 2 different forms of malate dehydrogenase (MDH), probably cytoplasmic (cMDH) and mitochondrial forms (mMDH). Laboratory survey revealed that m MDH locus was monomorphic and cMDH locus was polymorphic with 5 different electromorphs attributed to be governed by 4 codominant alleles. The c MDH appeared from third instar larval stage and m MDH from pupa onwards. However, no activity could be observed in the eggs and first 2 larval instars. Adult insects probably metabolize the pyruvate and NADH via mitochondria or through malate shuttle. cathodal (mitochondrial) MDH seems to be more suited to fuel the predominant flight activity. This locus seems to be switched on as an evolutionary necessity or an adaptive effort for the change from aquatic to terrestrial life. Tissue distribution indicated that the activity was predomenantly concentrated in the abdomen and no activity was detected in the head. Quantitatively, the activity increased continuously during the complete development. The higher activity in male stages could be due to their high energy requirements to lead more active life. Changes in isoenzymes and specific activity have been correlated with the various physiological and morphological events occurring during development.

Key Words : *Anopheles stephensi*, Development, malate dehydrogenase, isozymes.

INTRODUCTION

Studies on the specific gene-enzyme system during development promise for elucidating regulatory events underlying differential gene expression which forms the basis of eukaryotic development. Malate dehydrogenase (MDH, EC 1.1.1.37) is known to exist in 2 forms, cytoplasmic (cMDH) and mitochondrial MDH (mMDH) in many organisms. The oxidation of malate in the mitochondria by the action of NAD linked MDH to produce oxaloacetate forms the last reaction of Kreb's cycle. In addition, mMDH alongwith cMDH is involved in a malate shuttle process in some insect tissues (Sacktor 1974).

Qualitatively, there are only a few studies on the MDH activity during post-embryonic development and adult life of *Culex quinquefasciatus* (Narang & Narang 1975), *Aedes aegypti* (Mukiama 1980), *Apis mellifera* (Nunamaker & Wilson 1981, 1982) and *Caledia captiva* (Colgan 1986). Similarly, quantitative studies pertain to only *Tenebrio molitor* (Ludwig & Bursa 1958), *Cx. quinquefasciatus* (Narang & Narang 1975) and *Ae. aegypti* (Mukiama 1980). No systematic study seems to have been made on the developmental genetics of any particular insect species. Age and sex specific studies on the expression of MDH are also lacking.

The present study was undertaken with a view to examine the qualitative and quantitative expression of MDH during the complete development of *Anopheles stephensi* L., a vector of malaria on the Indian subcontinent (WHO 1982) where its control is hampered by insecticidal resistance (Subbarao et al. 1982). In addition, the effect of glucose and blood feeding on isoenzyme pattern during ageing in both the sexes have also been studied. The present study complements the detailed analysis (Vandana 1992, Gakhar & Vandana 1994) of developmental variations in the expression of house keeping genes.

MATERIALS AND METHODS

The culture of *An. stephensi* (Delhi strain : stock obtained from Malaria Research Centre, N. Delhi) was maintained in the laboratory at 28±2°C and 70-80% R.H. following the routine method of colonization. The adults were fed on 4% glucose solution soaked in cotton pads and females were allowed to feed on rat blood. The mosquitoes were sexed from third instar larva onwards on the basis of the presence of testes as the black spot on the sixth abdominal segment. The blood fed females had the age of about 25 d while the glucose fed females and males both had much lower life span of about 17 or 18 d. Whole bodies and also different regions (head, thorax and abdomen) were analysed for MDH activity. More than 3 replicates were used for each stage. Different developmental stages of known chronological age were pooled and stored at -20°C.

Isoenzymes of MDH were separated by horizontal starch gel electrophoresis. The gels were made from 11% starch in 0.01M Tris-0.0028M citric acid buffer, pH 7.0. The electrophoretic run was carried out at 250 V and 25 mA for 2 and 1/2 h. using 0.15M Tris-0.043M citric acid, pH 7.0. The gels after run were incubated at 37°C in the following staining solution (Brewer 1970). Phenazine methosulfate (PMS) was added after 45 min of incubation.

Staining solution : 100 ml 0.2M Tris-HCl buffer, pH8.2 + 30 mg malic acid + 20 mg nicotinamide adenine dinucleotide (NAD) + 15 mg thiazolyl blue tetrazolium bromide (MTT) + 5 mg PMS.

The quantitative activity was assayed at 340 nm using uv-visible spectrophotometer (CICEL - 534, double beam) by the rate of change in absorbance over a period of 5 min. The total volume of enzyme reaction mixture (containing 5-10 mg wet body wt.) in the cuvette was 3.0 ml. It contained 100µl of enzyme extract and other components i.e., 0.1M Tris-HCl buffer, pH 9.0 + 1 mM NAD + 25 mM malic acid.

Protiens were determined using the Bovine Serum Albumin (BSA) as the standard protein (Lowry et al 1951). All the data was subjected to student's t-test to determine the statistical significance.

OBSERVATIONS

The survey for MDH in the laboratory strain revealed 2 different zones of activity. The cathodal zone was designated as MDH-1 and anodal zone as MDH-2. The survey of electrophoretic variability revealed that MDH-1 was invariable and monomorphic while MDH-2 was polymorphic. A total of 5 electromorphs were attributed to be governed by 4 codominant alleles - Mdh^{90}, Mdh^{100} Mdh^{110}, Mdh^{120} present at MDH-2 locus (Fig. 1), Mdh^{100} being the most common allele.

The qualitative and quantitative variations in the pattern of MDH during the complete development and ageing of *A. stephensi* are presented in Figs. 2-4. Electrophoretically, no activity was observed in the eggs and first 2 larval instars. The cathodal zone, MDH-1 appeared in the pupae and was present subsequently throughout the adult life without any variation. The anodal zone, MDH-2 appeared from third instar larvae which also persisted during further development. This zone had only one band of broad area in pupae and adult males, however, in females 2 distinct bands were resolved. No sex-specific difference was present in the immature stages of development.

The maximum concentration of MDH was found in the abdominal region of all the stages examined. No activity could be detected in the larval head.

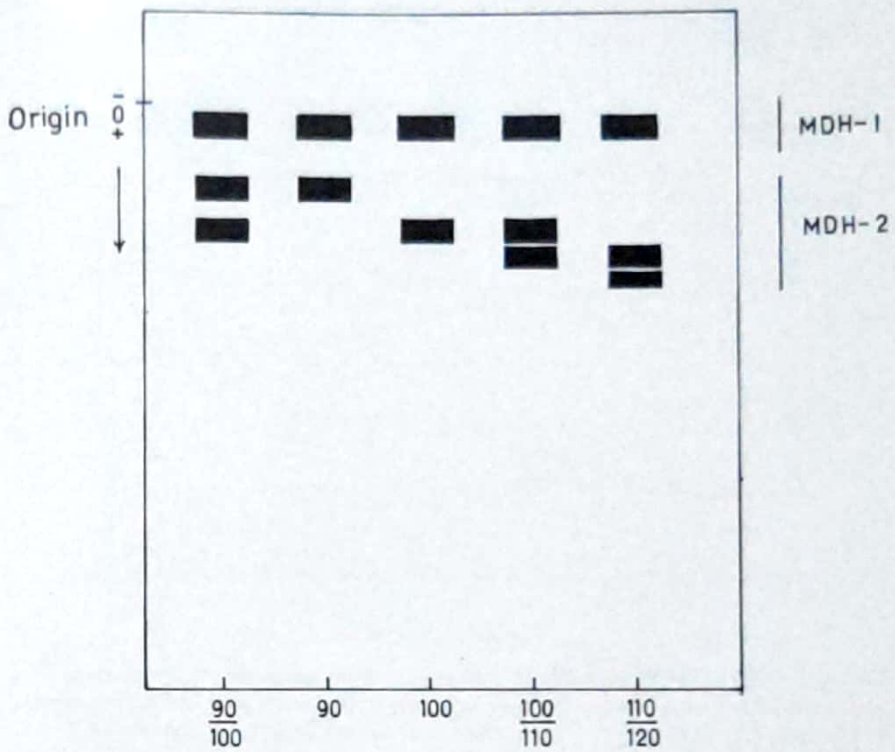

Fig. 1 : Electrophoretic phenotypes of MDH in *A. stephensi*.

The specific activity of MDH increased continuously during development. The activity appeared in first instar larvae (58 IU) and increased by more than 5 times during larval growth ($p<.001$). During metamorphosis, there was no significant change up to the mid-pupal stage, after which it almost doubled ($p<.001$) in the late pupae. Newly emerged adults of both the sexes had about 70% higher MDH level ($p<.001$) than their preceding stage. During adult life, the activity increased by more than 2.5 folds ($p<.001$) in both the sexes. The blood fed females showed a lower level of activity (by about 30% ; $p<.001$) than the glucose fed females of corresponding age. Regarding the sex-specific differences, the male larvae, pupae and adults always had higher level of activity than their female counterparts ($p<.001$)

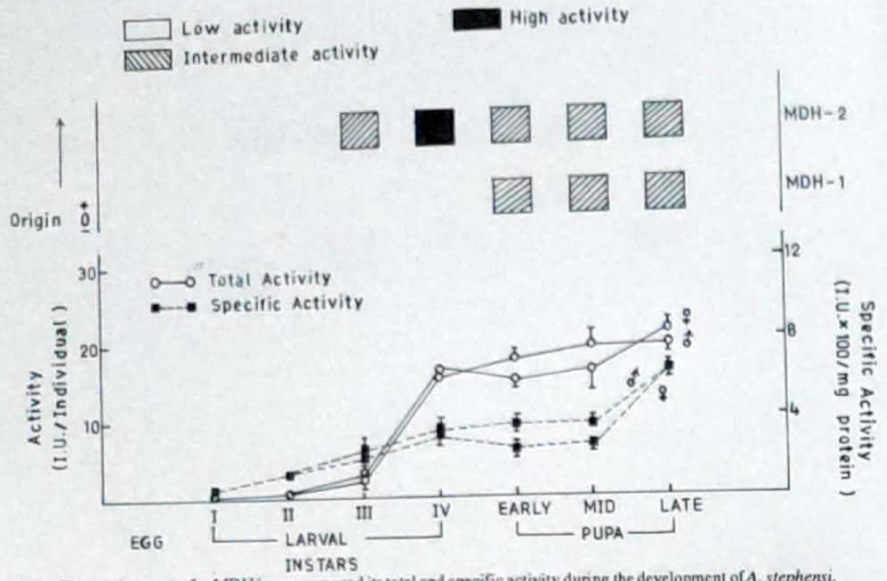

Fig. 2: Electropherogram for MDH isoenzymes and its total and specific activity during the development of *A. stephensi*.

DISCUSSION

The activity of MDH has been viewed in terms of physiological and physical environment of both immature and mature stages. Two zones of activity in the present study, presumably represent the cytoplasmic and mitochondrial forms. These 2 forms may control the malate shuttle (Sacktor 1974). The cytoplasmic enzyme (MDH-2) is involved in the cytoplasmic generation of NAD^+ and malate. The malate diffuses into the mitochondria, where it is involved in the energy producing reactions through MDH-1 isozymes (Everse & Kaplan 1975). Herrera & Mukherjee (1982) also revealed 2 multiple banded zones in 8 insect cell lines derived from different mosquito species. Contrary to earlier reports from Pakistan (Van Driel et al. 1987), we found this to be polymorphic in *A. stephensi*. However, our findings concur with Yong et al. (1985) who found cathodal MDH to be monomorphic and anodal MDH polymorphic in *Mansonia*. In the present study, 5 electromorphs seem to be governed by 4 codominant alleles. The most common allele, $MDH\text{-}2^{100}$ controls the phenotype with intermediate mobility. This phenomenon has been taken as supporting evidence that protein polymorphism is not primarily influenced by random genetic drift acting on a number of neutral isoalleles (Bullini & Colluzi 1972). Recently, this locus in *A. stephensi* has been assigned to the linkage group III (Adak et al. 1992).

The developmental sequence includes the switching on of MDH-2 locus (cytoplasmic form) from instar-III larva onwards and MDH-1 (mitochondrial form) from pupa onwards. The genetic

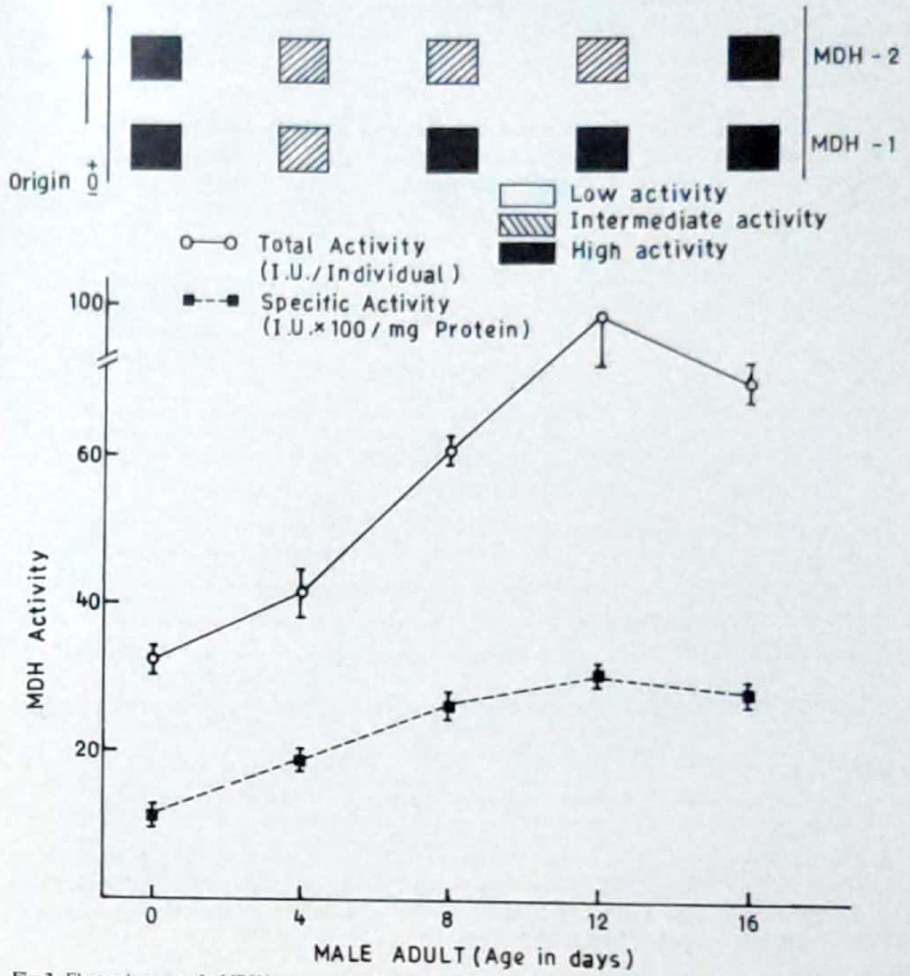

Fig. 3: Electropherogram for MDH isoenzymes and its total and specific activity in ageing male *A. stephensi*.

basis for this phenotypic variation may be due to differential regulation of separate genes. Like that in *Drosophilla* (O'Brien & Macintyre 1978), in *A. stephensi* also, the m-MDH and c-MDH forms seem to be controlled by different structural genes. The findings of separate genetic control for the 2 forms seem to be the rule as has been found in other species of diverse phylogenetic origin also

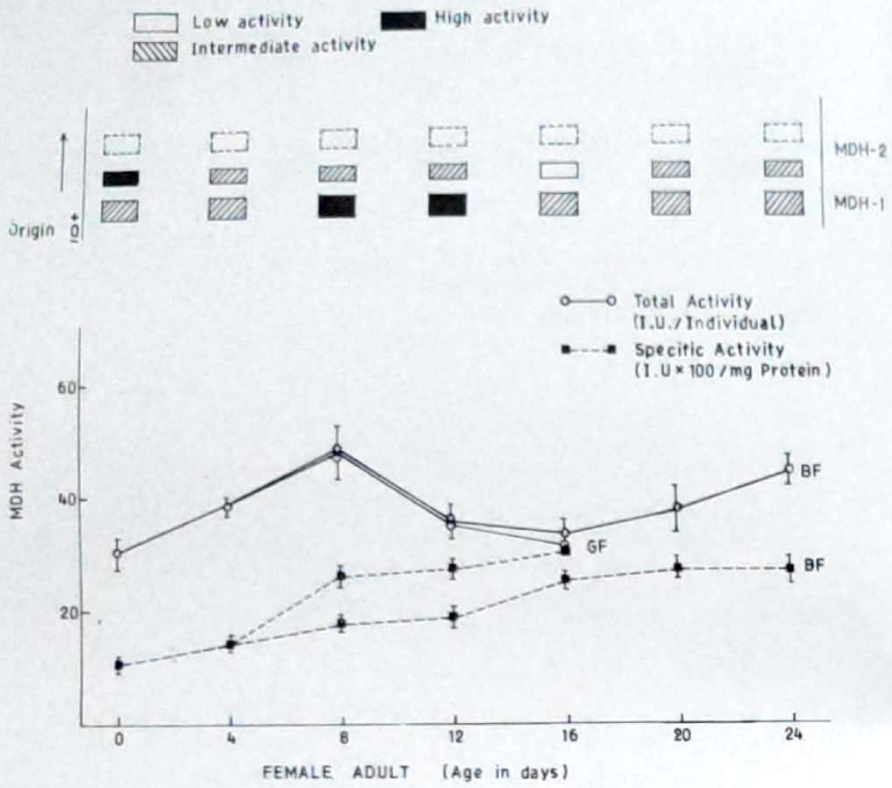

Fig. 4 : Electropherogram for MDH isoenzymes and its total and specific activity in ageing female *A. stephensi.*

viz., maize (Yang et al. 1977), mice (Shows et al. 1970) etc. However, the appearance of an additional isozyme band-c in MDH-2 zone in female mosquitoes only could be attributed to the epigenetic modifications. Further experiments concerning breeding and molecular genetics are in progress to confirm the above preposition.

The appearance of mitochondrial form from pupa onwards reflects the adaptive effort as an evolutionary necessity for the complete change from aquatic to the terrestrial habitat. This isoenzyme may be more suited for the high energy reactions in the mitochondria required for active adult life. The high level of activity in larvae as compared to other dehydrogenases (Vandana 1992) indicates the overall need for energy during this active growth phase.

The continuous increase in activity during metamorphosis also finds the support from Mukiama (1980) in *Ae. aegypti.* On the other hand, a U-shaped pattern was found in *Tenebrio*

molitor (Ludwig & Bursa 1958). Narang & Narang (1975) also found the maximum activity during the adult *Cx. quinquefasciatus.* The higher activity in male mosquitoes could be due to their high energy requirements to lead more active life. Interestingly, there was no significant difference in the activity when the females were fed on blood and glucose separately.

It could be far more advantageous for the adult insect to metabolise the pyruvate and NADH via mitochondria than utilize the LDH reaction to malate (Gakhar & Vandana 1994). Most probably, the malate shuttle takes over the available pyruvate and NADH, thus effectively competing with LDH reaction. This may explain the low activity of lactate dehydrogenase in adults (Vandana 1992).

The developmental profile of MDH is not the same as of *Aedes* (Mukiama 1980). General similarities do occur and generalizations have been suggested. However, dehydrogenase activity profiles during ontogenesis are most likely species-specific.

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KARYOMORPHOLOGICAL STUDIES IN *ALLIUM SATIVUM*

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SUMMARY

Karyomorphological studies have been carried out on 4 accessions of *Allium sativum* Linn. They are diploids with 2n=16. Heteromorphic pairs were observed in the eighth chromosome of accessions 2 and 4. Sattellited chromosomes were noticed in the second and fifth chromosomes of accession 4, whereas, it is absent in the other 3 accessions. The presence of satellited chromosomes in the second and fifth chromosomes of accession 4 is a deviation from the earlier reports. The absence of satellites in other accessions might be due to deletion of chromosome segment carrying satellite.

Key words : *Allium sativum*, karyotype, satellited chromosome.

INTRODUCTION

Like all species of *Allium*, *A. sativum* Linn. (garlic) has a very favourable chromosomes inviting detailed studies. But little work has been done especially in comparison with the classical chromosome material of *A. cepa*. However, a few investigators have made cytological studies in *A. sativum* from various geographic regions and found that different clones of this species differ in their karyomorphology (Levan 1935, Mensinkai 1939, Battaglia 1963, Ved Brat 1965, Konvicka & Levan 1972). The present paper embodies the results of detailed karyomorphological studies in 4 accessions of *A. sativum*.

MATERIALS AND METHODS

The bulbs of 4 clones of *A. sativum* were collected from Bangalore (Acc.1), Delhi (Acc. 2), Mysore (Acc. 3) and Thiruchirappalli (Acc. 4). Healthy root tips were excised, washed with water and pretreated separately with 0.002 M aqueous solution of 8-hydroxyquinoline (Tjio & Levan 1950) and kept at 12-16 °C overnight. Treated root tips were thoroughly washed with tap water and fixed in 3:1 ethyl alcohol and glacial acetic acid. A trace of ferric acetate added to the fixative prior to staining considerably increased the stainability of the chromosomes. Acetocarmine (2%) squash technique was followed for mitotic preparation. For chromosome morphology and classification, the system proposed by Abraham & Nagendra Prasad (1983) was followed.

OBSERVATIONS

The somatic complement of all clones agree in having median or submedian chromosomes. The details of individual chromosomes of the 4 accessions vary.

Accession 1

The bulbs had medium-sized cloves with white coats. The root tip cells have 16 chromosomes in each (Figs. 1,5). The longest pair is metacentric, the rest are all submetacentric. the

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TABLE 1 : Chromosome metrics in 4 accessions of *Allium sativum*.

Acc. No.	Characters	Chromosome pairs 1	2	3	4	5	6	7	8
1	AL	16.60	16.60	13.30	13.30	13.20	11.60	11.60	8.30
	LA	8.30	8.30	8.30	8.30	6.60	6.60	8.30	5.00
	SA	8.30	8.30	5.00	5.00	6.60	5.00	3.30	3.30
	AR	1.00	1.00	1.66	1.66	1.00	1.32	2.51	1.51
	CP	M	M	nm	nm	M	nm	nsm	nm
2	AL	14.90	13.20	13.20	11.60	11.60	11.60	9.90	8.30 6.60
	LA	8.30	6.60	6.60	6.60	6.60	6.60	6.60	5.00 5.00
	SA	6.60	6.60	6.60	5.00	5.00	5.00	3.30	3.30 1.60
	AR	1.25	1.00	1.00	1.32	1.32	1.32	2.00	1.51 3.12
	CP	nm	M	M	nm	nm	nm	nsm	nsm
3	AL	13.20	13.20	13.20	13.20	11.60	11.60	11.60	9.90
	LA	6.60	6.60	6.60	6.60	6.60	6.60	6.60	6.60
	SA	6.60	6.60	6.60	6.60	5.00	5.00	5.00	3.30
	AR	1.00	1.00	1.00	1.00	1.32	1.32	1.32	2.00
	CP	M	M	M	M	nm	nm	nm	nsm
4	AL	19.80	16.60	16.60	16.60	16.60	14.90	13.20	11.60 6.60
	LA	13.20	10.00	8.30	10.00	8.30	8.30	6.60	6.60 3.30
	SA	6.60	3.30 3.30	8.30	6.60	5.30 0.30	6.60	6.60	5.00 3.30
	AR	2.00	1.51	1.00	1.51	1.00	1.25	1.00	1.32 1.00
	CP	nsm	nm*	M	nm*	M	nm*	M	nm*

AL=Absolute length; LA=Long arm; SA=Short arm; AR=Arm ratio; CP=Centromeric position.

Accession 4

The bulbs had medium-sized cloves. 16 chromosomes were seen at metaphase (Figs. 4,8). The length of the chromosomes varied from 19.8 to 6.6 μm (Table 1). The second and fifth pair possessed *sativum*-type satellites in their short arm. These pairs also differed in arm ratios and sizes of satellites and of the chromatin segments between primary and secondary constrictions. The eighth pair was heteromorphic chromosome. The TCL and ACL were estimated to be 132.5 μm and 14.72 μm respectively. The length of the chromosomes varied from 19.8 μm to 6.6 μm.

DISCUSSION

Karyotypes in all cases indicate a common general pattern in their morphology proving that they form a homogenous assemblage originating from a single basic set (x=8). This agrees with the earlier reports of Levan (1935), Mensinkai (1939), Khoshoo et al. (1960), Battaglia (1963), Kovicka & Levan (1972), Howevver, on critical analysis the accessions of the present study show distinct karyotypic differences. Even though the different accessions share a few characters in common each has its own distinctiveness with respect to the morphology of certain individual chromosomes.

On analysis, each accession is different from the other in respect of chromosome types, TCL content and ACL value (Table 1). On the basis of centromeric position, accession 3 possesses more of symmetrical chromosomes followed by accessions 1, 4 and 2 (Table 1).

The lowest value of ACL is observed in accession 2 (11.21 μm) whereas, the highest in accession 4 (14.72 μm). The accessions also differ in their TCL content, lowest in accession 3 (97.5 μm) whereas the highest in accession 4 (132.5 μm) (Table 1).

The difference in TCL content may be possible due to the chromosomal aberrations possibly of deletion. The deletion of comparatively inert chromatin segments, the heterochromatin parts, might have occurred in nature resulting in diminution of the chromosome size. It is suggested that the reduction in the total TCL content observed in accession 3 might be due to deletion of heterochromatin from the complement. The genetic and evolutionary significance of heterochromatin has also been reported by Darlington & Mather (1950).

The secondary constriction in the chromosome serves as good marker which enables to understand the morphology of the chromosomes. Out of 4 accessions of *A. sativum* under study, secondary constriction in the short arm has been noted in accession 4. the second and fifth chromosomes in accession 4 possesses satellites. They differ in the size of short arm, chromatin segments between primary and secondary constrictions and also in the size of long arm. Normally the sixth and seventh chromosomes bear secondary constrictions on the short arms (Sharma et al. 1988, Konvicka & Levan 1972, Verma & Mittal 1976). All of them conform to the *sativum*-type of nucleolar chromosomes. The 2 nucleolar pairs differ in arm ratio, size of satellites and of chromatin segments. In the present study the second and fifth chromosomes possess satellites in accession 4. This shifting of satellites from sixth and seventh chromosomes to second and fifth chromosomes may be due to translocations. Satellited chromosomes show much more variation in arm ratio than the other chromosomes. They are liable to greater hazards leading to breaks and translocations. But, the accessions 1, 2 and 3 do not bear any satellites. The absence of satellites in these accessions may be due to deletion of satellites and subsequent loss during evolution. Satellites do not contain functional genes and hence these accessions survive in nature. Battaglia (1963) and Ved Brat (1965) also suggested that in *Allium* the origin of the variations in the morphology and its structural heterozygosity has been presumed to be due to the susceptibility of the nucleolar chromosomes to natural mutations.

Heterozygosity has been noticed among homologous chromosomes in accessions 2 and 4. Heterozygosity in nucleolar chromosomes is due to difference in length of the arms of the chromosomes. This may be possibly due to deletion in the satellite region. But the possibility of

unequalness of the homologue due to the difference in condensations cannot be completely ruled out as has been previously reported by Verma & Mittal (1978).

From the analysis of the nature and degree of karyomorphological difference in respect of different chromosomes of different accessions of *A. sativum* investigated here it appears to indicate that notable degree of chromosomal structural changes like translocations and/or pericentric inversions have operated during the course of evolution of the clones and structural alterations of chromosomes are powerful tools of evolution particularly among the vegetatively reproducing taxa.

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NON-HOMOLOGOUS ASSOCIATION OF PACHYTENE CHROMOSOMES IN SOME SPECIES OF MOSQUITOES

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SUMMARY

A comparative study of non-homologous association of pachytene chromosomes of 17 species has been made. Based on the extent and nature of non-homologous association they were classified into 3 groups: star-like, flanking and bouquet-like. Star-like and flanking types are prevalent in anopheline mosquitoes, whereas bouquet like configuration is evident in culicine mosquitoes.

Key Words: Pachytene chromosomes, centromeres, telomeres, association.

INTRODUCTION

The occurrence of non-homologous meiotic chromosome associations and the tendency of their coaseling at the centromere region to form a common chromocentre during earlier meiosis has been reported for a few animal and plant species (de Jong & Stam 1985, John 1976, La Cour & Wells 1970). In recent years, it has been implied that these associations are generally produced through heterochromatic segments located at the centromeric and paracentromeric regions (Drets et al. 1983, Driscoll et al. 1979) or at the telomeric regions resulting in chain-like associations or bouquet configurations (Ashley 1979, Sadasivaiah & Kasha 1971).

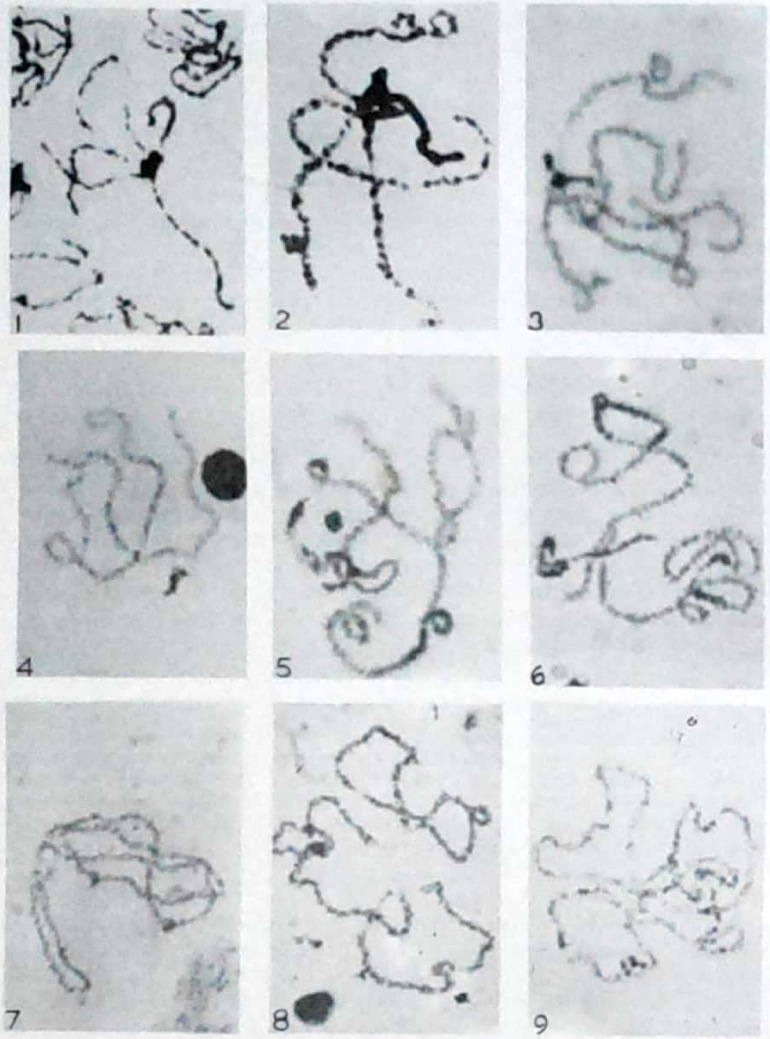
During the earlier studies on pachytene chromosome mapping for some mosquito species we came across the occurrence of non-homologous association of pachytene chromosomes either at centromeric or at telomeric region (Venkatachalaiah 1992, Chowdaiah & Venkatachalaiah 1989). In the present paper, observations made on heterologous association of pachytene chromosomes for each species are pooled and categorized based on their extent of association within the complement.

MATERIALS AND METHODS

The list of species analysed is cited elsewhere (Venkatachalaiah 1994). The hypotonic prefixation treatment and air-drying methods used in this study was followed by the modified technique of Hungerford (1971) for the preparation of pachytene chromosomes and these preparations were subjected to conventional Giemsa staining (Chowdaiah & Venkatachalaiah 1987).

OBSERVATIONS

Prolonged hypotonic pretreatment yielded several well spread pachytene cells. A pachytene cell contains 3 bivalents of variable length. A typical chromocentre was observed in pachytene complement of some anopheline species while telomeric associations resulting in bouquet-configurations was a common feature of culicine mosquitoes. In most of anopheline pachytene complement, the shortest bivalent is intensely heterochromatic and is known as sex-bivalent. The paracentromeric regions of the 2 autosomal bivalents are also distinctly heterochromatic in comparison with the chromomeric arms. No such heterochromatic stainability can be seen in culicines.



Figs. 1-9 : Representative Giemsa-stained pachytene chromosome complement of mosquitoes. 1. *Anopheles fluviatilis*. 2. *An. culicifacies*. 3. *An. subpictus*. 4. *An. aitkenii*. 5. *An. stephensi*. 6. *An. vagus*. 7. *Culex quinquefasciatus*. 8. *Aedes albopictus*. 9. *Ae. aegypti*.

In anophelines there are several modes of non-homologous associations of pachytene bivalents during the earlier meiotic stages. However, the occurrence of typical chromocentric formation is of special interest. These heterologous associations appear first in late zygotene and could be seen up to early diplotene. They are most prevalent at pachytene. At the typical pachytene stage, about 30-50% of pachytene cells of most anophelines exhibit complete association resulting in star-like configurations of heterochromatically coascled chromocentre formation with bivalent arms projecting out. During the later periods of pachytene, the fused connections start to detach resulting into 3 synapsed components (Figs.1-9).

In culicine species, the telomeric association of the 3 pachytene bivalents with a tangled association is a common feature. As they start condensing the typical bouquet picture emerges with the telomeric region beginning to segregate and orient towards one pole and the centromeric regions towards the other pole.

Depending upon the extent and nature of heterologous association in the complement, they are classified into 3 types: 1) star-like configuration, 2) flanking type and 3) bouquet type.

Star-like configuration is characterised by the presence of complete fusion of all 3 pachytene bivalents at the centromere region with 5 arms projecting out. Such configuration was commonly observed in the pachytene complements of *Anopheles fluvatilis, An. culicifacies, An. jeyporiensis* and *An. aconitus* all belonging to series Myzomia which belong to subgenus *Cellia* of genus *Anopheles* (Figs.1-3).

Flanking type is characterized by various modes of association at centromeric region of 3 bivalents in the complement. In a typical situation, 2 bivalents are associated completely at the centromere whereas the third one tends to lay near the other fused centromeres. This type was evident in species belonging to subgenus *Anopheles*; viz., *An. nigerrimus, An. aitkenii* and *An. gigas*. In some others, in pachytene complement the centromere of each bivalent tends to stay nearer to each other within a very narrow circular range. This type was evident in the case of anophelines belonging to neomyzomyia series of subgenus Cellia, i.e. *An. leucospyrus elegans* and *An. tessellatus*. However, while in some, the centromeres of each bivalent tends to lay on the outer circular range. This type was prevalent in the species belonging to series Pseudomyzomayia and Neocellia of the subgenus Cellia: *An. stephensi, An. annularis, An. pallidus, An. vagus* and *An. subpictus* (Figs.4-6).

Bouquet type is characterized by the complete telomeric association of the 3 bivalents. This type was seen in culicine mosquitoes: *Aedes albopictus, Ae. aegypti* and *Culex pipiens quinquefasciatus* (Figs.7-9).

DISCUSSION

An analogous situation in somatic and in meiotic chromosomes of some animal and plant examples has been reviewed in the light of new staining techniques by Yunis & Yasminch (1971), Yoon & Richardson (1978) and in a more comprehensive manner by Carpenter (1987). They have considered several other related aspects including the role of C-heterochromatin in such aggregation effect. A more detailed analyses of this decisive factor has been attempted in

Drosophila melanogaster females by Novitski & Puro (1978). Davring & Sunner (1975) and Nokkala & Puro (1976) demonstrated cytologically in *D. melanogaster* oocytes, the occurence of chromocentre during early meiosis. Driscoll et al. (1979) have studies non-homologous chromosome association and the influence of C-heterochromatin at human male meiotic prophases and imply of the influence of C-heterochromatin in the chromosome aggregation.

The results of present study pertaining to some anopheline examples in which the non-homologous chromosomes association at centromere resulting in incomplete or complete chromocentre formation at pachytene stage confirm the hypothesis of Novitski & Puro (1978). As opined earlier about the specific pairing of non-homologous chromosomes and of ectopic pairing observed in the salivary gland nuclei of certain dipterans (Barr & Ellison 1972, Cohen 1976, Mittal & Dev 1979). Based on these observations it is possible to ascribe these features due to some inherent biochemical property. Such commonness might facilitate in the cytological expression of flanking effect, as proposed by Driscoll et al. (1979).

The formation of bouquet configuration due to the telomeric association of the pachytene bivalents resulting in the telomeric and kinetochore polar clustering is highly specific to culicine group of mosquitoes. In the moderately condensed pachytene complements, due to telocentric connections, the bivalents appear as tangled bivalents held at the telomere region resulting in chain-like orientation. The present finding on culicine pachytene complements are also in agreement with observations made on both polytene and pachytene synaptonemal complex karyotypes (Fill 1978, Wandall & Svendsen 1983). Less information is available on possible association between non-centromeric regions. Ashley & Wagenaar (1974) in plants, Miklos & Nankivell (1976) in grasshoppers, Kirshc-volders et al. (1980) in humans reported specific telomeric associations. More recent studies on the molecular nature of these regions have revealed their possible existence of 2 major types of repetitive sequences: satellite-like, short, tandemly repeated sequences and more complex, moderately repetitive sequences (Biessmann & Mason 1992, Kenton 1991).

Pertaining to the results of the present study, it is possible to imply that in anophelines those of the postulated sequences are congregating in and around centromeric regions and in culicines they could have been distributed individually each into centromere-specific and telomere-specific sites.

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A PRELIMINARY ANALYSIS OF GENETIC POLYMORPHISM IN THE GENUS *CYAMOPSIS* (FABACEAE) USING ISOZYME AND RIBOSOMAL DNA MARKERS

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SUMMARY

Guar bean (*Cyamopsis tetragonoloba*) is a legume which is an economically important minor crop. The origin and domestication of the species have not been well understood. We analyzed the genetic variation in the 3 species of the genus using isozyme and ribosomal DNA markers. Our preliminary results did not show any clear pattern of relationship among the species. DNA markers are possibly more reliable than the isozymes.

Key Words: *Cyamopsis*, isozymes, ribosomal DNA, polymorphism.

INTRODUCTION

The genus *Cyamopsis* is represented by three species. *C. serrata* and *C. senegalensis* are of African origin and occur in east and southwest Africa. *C. tetragonoloba* is known only as a cultivated species mainly from the Indian subcontinent. It is grown as minor crop and is known as "Cluster bean" or "guar bean". Since the second World War, it is also being grown in experimental gardens in parts of Europe and the USA. The endosperm of the seed is a source of gum which is being extensively used in paper, textile and food industries. There is little information on the origin and domestication of *C. tetragonoloba*.

The use of isozyme markers in evolutionary studies has been well-documented (Liu & Furnier 1993). Eukaryotic ribosomal DNA (rDNA) exists as tandemly repeated clusters of long and similar repeating units. Each array includes a highly conserved coding region and a non-coding region. The non-coding region is also called the intergenic spacer region (IGS) since it separates 2 adjacent repeat units. rDNA polymorphisms arise mainly as a result of variation in the number of subrepeats in the IGS (Rogers & Bendich 1987). Length variants in the IGS are called spacer length variation and have been successfully used in recent years in a number of different species(for review, see Knaak et al. 1990). In the present study, we present some information on the genetic varition in the 3 species of the genus *Cyamopsis* using isozyme and ribosomal DNA markers.

MATERIALS AND METHODS

Four genotypes of *C. tetragonoloba* and 2 accessions of each of *C. serrata* and *C. senegalensis* were selected for the study. Seeds of *C. tetragonoloba* were collected in India and those of the other 2 species were obtained from the USDA, Beltsville, Maryland, USA. Plants were grown in the greenhouse of the University of Calgary.

Starch gel electrophoresis, as outlined by Soltis et al. (1983), was followed for the isozyme studies. Nine enzyme systems, namely, aminoacyl transferase (AAT), aconitase (ACO), isocitrate dehydrogenase (IDH), leucine amnio peptidase (LAP),

malate dehydrogenase(MDH), phospho glycero isomerase (PGI) and phospho glucodehydrogenase (PGD), phospho gluco mutase (PGM) and shikimate dehydrogenase (SKDH) were studied.

Genomic DNA from the three species was extracted using a modified CTAB procedure (Ramamoorthy et al. 1994). Five restriction enzymes, namely, Bam HI, Eco RI, Eco RV, Hind III and Dra I were used to restrict the genomic DNA. Approximately 10µg of the genomic DNA was digested with 10 units of restriction enzyme at 37°C overnight. Nylon membranes from Amersham were used for Southern blotting and was carried out overnight according to the alkaline transfer method of Reed & Mann (1985). Membranes were baked at 100°C for an hour after a brief UV cross-linking.

rDNA from soybean (*Glycine max*) was used as probe and a non-radioactive labeling and detection procedure using Digoxygenin-dUTP (Boehringer-Mannheim) was followed. Labeling, hybridization and detection were done using the random primer labeling kit according to manufacturer's recommendations. Restriction fragment polymorphisms were visualized using Lumigen™ PPD (Boehringer-Mannheim) within few hours. Shannon-Weaver diversity values (Bowman et al. 1971) were calculated using the isozyme and ribosomal DNA phenotypic frequency data from the three species.

RESULTS AND DISCUSSION

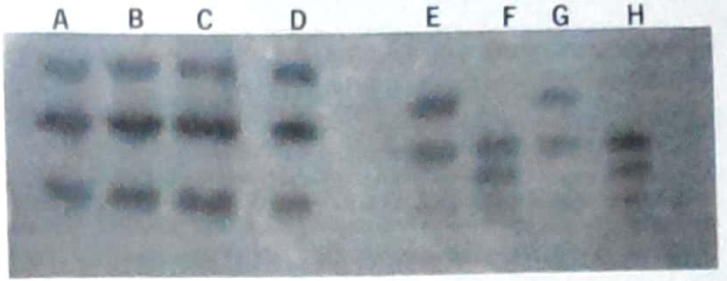
Five of the 9 isozyme systems were polymorphic in the 3 species of *Cyamopsis*. Maximum variation was observed for PGD followed by MDH and LAP. Shannon-Weaver diversity values for different enzymes and different species are presented (Table 1). Fig. 1 shows the PGI pattern of *C. tetragonoloba, C. senegalensis* and *C. serrata*. Overall, *C. tetragonoloba* exhibited maximum diversity followed by *C. senegalensis*.

TABLE 1: Shannon-Weaver diversity values using isozyme phenotypic frequencies in *C.tetragonoloba, C.senegalensis* and *C.serrata*.

Species/Enzyme	PGD	PGI	LAP	PGM	MDH	OVERALL
C. *tetragonaloba*	0.6	0.5	0.6	0.0	0.4	1.04
C. *selegalensis*	0.5	0.3	0.3	0.3	0.5	0.69
C. *serrata*	0.3	0.3	0.3	0.0	0.5	0.44

Fig. 2 shows the rDNA pattern of the 3 species after digestion with 5 restriction enzymes and hybridization with soybean rDNA probe. Table 2 summarizes the restriction fragment patterns of *C. tetragonoloba, C. senegalensis* and *C. serrata*. Of the 3 species, *C. senegalensis* exhibited variation for 2 of the 5 enzymes whereas in *C. tetragonoloba* and *C. serrata*, variation was observed only for one of the 5 enzymes.

Isozyme data suggests that *C. tetragonoloba* is the most variable followed by *C. senegalensis*. Results from rDNA data show that *C. senegalensis* is the most variable species of the 3. Overall, our preliminary results show that the 3 species are not related to each other. A comprehensive study using more number of probes as well as a new marker such as random amplified polymorphic DNA (RAPD), is under way to understand the evolutionary relationships between the 3 species of *Cyamopsis*.



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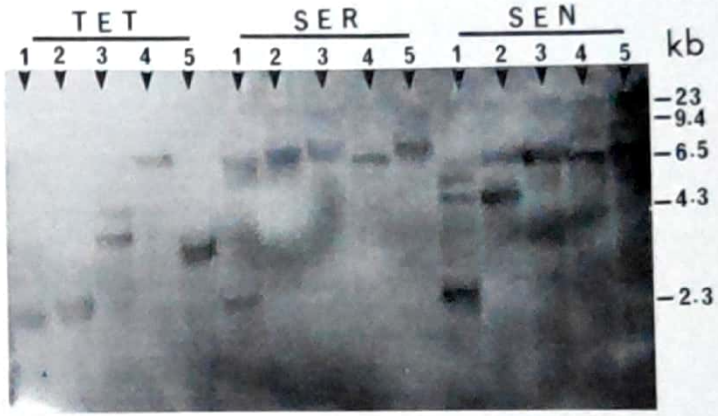

2

Figs. 1 & 2: 1. Starch gel electrophoretic banding patternss of PGI in *Cyamopsis*. Lane A-D represent accessions of *C. tetragonoloba*, lanes E and G - *C. serrata* and lanes F and H represent *C. senegalensis*. 2. rDNA restriction fragment length polymorphism in three species of *Cyamposis* after hybridization with soybean rDNA probe. TET denotes *C. tetragonoloba*, SER-*C.serrata*, and SEN- *C.senegalensis*. Lane 1 for each species represents Bam HI, Lane 2 - Eco RI, Lane 3 - Eco RV, Lane 4 - Hind III and Lane 5 - Dra I.

TABLE 2 : Restriction fragment data for rDNA in *C.tetragonoloba, C.senegalensis* and *C.serrata*.

Restriction enzyme	*C.tetragonoloba*	*C.serrata*	*C.senegalensis*
Bam HI	2.0 kb	2.2 kb	2.4 kb
		5.8 kb	4.3 kb
			5.3 kb
Eco RI	2.1 kb	6.5 kb	4.3 kb
			6.5 kb
Eco RV	3.3 kb	7.5 kb	6.5 kb
	3.8 kb		
Hind III	5.8 kb	6.5 kb	6.5 kb
Dra I	2.9 kb	7.5 kb	7.5 kb

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CHROMOSOME NUMBERS OF SOUTH INDIAN CUCURBITACEAE AND A NOTE ON THE CYTOLOGICAL EVOLUTION IN THE FAMILY

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SUMMARY

Chromosome numbers of 41 taxa belonging to 33 species in 19 genera of Cucurbitaceae are determined. The chromosome counts of *Trichosanthes anaimalaiensis* (n=11, 2n=22), *T.nervifolia* (n=11), *Gymnopetalum wightii* (n=12) and *Momordica denudata* (n=14) are reported for the first time. The family is found to have several basic numbers such as, x=7,8,9,11,12,13,14,15,16 and 20 of which x=12 is the most frequent number. It is suggested that x=14 is the original basic number of the family, derived from x=7 through tetraploidy. Other basic numbers, x=13, 12 and 11 are evolved from x=14 by aneuploid reduction and x=15 and 16 by aneuploid increase. Basic number x=7 in *Cucumis sativus* is the outcome of reversion to the diploid level. Highest basic number x=20 is suggested as secondary polyploid. Among the cytologically known members 26% are polyploids, of which 75% are tetraploids. Infraspecific polyploidy has been encountered in *Trichosanthes bracteata* and *Solena amplexicaulis*. A positive correlation between polyploidy and vegetative reproduction is suggested. Incidence of aneuploidy is met in species of *Cucumis, Momordica* and *Mukia*. In *Sechium* infraspecific aneuploidy is indicated.

Key Words : Cucurbitaceae, cytology, basic number

INTRODUCTION

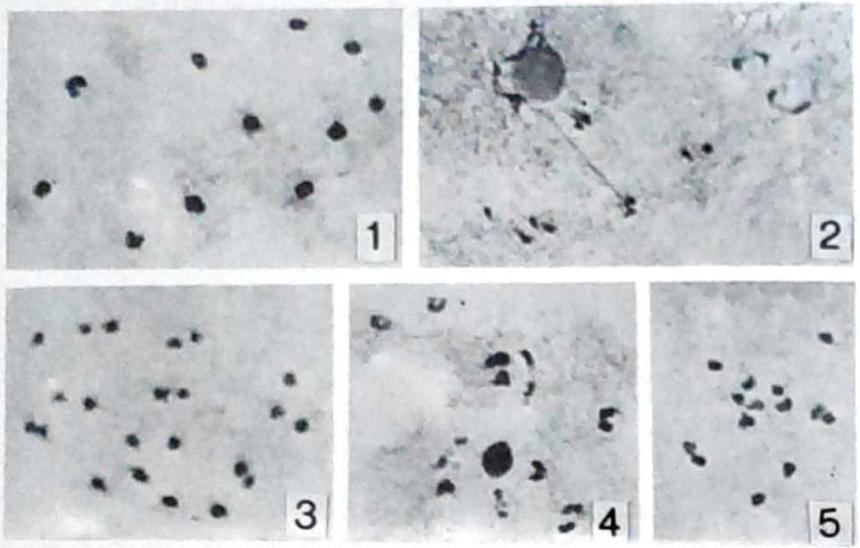
The family Cucurbitaceae is a moderately large family with 108 genera and 825 species (Jeffrey 1980, 1990). In South India, Cucurbitaceae is represented by 52 species under 24 genera (Chakravarthy 1982). Considerable amount of investigation has already been done on the chromosomal analysis and inter-relationships between various taxa of the family. But there are differences of opinion regarding the origin of basic numbers and their evolution. The total chromosome number reports available accounts for 141 species in 41 genera. This includes the chromosome determination in species belonging to 20 genera from South India. The present paper reports the cytology of 33 species belonging to 19 genera from South India of which four are new chromosome counts. The aim of the study is to review and analyse the chromosomal information in order to have an insight into the evolutionary processes operative during the cytological evolution of the family.

MATERIALS AND METHODS

Materials for the present study were collected from different localities of Kerala and Tamil Nadu. In the case of a few cultivated species seed materials procured from a seed firm, Pocha seeds, Poona were also utilized. The materials for cytological studies were fixed in 3:1 ethanol-acetic acid mixture. Two per cent acetocarmine was used for both smear and squash preparations.

OBSERVATIONS

Chromosome counts of 41 taxa belonging to 33 species under 19 genera carried out in this study are listed in Table 1.



Figs.1-5: 1. *Trichosanthes nervifolia*, PMC showing 11 bivalents at metaphase I (x 1500). 2. *T. anaimalaiensis*, PMC showing 11 bivalents at diakinesis (x 1000). 3. *T. anaimalaiensis*, somatic chromosomes (2n=22) (x 1500). 4. *Gymnopetalum wightii*, PMC showing 12 bivalents at diakinesis (x 1000). 5. *M. denudata*, PMC showing 14 bivalents at metaphase I (x 1300).

TABLE 1 : Chromosome numbers of different taxa of the Cucurbitaceae.

Species	Locality	Chromosome number		
		n	2n	
Trichosanthes anguina L.	Trivandrum	11	22	
T. cucumerina L.	Quilon	11	22	
**T. nervifolia* L.	Upper Kodayar	11	-	(Fig.1)
T. lobata Roxb	Upper Kodayar	11	-	
T. bracteata var. *bracteata*				
Cytotype 1	Trivandrum	11	22	
Cytotype 2	Trivandrum	22	-	
**T. anaimalaiensis* Bedd.	Braemore	11	22	(Figs.2&3)
**Gymnopetalum wightii* Arn.	Nelliampathy	12	-	(Fig.4)
Lagenaria siceraria (Mol.) Standl.	Trivandrum	11	-	

(Table 1 Contd.)

Table 1 : (Concluded)

Luffa acutangula var. *acutangala*	Trivandrum	13	-	
L. acutangula var. *amara* (Roxb.) C.B.Cl.	Nelliampathy	13	-	
L. cylindrica var. *minor* Chakravarty	Coimbatore	13	-	
Benincasa hispida (Thunb.) Cogn.	Pocha seeds, Poona	12	-	
Momordica charantia L.	Trivandrum	11	22	
M. dioica Roxb. ex willd	Adimali	14	-	
M. cymbaalaria Hook.f.	Tirunelveli	11	-	
**M. denudata* (Thwaites) C.B.Cl.	Sherthalai	14	-	(Fig.5)
Cucumis pubescens Willd.	Tirunelveli	12	-	
C.trigonus Roxb	Tirunelveli	12	-	
C. melo var. *melo*	Pocha seeds, Poona	12	24	
C. melo var. *agrestis* Naud.	Pocha seeds, Poona	12	24	
C. sativus L.	Pocha seeds, Poona	7	14	
C. sativus var. *hardwickii* (Royle) Alef.	Ponmudi Hills	7	14	
C. prophetarum L.	Sholayar	-	24	
Citrullus colocynthis (L) Schrad.	Ambasamudram	11	-	
C. lanatus (Thunb.) Mats & Nakai	Pocha seeds, Poona	11	-	
Praecitrullus fistulosus (Stocks.) Pangalo	Pocha seeds, Poona	12	24	
Coccinia grandis (L.) Voigt	Trivandrum	12	-	
Cucurbita maxima Duch. ex lam.	Trivandrum	20	-	
Diplocyclos palmatus (L.) C.Jeffrey	Trivandrum	12	-	
Mukia maderaspatana (L.) M.J.Roem	Trivandrum	12	-	
Zehnaria maysorensis				
Var. 1	Peechi forest area	24	48	
Var. 2	Braemore	24	-	
Solena amplexicaulis (Lam.) Gandhi				
Cytotype 1	Trivandrum	12	24	
Cytotype 2	Vadakkankulam	24	-	
Kedrostis foetidissima (Jacq.) Cong.	Vadakkankulam	13	26	
Corallocarpus epigaeus (Roettl) C.B.Cl.	Coimbatore	1326		
Ctenolepis garcinii (Burm.f.) C.B.Cl.	Palayamkottai	12	-	
Sechium edule (Jacq.)SW.				
Cytotype 1	Upper Kodayar	13	-	
Cytotype 2	Ponmudi Hills	-	28	

* New chromosome counts

DISCUSSION

The available data on chromosome numbers in the family reveal the presence of basic numbers x-7,8,9,11,12,13,14,15,16 and 20 (Darlington and Wylie 1955, Ayyangar 1967, Federov 1969, Moore 1970, Goldblatt 1981, 1984, 1985, 1988, Virendra Kumar and Subramanian 1986). It is found that most of the genera are monobasic, while *Cucumis* (x=7,12), *Ibervillea*, *Mukia* and *Gymnopetalum* (x=11,12), *Cyclanthera* (x=8,11), *Momordica* (x=11,14) and *Corallocarpus* (x=12,13) are dibasic. An analysis of the frequency of various basic numbers among the genera and species of the family reveals that x=12 is the most prevalent basic number in the family, occurring in 41% of the species followed by x=11 which occurs in 22.7%. Other basic numbers occur in a low

percentage of the genera. The lowest basic number x=7 is found only in one genus *Cucumis* along with x=12 and the highest number x=20 is found in species of *Cucurbita*.

Various suggestions have been made with regard to the origin and evolution of basic numbers of the family. Thakur et al. (1969) and Thakur & Sinha (1973) suggested x=11 as the most primitive basic number whereas Varghese (1973) considered x=5 as the ancestral basic number of the family. However, Trivedi & Roy (1970), Singh & Roy (1974), Sen (1976), Sen & Datta (1978) and Yadava, Singh & Arya (1984) opined x=12 as the primitive basic number from which the other basic numbers have evolved. According to Roy et al. (1983) and Sinha et al. (1983) x=11 and 12 are the most widely represented numbers from which the other numbers have evolved. Stebbins (1967) and Ehrendorfer et al. (1968) have suggested that x=7 is the original basic number of the angiosperms. After reviewing the chromosome numbers in Cucurbitaceae, Raven (1975) indicated x=14 or x=13 might be the original basic chromosome number of the family and that these numbers in turn have originated from x=7, the base number of the order Violales by tetraploidy and aneuploidy preceeding the origin of the family. The occurrence of x=14 in three genera viz., *Dimorphochlamys, Apodanthera, Sechium* and x=14 and 11 in *Momordica* and the presence of other combinations of aneuploid basic numbers such as x=13 and 12 and x=12 and 11 in at least four other genera of the family indicate that, basic numbers x=13, 12 and 11 seen in 71.2% species are derived from the original basic number x=14 by stepwise dysploid reduction.

There are conflicting views regarding the origin of basic numbers x=7 and 12 in the genus *Cucumis*. Whitakar (1933), Bhaduri & Bose (1948) and Ayyangar (1967) have suggested that x=12 in *Cucumis* has derived from x=7 by fragmentation whereas Trivedi & Roy (1970), Singh & Roy (1974) and Sen & Datta (1978) considered that the basic number x=7 has evolved from x=12 by fusion of chromosomes. Ramachandran & Sheshadri (1986) refused both interpretations. In their opinion the two subgenera are not closely related phylogenetically because of differences in number, size, organization and behaviour of chromosomes and geographical distribution. The diversity in origin of subgenus *Cucumis* (x=7) in India and the subgenus *Melo* (x=12) in Africa (de Candolle 1882, Purseglove 1968, Jeffrey 1980, Zeven & de Wet 1982) points to the possibility of their different evolutionary pasts. It is therefore likely that subgenus *Cucumis*, endemic to the Indian subcontinent has reverted to the diploid level from the original polyploid base number x=14. The subgenus *Melo* with x=12 has evolved from x=14 by aneuploid reduction. The reversibility of tetraploids to diploid cytological condition is an established phenomenon in nature (Ornduff 1960, Raven & Thompson 1964, de Wet 1971). It is through the derivation of functional diploids from polyploids as a result of parthenogenetic development of unfertilized gametes. De Wet (1971) and Harlan & De Wet (1975) after reexamining the possible evolutionary implication in the light of a few described cases of polyhaploidy established the evolutionary potential of such taxa formed by reversion of tetraploidy.

The occurrence of gametic numbers n=8 and n=11 in *M. cymbalaria* besides the gametic numbers n=14 and n=11 in other species of *Momordica* strongly suggests that x=9 and 8 are part of the aneuploid reduction series from x=14. The origin of low basic numbers through stepwise reduction from high chromosome numbers is a common feature in polyploids (Jones 1970), The basic numbers x=15 and x=16 reported in *Zanonia* and *Gomphogyne* respectively might be the result of

aneuploid increase from x=14. Moreover *Zanonia* is a genus of only two species and *Gomphogyne* is monotypic. This indicates that they are not the starting point of any fervent speciation. This would suggest an aneuploid rather than hybrid origin of the basic numbers x=15 and x=16. The highest base number x=20 observed in *Cucurbita* is in all probability a secondary polyploid derived from the aneuploid basic number x=10.

Of the cytologically known genera only 26% are polyploids. Moreover majority of polyploids are tetraploids. Polyploids based on the lowest haploid numbers above the tetraploid level are met with only in the genera *Trichosanthes* and *Cucumis*, the highest chromosome number reported in the Cucurbitaceae being 2n=72 (hexaploid) in *Cucumis figarei* (Dane and Tsuchiya 1979). The two cytotypes in *Trichosanthes bracteta* (n=11 and n=22) and *Solena amplexiculis* (n=12 and 24) observed during the present study give evidence to the evolution of infraspecific polyploidy in the family.

Members of the Cucurbitaceae are annual or perennial climbers. In *Trichosanthes* where most of the species are annuals, those species exhibited polyploid nature have thick tuberous roots for vegetative propagation. The polyploid taxa of *Zehneria* studied presently also showed tuberous perennial habit. Dane & Tsuchiya (1979) in their study found that all polyploid species of *Cucumis* are perennials. The data on the incidence of polyploidy among annuals and perennials strongly suggest that there is a fair degree of correlation between polyploidy and vegetative reproduction also.

Since x=14 is suggested as the original basic number of the family, the basic number x=12 seen in the majority of the genera in the family can be considered as an aneuploid derivative of x=14. It is likely that the other numbers are also products of aneuploid changes either from x=14 or x=12.

Infrageneric aneuploidy has been observed in a few genera such as *Cucumis* (n=7 and 12) *Momordica* (n=8, 11 and 14) *Sechium* (x=12, 13 and 14) and *Mukia* (n=11 and 12). The occurrence of the haploid number n=7 along with n=12 in the genus *Cucumis* supports the fact that n=7 has its relationship to n=12 through polyploidy and aneuploid decrease. Reports on species of *Momordica* indicate the occurrence of the gametic numbers n=14, 11 and 8. It is likely that n=14 is the original situation in the genus and from which n=11 and n=8 have originated through aneuploid reduction. The existence of haploid numbers 11 and 12 in *Melothria maderaspatana* indicates aneuploid variation in chromosome number within the species. It is possible that the chromosome numbers n=12, 13 and 14 in *Sechium edule* are aneuploid derivatives of n=14.

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CYTOMIXIS IN *PLUMERIA RUBRA*

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SUMMARY

The process of cytomixis occurs spontaneously in *Plumeria rubra* Linn. from very early stage of meiosis. Cytoplasmic connection between adjacent pollen mother cells with or without migrating chromatin material was noticed during meiosis. A correlation between temperature and cytomixis has been observed. It is suggested that the physiological change due to higher temperature brings about cytomixis and related abnormalities in *Plumeria rubra*.

Key Words: *Plumeria rubra*, cytomixis, temperature

INTRODUCTION

The phenomenon of cytomixis was defined by Gates (1911) as the passage of chromatin materials from one pollen mother cell to the adjacent one through cytoplasmic connections. Since then, a number of investigators have reported this phenomenon both in mitotic (Sarvalla 1958, Bowes 1973) and meiotic cells of different plant species. (Lakshmi & Raghavaiah 1981, Datta 1982, Bahl & Tyagi 1988, Lakshmi et al. 1989, Koul 1990, Soman & Bhavanandan 1993) Gottschalk (1970) pointed out its occurrence limited to genetically unbalanced types such as haploids, triploids and other genetically disturbed plants. Certain other investigators reported that this process could be induced by the use of mutagen, clastogen and carcinogen (Morisset 1978, Sasikumar & Susan Abraham 1993).

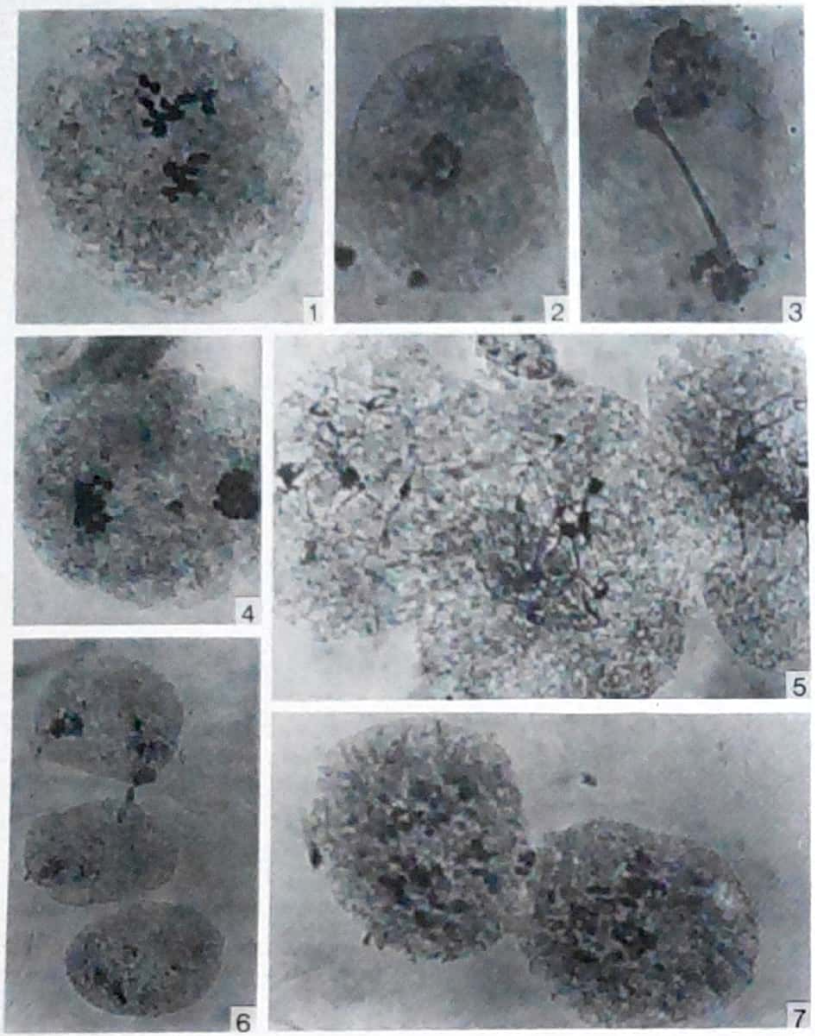
While studying the cytology of Apocynaceae from South India, extrusion of chromatin material from one PMC to the adjacent PMC was observed in *Plumeria rubra* collected from different localities at Trivandrum. The present paper embodies the results on cytomixis observed at different temperatures in *P. rubra*.

MATERIALS AND METHODS

For meiotic studies, the young buds collected from different localities at Trivandrum during different seasons (monsoon season with maximum day temperature between 23-27°C, post-monsoon (normal temperature) with temperature ranged from 31 to 33°C and summer season with maximum day temperature above 33°C), were fixed in Carnoy's fluid (1:1:3) (acetic acid : chloroform : ethyl alcohol) and squashed in 2% acetocarmine.

OBSERVATIONS

Meiosis was regular in 75-80% of the PMCs with n=18 during post-monsoon season (Fig.1) with a little amount of abnormalities like cytomixis, univalents, laggards etc. As the temperature increases a gradual reduction in the bivalent formation and increase in the frequency of the above mentioned abnormalities were observed in the PMCs (Table 1). Chromosome clumping, sticky bridges and lagging chromosomes were more frequent during summer season (Figs.2-4). The incidence of cytomixis and other irregularities were at maximum (27%) in summer. The meiocytes were found to contain chromatin bodies in the cytoplasm in leptotene and diakinesis.



Figs.1-7: Cytology of *Plumeria rubra* 1. PMC showing n=18. 2. PMC showing clumping of chromosomes. 3. PMC showing sticky bridges at anaphase I. 4. PMC showing lagging chromosome. 5. Cytoplasmic connections between PMCs. 6&7. PMCs showing the movement of condensed chromatin material from one PMC to the other PMC. (All Figs x 1500)

During cytomixis, the PMCs were connected either by direct cytoplasmic connections or by cytoplasmic bridges (Figs.5,6). In both cases, the chromatin materials in a condensed stage moved from one PMC to the other (Fig.7). The PMCs involved in cytomixis were in a uniseriate row, and the flow of the chromatin material was always in a unidirectional manner. All these meiotic irregularities led to high pollen sterility (56%).

TABLE 1 : Cytomixis and other meiotic irregularities (%) at different seasons.

Max. atmospheric temperature	Total PMCs analysed	Cytomixis	Other irregularities	Sterile pollen	Cytomixis	Other irregularities	Sterility
27 - 31°C (Monsoon)	110	3	8	41	207	7.3	37
31 - 33°C (Normal tem. Post monsoon)	120	4	11	52	3.3	9.2	43
Above 33°C (Summer)	150	40	38	84	27	25	56

DISCUSSION

The materials from different populations of *Plumeria rubra* in the present study showed normal meiosis at normal temperature. As the atmospheric temperature increases a corresponding increase in the frequency of cytomixis is also noticed. The relationship between cytomixis and fluctuating atmospheric temperature has been reported earlier in different plant species like *Urochloa panicoides* (Basavaiah & Murthy 1987), *Jasminum* (George & Geethamma 1983), *Capsicum anuum* (Lakshmi et al. 1989) and *Helicanthus elastica* (Soman & Bhavanandan 1993).

Cytomixis is usually presumed to be the result of unknown physiological disturbances, which may themselves be associated with other meiotic irregularities or with hybridization (Bell 1964). However, based on the occurrence of cytomixis in meiotically normal plants of *Clitoria ternatea*, Banerjee et al. (1988) suggested that meiotic irregularities may not be the sole criteria of cytomixis.

There exist differences of opinion among cytologists regarding the origin and significance of cytomixis. Woodworth (1931) and Sarvella (1958) explained cytomixis as due to mechanical injury. Several other reasons are also there like faulty fixation and handling (Linnert 1955, Kamara 1960) and nutritional deficiency (Milajajev 1967). Some others have the opinion that cytomixis is due to pathological phenomenon (Maheshwari 1950, Morisset 1978) or genetic mechanism (Brown & Bertke 1974, Omara 1976). Koul (1990) suggested that some changes in the biochemical process are responsible for cytomixis. Soman & Bhavanandan (1993) suggested that cytomixis occurs as a natural phenomenon. They further suggested that some bio-chemical changes might have initiated at high atmospheric temperature which ultimately culminate in a deviation in the physiological process.

Contradicting views were put forward by different authors regarding the role of cytomixis in evolution. Sarvella (1958) suggested that aneuploid plant could be originated by cytomixis. Soman

& Bhavanandan (1993) suggested that cytomixis may result in hyperploid plants. It may be noted that all the species of *Plumeria* so far reported are diploids with 2n=36 on the basic chromosome number x=18. However, there is every possibility of the origin of aneuploids due to cytomixis. Repeated observations in 2 successive years by the present authors led to suggest that transmigration of chromatin could be attributed to some physiological changes in *P. rubra* to initiate cytomixis, and the frequency is enhanced by increased atmospheric temperature.

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CHLOROPHYLL MUTANTS OF ONTOGENETICALLY DIFFERENT TILLERS IN RICE

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SUMMARY

The mutagenicity of sodium azide in ontogenetically different tillers of rice with reference to the effect of concentration and presoaking time in inducing chlorophyll mutations was studied. Dry seeds of 2 rice cultivars, Heera and Ravi were presoaked in distilled water for 0, 8, 16, 24 and 32 h and treated with 0.1, 0.3 and 0.6% concentrations of azide solutions. The tillers of M_1 plants were marked as they developed. In both the cultivars 32 h period induced high frequency of mutants. Secondary tillers in Heera and primary tillers in Ravi produced maximum number of chlorophyll mutants. In both, xantha mutants dominated the spectrum.

Key Words : Sodium azide, presoaking period, ontogenetically different tillers.

INTRODUCTION

In rice, every auxiliary bud is a potential tiller. Primary or first-order tillers appear in acropetal succession on the main culm and give rise, in due course, to secondary or second-order tillers, the latter in turn produce third-order tillers and so on. Earliness and duration of tillering vary with variety. Tillering capacity determines ear number which is a yield component. Environmental factors profoundly influence the proportion of total tiller number that ultimately produces ears. Chlorophyll mutations are used to evaluate the genetic effects of various mutagens and are widely used as genetic markers since they can often be identified in the seedling stage. Experiments with irradiated rice seeds have shown the highest rate of mutations in the main tiller (Osone 1963). Reddy & Reddy (1971) reported in dES treated rice, maximum frequency of chlorophyll mutations in the secondary tillers compared to other tiller types. In the light of these reports, the present study evaluates the mutagenic effect of sodium azide in ontogenetically different tillers of rice based on the frequency of chlorophyll mutants in M_2 generation with an emphasis on presoaking period.

MATERIALS AND METHODS

The cultivars of the present study Heera and Ravi have medium (15-20) and high tillering (40-45) ability. Seeds of these 2 cultivars were presoaked in distilled water for 0, 8, 16, 24 and 32 h and treated with 0.1, 0.3 and 0.6% concentrated solutions of azide for 6 h. In M_1 generation, in each plant, 4 tiller types viz., the main, primary, secondary and tertiary were marked depending on their emergence. Selfed M_1 seed was harvested separately from each tiller and sown as tiller progenies in nursery beds. Chlorophyll deficient mutants were scored from one week old M_2 seedlings. Mutation frequency and spectrum were worked out on the basis of M_1 pancile progeny and M_2 seedlings.

OBSERVATIONS

Presoaking the seeds prior to mutagen treatment had differential response in both the cultivars in the induction of chlorophyll mutants. On the basis of M_1 panicle progeny, in Heera, highest rate of mutated panicles was observed in 32 h period followed by 24 and 8 h, whereas in Ravi, zero

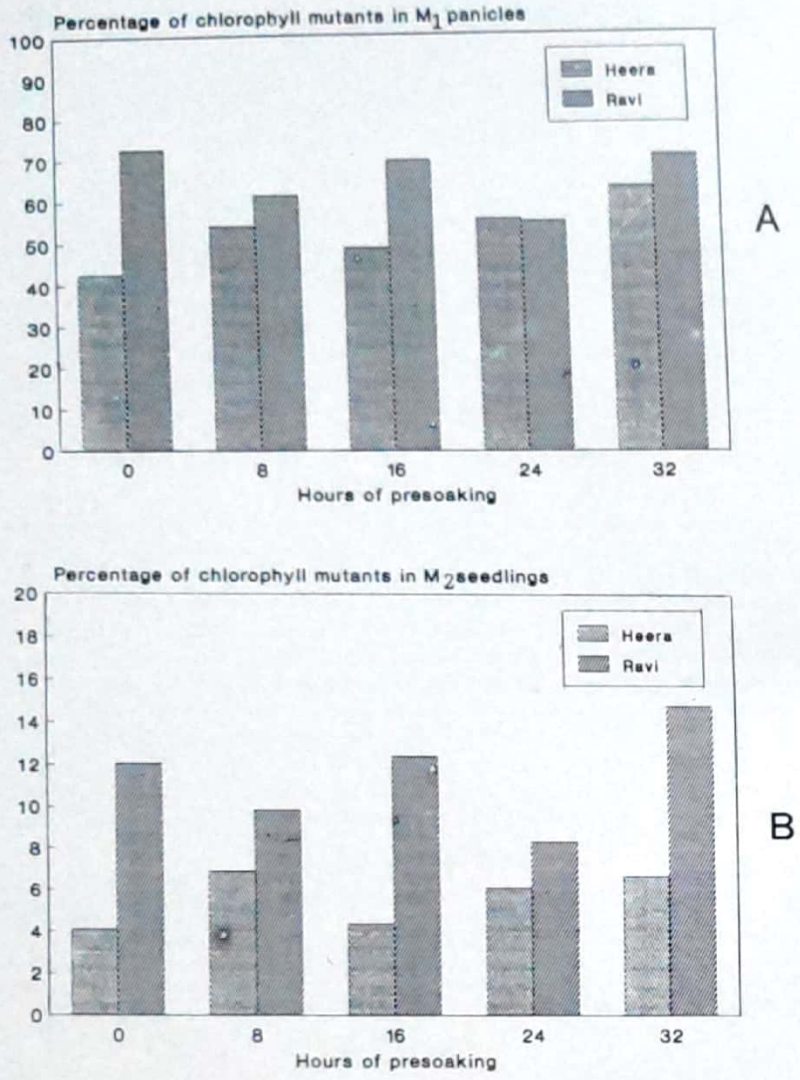

Fig. 1: Effect of different presoaking periods on rice cultivars in producing chlorophyll mutants. A. M_1 panicles. B. M_2 seedlings.

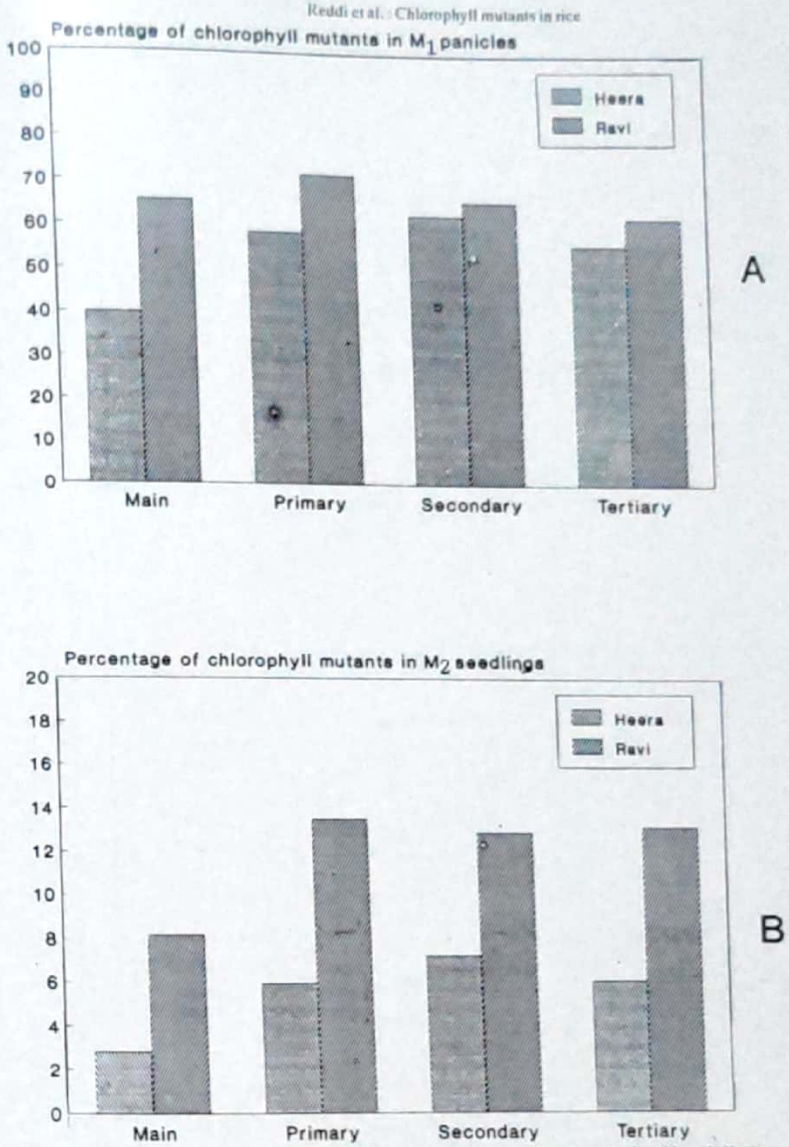

Fig. 2: Differential response of ontogenetically different tillers of rice cultivars in producing chlorophyll mutants. A. M_1 panicles. B. M_2 seedlings.

Fig. 3: Effect of sodium azide concentration in the induction of chlorophyll mutants. A M_2 panicles, B M_2 seedlings

TABLE 1: Frequency and spectrum of chlorophyll mutants among different tillers in M_2 generation.

Tiller Type	HEERA					RAVI				
	Albino	Viridis	Xantha	Alboviridis	Total	Albino	Viridis	Xantha	Alboviridis	Total
Control	-	-	-	-	-	-	-	-	-	-
Main	1.07 (13.02)	3.28 (39.90)	3.42 (41.61)	0.45 (5.47)	8.22 (100.00)	-	4.53 (39.77)	6.15 (54.00)	0.71 (6.23)	11.39 (100.00)
Primary	0.38 (4.94)	2.21 (28.70)	5.11 (66.36)	-	7.70 (100.00)	-	3.55 (20.50)	12.57 (72.62)	1.19 (6.88)	17.31 (100.00)
Secondary	1.40 (15.42)	3.36 (37.00)	4.32 (47.58)	-	9.08 (100.00)	0.56 (3.41)	4.44 (27.09)	11.39 (69.50)	-	16.39 (100.00)
Tertiary	0.65 (7.26)	3.76 (42.01)	2.78 (31.06)	1.76 (19.67)	8.95 (100.00)	2.23 (9.96)	4.16 (18.57)	11.01 (49.15)	5.00 (22.32)	22.40 (100.00)

Figures in parentheses indicate percentage of individual mutants in total mutant population.

hour period was followed by 32 and 16 h (Fig. 1A). High frequency of chlorophyll mutants was observed in secondary and primary tillers in Heera and Ravi respectively (Fig. 2A). In both the cultivars, highest concentration of azide (0.6%) induced high mutation frequencies (Fig. 3A).

Mutation frequency expressed on the basis of M_2 seedling population is more reliable and has been used to test the effectiveness of particular mutagen. Among M_2 seedlings, 8 and 32 h periods in Heera and 32 and 16 h periods in Ravi produced high frequency of mutants (Fig. 1B). Lowest mutation frequency was observed in zero and 24 h in Heera and Ravi respectively.

Secondary tillers in Heera and primary tillers in Ravi produced highest frequency of mutated seedlings. In both the cultivars, lowest frequency was recorded in main tiller (Fig. 2B). Mutation frequency progressively increased with increase in concentration of the mutagen in both the cultivars (Fig. 3B).

Xantha mutants dominated the frequency of mutants in main tiller and declined gradually in other tillers in Heera (Table 1). In Ravi, higher frequency of xantha mutants were observed in primary tillers and their frequency is very low in main tillers. Viridis mutants increased gradually from main to tertiary tillers in Herra. In Ravi, main tillers showed maximum frequency of viridis over the tertiary, secondary and primary tillers. Albinos were at their maximum in main and tertiary tillers in Heera and Ravi respectively. In both, maximum frequency of alboviridis was observed in tertiary tillers. The spectrum of mutants induced include xantha, viridis, albino and complex phenotype alboviridis. In Heera, xantha mutants dominated the spectrum followed by viridis, albino and alboviridis and in Ravi the order is xantha, viridis, alboviridis and albino.

DISCUSSION

Hydration of seeds through presoaking in water was known to facilitate rapid mutagen infusion as well as to increase the sensitivity manifold. According to Gopal-Ayengar et al. (1969) the first onset of DNA synthesis in rice seeds was found to be initiated between 24 and 32 h after hydration. Mutagenic treatments given to presoaked seeds at highly metabolic stages of G_1, S and G_2 results in increased sensitivity, high mutation frequency and spectrum. In both the cultivars, 32 h presoaking period was found to be most effective in producing the chlorophyll mutants. Differences due to azide treatments to the seeds at pre-DNA synthesis stages (i.e., 8 and 16 h periods) might be due to the physiological condition of the treated plant at the time of mutagen treatment. Sarma et al., (1979) reported azide induced maximum mutations in rice seeds presoaked in water for 4-12 h.

The observed differences in the mutation frequencies of ontogenetically different tillers might be due to variation in the degree of differentiation of various shoot meristems (Reddy & Reddy 1971). The higher mutation frequency observed in secondary tillers in Heera might be due to more severe diplontic selection in the meristems of main and primary tillers after mutagenic treatment. Similar type of diplontic selection was observed in the main tillers of Ravi. The frequency and spectrum of chlorophyll mutants were higher in Ravi than in Heera. This differential sensitivity of the cultivars in producing chlorophyll mutants suggests an influence of genome on the effect of the mutagen. Such variation in the cholorphyll mutation frequencies among rice cultivars was reported earlier by Reddi & Rao (1988) and Reddi & Suneetha (1992). Konzak et al. (1965) attributed the

diversity of the chlorophyll mutation to variations in the intragenic effects of the mutagen. From these observations it may be concluded that in practical mutation breeding, especially in evaluating the genetic effects of the mutagens like sodium azide, we should not only aim at getting ontogenetically older panicles but also collect as many panicles from primary and secondary tillers so as to maximize the mutation frequency.

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NON-MUTAGENICITY OF TWELVE SYSTEMIC PESTICIDES IN *SALMONELLA TYPHIMURIUM*

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SUMMARY

In the present investigation, 12 systemic pesticides, namely, anthio, benomyl, carbaryl, 2,4-D, derosal, dimecron, ekatin, metasystox, monocrotophos, rogor, thimet and vitavax were examined for their mutagenicity following plant and animal activation employing Ames' *Salmonella* assay in TA97a and TA100 strains. It was observed that none of the 12 pesticides tested showed mutagenicity under any of the experimental conditions.

Key Words: Pesticides, mutagenicity, *Salmonella typhimurium*, plant activation.

INTRODUCTION

The fact that some of the pesticides being mutagenic, coupled with their extensive, widespread and indiscriminate use have subjected them to close scruity for genotoxicity (Shirasu et al. 1976, Moriya et al. 1983, Grover & Malhi 1985, Malhi & Grover 1987, Adhikari & Grover 1988). India being predominantly an agricultural country, used more than 100,000 MT of pesticides in the year 1990 and its use is rising by an approximate rate of 3%. Literature survey revealed that many of the pesticides either have not been studied or the study is incomplete. Moreover, when a pesticide, especially systemic one, is sprayed over, the crops undergo transformation and in certain cases an ostensibly innocuous pesticide may metabolize into a product which may be genetoxic (Gentile et al. 1977, Gentile & Plew 1982). Such transformations by plant metabolism are referred to as "plant activation". Wildeman & Nazar (1982) proposed a protocol where S14 fraction from a few days old seedlings is supplemented with a chemical to be examined for genotoxicity. This protocol is a rather modification of standard *Salmonella*/microsomal assay, where a test chemical is supplemented with S9 liver homogenate (from rats/mice) which endowed mammalian potentiality to metabolize a chemical in in vitro condition. Wildeman & Nazar (1982) reported that several pesticides, such as atrazine, simazine, propachlor, heptchlor, captan, etc., got activated by a large number of pesticides have not been examined by plant activation assay. As the plants are the first target of any exposure to pesticide, the resultant data are of immense value. This lacuna prompted us to examine critically the mutagenicity of pesticides, especially systemic ones, employing Ames assay in *Salmonella* as modified by Wildeman & Nazar (1982).

MATERIALS AND METHODS

The commercial formulations of 12 pesticides procured from local market, were used for the present investigation (Table 1). Seven log concentrations (1, 5, 10, 50, 100, 500 and 1,000 µg/plate) of each pesticide, dissolved in dimethyl sulphoxide (DMSO), were examined for its mutagenicity using plate incorporation assay (Maron & Ames, 1983) and pre-incubation assay (Yahagi et al. 1975) employing TA97a and TA100 strains of Salmonella typhimurium. The detailed procedure, as given by Wildeman & Nazar (1982), for testing the efficacy of plant homogenate (S14) was used. Each pesticide is examined in the following conditions : (i) without supplementation of S9, (ii) without supplementation of S14 (maize) or S14 (Brassica), (iii) with

supplementation of S9 (Rat), (iv) with supplementation of S14 (maize), and (v) with supplementation of S14 (Brassica). These experiments are run concurrently with negative and positive controls. The exclusion of pesticide, but the addition of corresponding volume of solvent (DMSO) for all the above mentioned combinations, constituted negative control. Several kinds of positive controls were also set up as detailed below.

Mutagen	Dependence (S9 or S14)	Strain of *Salmonella typhimurim*
4-nitro-o-phenylenediamine (NPD)	S9-independent	TA97a
Sodium azide	S9-independent	TA100
2-aminofluorene (2AF)	S9-dependent	TA97a, TA100
Captan	S14-dependant	TA97a
Ethylidibromide (EDB)	S14-dependent	TA100

For each experiment, at least three plates with or without S9 or S14, as specified above, were run concurrently with the corresponding positive and negative control. Each experiment was repeated at least once and the data were pooled. All mutagens and DMSO were obtained from M/s Sigma Chemical Co., St. Louis (USA). S9 mix was prepared according to standard procedure given by Maron & Ames (1983) using Wistar rats induced with 0.1% phenobarbitol in drinking water. Five day old seedlings of maize/*Brassica campestris* were used to have cell-free S14 homogenate employing the protocol of Wildeman & Nazar (1982).

RESULTS AND DISCUSSION

All pesticides were examined directly and with either mammalian or plant metabolic activation system. The range of spontaneous reversion for TA97a and TA100 was found to fall between 110 to 185 and 120 to 200, respectively. It was significant to note that none of the pesticides induced any significant increase in his$^+$ revertants. The reversion rate with diagnostic mutagens, like 4-nitro-o-phenylenediamine (NPD) and sodium azide, was found to be ranging from 937 to 1032 and 2199 to 2473 in TA 97a and TA 100, respectively. 2-aminofleurino (2AF), which is S9-dependent mutagen, induced his+ auxotrophs varying from 772 to 1005 and 808 to 883 in TA97a and TA100, respectively. Captan and EDB, which get activated with S14, was found to induce his$^+$ revertants from 319 to 374 in TA97a and from 411 to 533 in TA100. In TA97a strain, maximum his$^+$ revertants were found to be 208.00±9.78 at a concentration of 5 µg/plate of anthio. The supplementation of S9 or S14(B) or S14(M) did not enhance his+ revertants. However, the lethality noticed at a concentration of 1,000 ug/plate was overcome with supplementation of S9 or S14(B) or S14(M). With benomyl, maximum his+ revertants (209.33±7.45) were encountered with S14(B) supplementation. Both S14s overcame the lethality at a concentration of 1,000µg/plate. Similarly, the maximum number of his$^+$ revertants, i.e. 222.00±5.78, 228.00±10.42, 215.66±3.67, 183.33±9.53, 184.33±2.96, 191.00±2.64 and 214.66±3.71, were found with carbaryl, 2,4-D, derosal, dimecron, ekatin, metasystox and rogor, respectively. The supplementation of S14 or S9 did not enhance revertants significantly. With rest of the pesticides, at certain concentrations, the induced revertants were less than the spontaneous number. It is significant to note that none of the induced revertant values was more than the expected 2-fold increase of spontaneous revertants of a chemical depicting mutagenicity. Similarly, it was observed that even in TA100, which is base pair substitution strain, the his$^+$ revertants noticed following pesticide treatment with or without S9/S14 did not cross 2-fold increase over spontaneous value.

TABLE 1: Trade/Common and chemical names of pesticides.

Trade/Common name(s)	Chemical name (CAS number)
Anthio, Formothion	S-2-(formylmethyl amino)-2-oxo-ethyl 0,0-dimethyl phosphorodithioate (2540-82-1)
Benomyl	Methyl-1-(butyl amino)carbonyl-IH-benzimidazole-2-yl-carbamate (17804-35-2)
Carbaryl, Sevin	1-naphthylenylmethyl carbamate (63-25-2)
2,4-D	2,4-dichlorophenoxy acetic acid (94-75-7)
Derosal, Carbendazim	Methyl-IH-benzimidazole-2-yl-carbamate (10605-21-7)
Dimecron, Phosphamidon	2-chloro-3-(diethyl amino)-1-methyl-3-oxo-1-propenyldimethyl phosphate (13171-21-6)
Ekatin, Thiometon	S-2-(ethylthio)ethyl 0,0-dimethyl phosphorodithioate (640-15-3)
Metasystox, Oxydemetonmethyl	S-2-(ethyl sulfinyl)ethyl 0,0-dimethyl phosphorothioate (301-12-2)
Monocrotophos	Dimethyl (E)-1-methyl-3-(methyl amino)-3-oxo-1-propenyl phosphate (6923-22-4)
Rogor, Dimethoate	0,0-dimethyl S-2-(methyl amino)-2-oxoethyl phosphorodithioate (60-51-5)
Thimet, Phorate	0,0-diethyl S-ethylthiomethyl phosphorodithioate (298-02-2)
Vitavax, Carboxin	5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3 carboxamide (5234-68-5)

Out of the 7 organophosphates found to be negative in the present study, 5 viz. anthio, ekatin, monocrotophos rogor and thimet, have been found negative too in the Ames test by different workers (Shirasu et al. 1976, Simon 1976, Simon et al. 1978, Genetile & Plewa 1982, Waters et al.1982, Wildeman & Nazar 1982, Klopman et al. 1985). However, contradictory to the present results, both metasystox and dimecron were found to be positive in *Salmonella* with and without S9 and S14 (Wildeman & Nazar 1982, Pandita 1983, Vishwanath & Jamil 1986).

The reports of the non-mutagenicity of three carbamate pesticides, namely benomyl, carbaryl and derosal, are also available in the Ames test following animal and plant activation (Marshall et al. 1976, Shirasu et al. 1976, Carere et al. 1978, Wildeman & Nazar 1982, Moriya et al. 1983). Vitavax and 2, 4-D have also been found to be negative in *Salmonella* strains after mammalian activation (Moriya et al. 1983, Klopman et al. 1985).

The relatively small effect observed with S14s, in the present investigation, is in conformity with the observations of Wildeman & Nazar (1982), who concluded that since the cell-free extracts used are very crude, they may also show an increased activity after appropriate optimization of both the preparative and assay conditions. Another possible reason of the failure of the pesticides to

elicit positive response might be attributed to the deactivation of their electrophility by conjugation with glutathione. However, before drawing any conclusion, the exact genotoxic potential of the pesticide should be assessed in in vivo mammalian systems.

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HERITABLE COMPONENTS OF QUANTITATIVE CHARACTERS IN FRENCH MARIGOLD*

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SUMMARY

Information on variability, heritability and expected genetic gain (genetic advance as per cent of mean) were obtained for 9 characters including yield per plant in 12 genotypes of French marigold (*Tagetes patula* L.). High variability was recorded for all the characters. In general, heritability estimates were quite high for all the characters. Maximum heritability coupled with higher genetic advance were obtained for number of lateral branches per plant, number of flowers per plant and total yield per plant. Number of main branches per plant, average weight of flower and yield per plant had a high genotypic coefficient of variation.

Key Words : Variability, heritability, genetic advance, genotypic coefficient.

INTRODUCTION

French marigolds (*Tagetes patula*) are amongst the most popular flowers and are ideal for garden display and loose flower purpose. High variability for both quantitative and qualitative characters are available in this crop (Fig.1). The extent of variability in metric trait is the basic requirement in formulating a successful breeding programme of any crop. Therefore, the present experiment was conducted to generate information on above aspects.

MATERIAL AND METHODS

Twelve varieties were grown at spacing of 30 cm between rows and between plants in randomized block design with 3 replications at IIHR, Bangalore during 1991-92. The cultural operations were uniform for all the varieties. Five plants per variety in each replication were selected at random and observations were recorded on 9 quantitative traits, viz., days to flower, plant height (cm), plant spread (cm), number of main branches per plant, number of lateral branches per plant, flower size (cm),flower weight (g), number of flowers per plant, total yield per plant (g). The data were subjected to statistical analysis. The analysis of variance, coefficient of variation (c.v.) were estimated according to the methods of Panse & Sukhatme (1967). The phenotypic and genotypic coefficients of variation were calculated according to the formula of Burton (1952). Heritability in broad sense, genetic advance as percentage of mean were worked out as suggested by Burton & De Vane (1953) and Johnson et al. (1955).

RESULTS AND DISCUSSION

Range, mean, coefficient of variation, phenotypic and genotypic coefficients of variation, heritability and genetic advance for various characters are presented in Tables 1 and 2. The varietal differences in respect of all the characters were highly significant. Variation was high for number of main branches per plant, number of lateral branches per plant and number of flowers per plant as shown by range and coefficient of variation. The genotypic coefficient of variation was highest for number of lateral branches per plant followed by number of flowers per plant and total yield per plant. Though genotypic coefficient of variation helps to measure the genetic variability present in

* IIHR contribution No.29/1994.

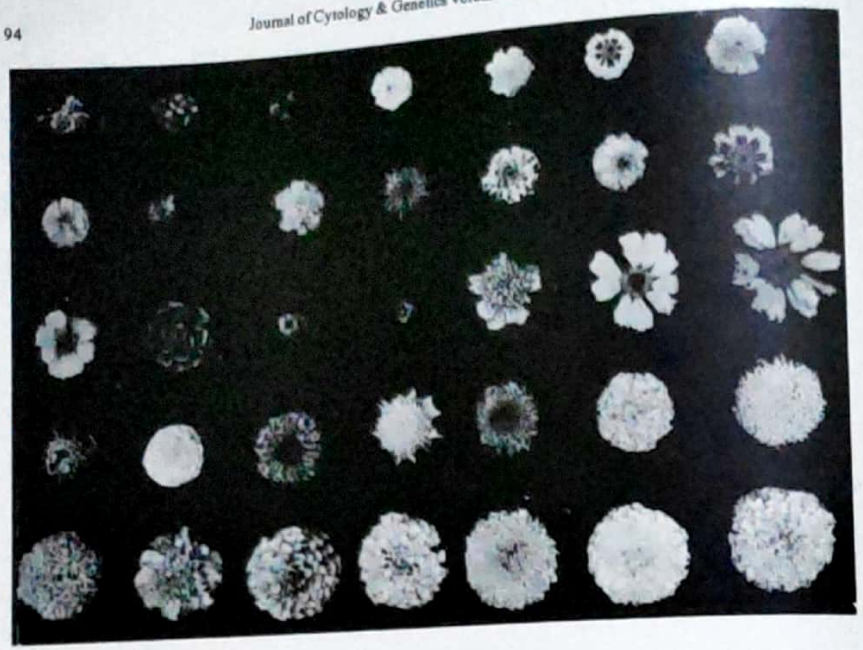

Fig.1: Floral variability in marigold

different traits, it is not possible to partition the heritable components of variation with this alone (Swarup & Chaugale 1962). Burton (1952) has suggested that a genotypic coefficient of variation together with the heritability estimates would give the best picture of the amount of advance to be expected from selection. The heritable portion of the variation thus, worked out with the help of the heritability estimates. High heritable values were observed for plant height, number of lateral branches per plant, flower size, flower weight, number of flowers per plant and total yield per plant, whereas the heritability values were medium for days to flower, plant spread and number of main branches per plant. Heritability along with the genetic gain is more useful in expecting the resultant effect for selecting the best individuals Johnson et al. (1955). In the present study, number of lateral branches per plant, number of flowers per plant and total yield per plant showed high genetic advance as percentage of mean. According to Panse (1957), inheritance governed by nonadditive gene action results into a low genetic gain, while that determined by additive gene action gives a high genetic gain. Therefore, selection for number of lateral branches per plant, number of flowers per plant would be very effective as these characters have both high heritability values and genetic gain and are controlled by additive genes. In the present investigation, high heritability along with medium genetic gain was observed for flower weight, so selection for this trait would also be effective. High heritability and low genetic advance were found for days to flower, plant height, plant

TABLE 1 : Analysis of variance for different quantitative characters in French marigold.

Character	Range	Mean	C.V.(%)	'F' value	C.D. at 5%
Days to flower	41.50 - 67.86	47.00	9.44	7.30	7.52
Plant height (cm)	16.06 - 32.90	23.37	11.37	8.96	4.50
Plant spread (cm)	19.52 - 37.06	26.35	113.76	4.87	6.14
No. of main branches per plant	6.60 - 11.72	8.37	19.99	3.75	2.83
No. of lateral branches per plant	33.88 - 112.97	58.87	15.22	19.01	15.17
Flower size (cm)	2.67 - 4.41	3.61	4.67	25.91	0.28
Flower weight (g)	0.91 - 2.15	1.48	8.54	25.34	0.21
No. of flowers per plant	70.13 - 249.00	120.23	16.45	15.53	33.48
Total yield per plant (g)	117.46 - 304.24	174.63	13.29	20.04	39.31

TABLE 2 : Variance, coefficient of variation, heritability and genetic advance for different quantitative characters in French marigold.

Character	Variance		Coefficient of variation		Heritability	Genetic advance
	Phenotypic	Genotypic	Phenotypic	Genotypic	(%)	as % mean
Days to flower	61.16	41.43	16.63	13.68	67.73	24.48
Plant height (cm)	25.82	18.75	21.74	118.52	72.63	35.52
Plant spread (cm)	30.14	16.97	20.83	15.63	56.31	23.24
No. of main branches per plant	5.36	2.56	27.67	19.13	47.81	24.15
No. of lateral branches per plant	562.48	482.14	40.28	37.29	85.71	84.40
Flower size (cm)	0.26	0.23	14.27	13.48	89.25	31.76
Flower weight (g)	0.14	0.113	25.80	24.34	89.02	57.22
No. of flowers per plant	2285.62	1894.42	39.76	36.20	82.88	79.21
Total yield per plant (g)	3951.23	3421.93	36.04	33.49	86.38	76.39

spread, number of main branches per plant and flower size. These traits with high or medium heritability along with low genetic gain exhibited a predominance of nonadditive gene action. Hence, there is a lot of scope for the improvement of these characters in french marigold by selection.

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MORPHOLOGICAL AND BIOCHEMICAL DIVERGENCE OF PRIMARY TRISOMICS OF *PETUNIA AXILLARIS* (LAM.) B.S.P.

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SUMMARY

Six of the primary trisomics of *Petunia axillaris* (Lam.) B.S.P. obtained from the progenies of an autotriploid showed morphological divergence from each other and from diploid control line. Morphological distinction was clear right from seedling stage in terms of plant stature in general and leaf shape in particular. An attempt was made to determine protein differences of the leaf extracts of the 6 primary trisomic and the disomic control lines by polyacrylamide slab gel electrophoresis. Although the trisomic lines closely resembled each other with respect to the number of protein bonds, differentiation among the different lines was possible based on the differential staining patterns of the particular bands.

Key Words: Primary trisomics, *Petunia*, electrophoresis.

INTRODUCTION

The usefulness of trisomic series in cytogenetics and breeding researches of diploid species is well-known and has been adequately discussed by Burnham (1962), Hermsen (1970) and Khush (1973). Trisomic phenotype is generally characterised by altered cellular processes and developmental patterns because of genic imbalance.

Addition of an extra chromosome may induce protein variations which can be revealed by electrophoresis and such electrophoretic patterns could be used as genetic markers for identification of trisomics. Electrophoretic techniques have been used to characterize trisomics in crop plants such as, barley (McDaniel & Ramage 1970), *Datura* (Carlson 1972, Smith & Conklin 1975), *Sorghum* (Suh et al. 1977) and pearl millet (Sidhu et al. 1984). In the present study, an attempt is made to distinguish 6 of the primary trisomics of *Petunia axillaris* (Lam.) B.S.P. on the basis of morphological phenotype as well as profile of total proteins separated by polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

The primary trisomics employed in the present study were isolated from the progenies of induced autotriploids of *Petunia axillaris* (Lam.) B.S.P.

For protein extraction 200 mg of fresh leaves (the 2 subtending leaves of the flower just before anthesis were thoroughly macerated with 1 ml of 0.1 M phosphate buffer (0.25 M sources) at pH 7.2 and centrifuged for 15 min at 10000 rpm. The supernatant was dialyzed overnight at 4°C against several changes of 0.1 M Tris-glycine (pH 8.3). The dialysate is centrifuged for 30 min at 15000 rpm. The pellet is discarded and sucrose is added to the supernatant to a final concentration of 0.5 M and it was taken as a protein sample. Bromophenol blue was added to one of the gels as a marker. The soluble proteins obtained were separated on polyacrylamide gels as per the procedure outlined by Moore (1981). Zymograms were prepared on the basis of protein mobility expressed in Rf values which represent the distance travelled by the protein band to the distance travelled by the indicator. The relative intensity of the bands were judged visually.

OBSERVATIONS

Six of the primary trisomics (2n+1=15) isolated from triploid progenies could be distinguished from the disomic (2n=14) by their late germination, poor seedling vigour, gross morphology and plant stature and leaf characters. Since leaf shape, size and texture showed visible differences in the trisomic lines, classification and nomenclature of the trisomics were made based on these features. They were provisionally named as 'Oval', 'Semi', 'Slender', 'Pseudonormal', 'Arrow' and 'Narrow'. The classification and description of the trisomics is in agreement with that of Reddi & Padmaja (1982).

The resolution pattern of proteins of disomic and 6 of the primary trisomic lines by polyacrylamide gel electrophoresis is presented in the form of zymograms (Fig.1). A total of 8 anodal

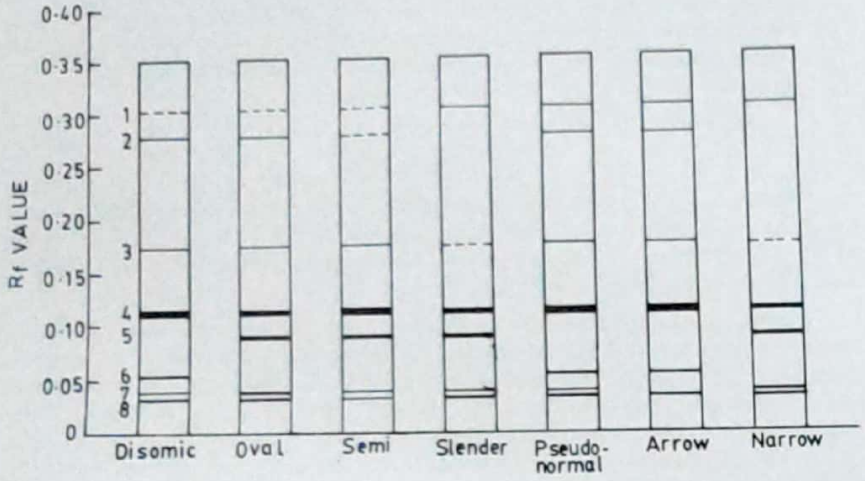

Fig.1: Zymograms showing protein profiles on polyacrylamide gel electrophoretic separation in disomic and trisomics of *Petunia*.

protein bands were observed with a range of Rf value between 0.031 and 0.303 in the different lines. Both qualitative and quantitative differences were observed in the protein bands. The control disomic plant showed 7 bands while in trisomic lines the number of bands were either 6 or 7. Further, particular trisomic lines possessing equal number of bands differed with respect to 1 or 2 individual bands present in them.

Oval and Semi trisomics were characterised by the absence of band number 6 and appearance of a novel band (Band No.5). On the other hand, Slender and Narrow trisomics showed absence of band number 2 and 6 and appearance of a novel band (Band No.5). However, Slender trisomic could be differentiated from Narrow by the decreased intensity of band number 7.

Pseudonormal trisomic, apparently resembling that of the disomic was characterised by increased intensity of band number 1. Arrow trisomic was characterised by the absence of band number 7 which was present in all trisomic and control disomic lines.

DISCUSSION

The advantage of electrophoretic analysis is that variations in the banding pattern can usually be related to variations in genes coding for the variant proteins. The various proteins present in the disomic and trisomics differ in their charges, as a result of altered amino acid composition. The chromosome related amino acid composition and enzyme differences might alter the net charge of some proteins by modifying side chains or auxillary groups. The presence of extra chromosome in the complement may alter the cellular concentration of individual proteins by the dosage effect of the structural genes or by the repressor action of the regulatory gene or by indirect action of the primarily altered protein on the degradation of other protein.

In the present study, there was an increase in the activity of particular gene/genes, decrease in the activity of genes at other sites as indicated by faintly stained bands and in still others some of the bands were totally missing or novel bands appeared. Band number 1 in 4 of the trisomics viz., Slender, Pseudonormal, Arrow and Narrow was more intensely stained than in the disomic. Alleles responsible for the production of this higher intensity protein band appear to specify the product in an additive manner. Carlson (1972) and Smith & Conklin (1975) localised 9 enzyme loci within the genome of *Datura stramonium* by observing proportional dosage responses in primary trisomics. McDaniel & Ramage (1970) also reported increase and decrease of structural protein quantities in different primary trisomics of *Hordeum vulgare*.

In the case of Arrow trisomic disappearance of one of the bands (Band No.7) was noted, which may be due to the reduced activity of the enzymes at the particular site, resulting in poor resolution so as to be not detectable in the gels. An increase in the dosage of regulatory genes through trisomy is accompanied by decreased action of one or more specific enzymes. In the disomic condition, the balance between the regulatory genes and structural genes result in a normal expression of the latter, while in particular trisomic, action of the third allele for the regulatory gene could cause genic imbalance of an unspecified nature.

Slender and Narrow trisomics are characteristic in that they differ from disomic in more than one respect. Disappearance of 2 of the bands as well as appearance of a novel band is encountered here. If the particular protein resolving at the given Rf value is a polymer, comprising of more than one polypeptide subunit, they could be specified by different alleles. Genetic differences between alleles involving only a single base pair may act through trisomy to specify a novel protein. Thus a novel band number 5 could be seen in the particular trisomic lines with the concomitant disappearance of band number 6.

In the present study 4 of the trisomics, viz., Slender, Narrow, Oval and Semi display this type of gene action, indicating that minor differences in the polypeptide subunits probably show altered mobility in the trisomic condition, rather than in disomic condition.

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GENETIC DIVERGENCE IN BREAD WHEAT

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SUMMARY

D^2 - analysis was carried out to estimate the genetic divergence in 45 genotypes of wheat. Analysis of variance revealed significant differences among genotypes for all the 11 quantitative characters. The 45 genotypes were grouped into 7 clusters. No definite trend regarding cluster means for different clusters was available. On the basis of inter-cluster distances, genotypes were identified to be included in hybridization programme.

Key Words : Genetic divergence, D^2-analysis, wheat.

INTRODUCTION

The importance of genetic diversity has already been emphasized by several workers in choosing parents for recombination breeding. Moll et al.(1965), Murthy & Anand (1966) and Jatasra & Paroda (1983) reported that degree of heterosis was related to the magnitude of genetic divergence between parental lines. Earlier, the geographic diversity was considered to be the measure of genetic divergence. Murthy & Arunachalam (1966), Anand & Murthy (1968), Bhatt (1970) and Garg & Gautam (1988) reported lack of relationship between the geographic and genetic diversity. Further, Yadav et al. (1974) and Garg & Gautam (1988) also reported the lack of correspondence between ploidy level of genotypes and the genetic diversity. In view of these facts, data on genetic divergence in a population may be helpful in selecting parents for hybridization programme aiming to exploit hybrid vigour. D^2-analysis (Mahalanobis 1936) has been effectively used as a tool in quantifying the genetic divergence in several crop species. The present study was conducted to assess the extent of genetic diversity among 45 semi-dwarf cultivars of spring wheat.

MATERIALS AND METHODS

Fortyfive genotypes of wheat (Table 1) were grown in a randomized block design block with 3 replications at experimental research farm, Department of Agricultural Botany, Ch. Charan Singh University, Meerut. Each genotype was represented by a 2 row plot of 3 m length, spaced 30 cm apart with plant to plant distance maintained at 10 cm. Data were recorded on 10 randomly selected competitive plants for the following characters : Days to flowering, Plant height (cm), Number of tillers/plant, Ear length (cm), Number of spikelets/ear, Number of grains/ear, Number of ears/spikelet, Grain yield/ear (g), 100 grain weight (g), Biological yield (g) and Grain yield/plant (g).

Plot means for different characters were processed using Mahalanobis D^2-statistic (Mahalanobis 1936). Tocher's method as described by Rao (1952) was used to classify genotypes into different clusters.

RESULTS AND DISCUSSION

Analysis of variance for yield and other characters indicated significant differences among genotypes for all the 11 quantitative characters studied.

On the basis of D^2-values, 45 genotypes were grouped in 7 different clusters (Table 1). The maximum number of genotypes (34 genotypes) were accommodated in cluster I, followed by cluster

TABLE 1 : Clustering pattern of genotypes

Cluster number	Number of genotypes	Name of genotypes
I	34	WH 541, CPAN 1973, HD 2189, HD 2329, UP 2122, CPAN 3051, UP 2294, Raj 3520, PBW 225, HUW 330, HUW 327, HP 1102, PBW 227, HD 2336, MUW 120, MUW 89, HD 1209, MUW 88, HD 2327, PBW 154, K 8027, PBW 62, HI 7080, UP 2294, CPAN 2038, CPAN 1905, CPAN 1734, MUW 221, HD 2237, CPAN 3067, HD 2177, PBW 288, HD 2285
II	2	UP 2121, UP 2009
III	4	HUW 257, CPAN 2092, HD 2281, HP 1578
IV	2	VL 404, HD 2397
V	1	PBW 11
VI	1	HUW 139
VII	1	HU 2001

TABLE 2 : Inter- and Intra-cluster distances of different clusters

Cluster No.	I	II	III	IV	V	VI	VII
I	(24.20)*	47.45	36.79	30.90	56.76	39.14	33.98
II		(9.34)	24.15	45.31	29.84	46.20	97.51
III			(16.63)	41.37	39.69	26.62	72.88
IV				(14.74)	56.12	63.02	28.77
V					(0.00)	61.82	100.91
VI						(0.00)	65.63
VII							(0.00)

* Values in parenthesis are intra-cluster distances

TABLE 3 : Cluster mean values for 11 characters in wheat

Cluster	Days to flowering	Plant height	No. of spikelets	Ear length /ear	No. of grains /ear	No. of grains /spikelet	No. of grains /ear	Grain yield	100 grain weight	Biological yield /plant	Grain yield
I	86.80	87.78	7.88	9.44	16.40	41.69	2.52	2.06	4.87	34.54	11.03
II	92.00	85.48	8.48	9.38	119.40	53.07	2.67	2.27	4.77	48.87	14.79
III	91.08	91.99	6.86	9.62	18.44	53.54	2.89	2.17	4.63	31.89	10.69
IV	79.84	84.19	7.80	9.04	17.44	3.82	2.26	1.85	4.33	29.67	8.23
V	94.00	76.77	7.10	10.93	19.33	44.50	2.30	2.08	4.97	33.33	10.45
VI	89.67	95.67	5.27	9.91	17.00	45.93	2.69	2.48	5.07	34.43	14.28
VII	77.67	89.13	7.93	9.07	14.90	31.93	2.00	1.75	4.67	26.00	8.54

III (4 genotypes) and clusters II and IV (2 genotypes each). Clusters V, VI and VII included single genotype each. These genotypes were extraordinary for one or more characters. Diverse and desirable genotypes were selected from the clusters which were characterized by maximum inter-cluster distances. Performance of the particular genotypes in the selected clusters, was also considered.

Intra- and inter-cluster distances are presented in Table 2. The intra-cluster distances ranged from 9.34 (cluster II) to 24.20 (cluster I) indicating that genotypes in cluster II were more similar in morphological features and performance than the remaining characters. The member of cluster V and VII exhibited maximum inter-cluster distance (100.91), suggesting that their entries were genetically more divergent from each other and it would be desirable to attempt crosses among the members of these 2 clusters for realising better heterotic effects and desirable segregates following hybridization between genotypes accommodated in clusters V and VII. Conversely, the members of clusters II and III were least divergent as indicated by their minimum inter-cluster distance (24.15).

A perusal of Table 3, revealed that, the cluster II had the highest mean values for the characters, number of tillers, ear length, number of spikelets/ear, number of grains/spikelets, yield/plant. In the same cluster, moderate mean values were found for days to flowering, plant height and number of grains/ear. Moreover, the genotypes of this cluster were more divergent relative to those of the other clusters. Therefore, this cluster was deemed, "best" for selecting genotypes.

In view of the above, genotypes UP 2121 and UP 2009 from cluster II; PBW 11 from cluster V; HUW 139 from cluster VI and HU 2001 from cluster VII were selected. Crossing programmes involving these genotypes are, therefore, likely to provide heterosis in the progenies and the opportunities may be greater for obtaining rare but superior segregates.

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Short Communication :

THE OCCURRENCE OF B-CHROMOSOME IN *PETUNIA AXILLARIS*

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SUMMARY

A supernumerary unpaired chromosome of about one-third the length of the smallest A chromosome was recorded among the selfed progeny of 'Oval' type primary trisomic (Triplo-1) of *Petunia axillaris* (Lam.) B.S.P. Mean A-chromosome chiasma frequency per cell was not significantly altered but increase in per cell chiasma variance was observed. Based on the morphological resemblance of the plants carrying the 'incipient' B-chromosome to the Oval trisomic, probable trisomic origin of the B-chromosome is suspected.

Key Words : B-chromosome, *Petunia*, trisomic.

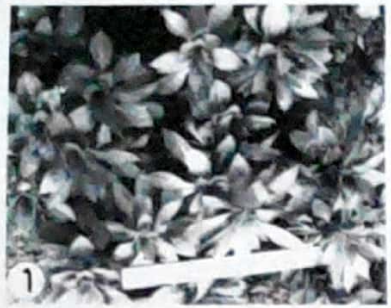
B-chromosome is the name given to accessory or supernumerary chromosome present in some cells of certain plants in addition to the normal chromosome complement. The effects of B-chromosome on the phenotype are manifold and often pronounced. Under certain circumstances these effects are adaptive conferring a superior fitness upon individuals or upon populations.

In *Petunia*, B-chromosomes were recorded in *P.parodii* (Jones 1975) and *P.hybrida* (Gohil & Kaul 1980). The present report of B-chromosome in *P.axillaris* (Lam.) B.S.P. (Solanaceae) is the first in this species. While much literature has accumulated and reviewed on different aspects of B-chromosomes, their origin continue to be conjectural (Jones & Rees 1982). In the present report on cytomorphological study over three successive generations of the Oval primary trisomic progeny enabled to trace the origin of accessory chromosome in *P.axillaris*.

The primary trisomics were obtained from the progenies of induced autotriploids of *Petunia axillaris* and maintained at experimental farm, Andhra University. Among the selfed progeny of an Oval type of primary trisomic (Triplo-1), one plant had apparently the same morphology as the parent trisomic. It was crossed with a disomic (2n=14) sibling and 2 plants of the progeny showed 2n=14+1B in some pollen mother cells at diakinesis. Both the plants with 2n+1=15 resembled the parental Oval trisomic in morphology (Fig.1), but were weak in stature; and were characterised by prolonged and delayed flowering compared to disomics. Leaves were narrow and there was marked increase in pedicel length as well as corolla tube height.

Out of 145 PMCs examined at diakinesis, the extra chromosome was present in 28 cells (19.3%) (Table 1). The extra chromosome was about one-third the size of the smallest A-chromosome and was not distinctly heteropycnotic and remained as a univalent in all the cells examined (Fig.2). The pachytene observations included instances of apparent 'fold-back' pairing and a

primary constriction. Yet another similarity with typical B-chromosomes was that the extra chromosome was absent in some cells; i.e.,the range was zero to one. With reference to A-chromosome chaisma frequency a marginal increase was noticed. However, the mean A-chromosome chiasma frequency per cell was not significantly altered in the B-material. Errant behaviour at first meiotic division was occasionally observed; chromosome stickiness (Fig.3) and presence of micronuclei were observed in 13.15% and 7.89% of the cells respectively at anaphase I.



Figs.1-3: Morphological and cytological features of B-chromosome plant. 1. B-chromosome plant just before flowering. 2. Diakinesis in B-plant showing 7 bivalents and an unpaired B-chromosome (arrow). 3. PMC showing stickness of chromosomes in B-plant. (Scale Bar = 10 µm)

TABLE I : Chromosome pairing and chiasma frequencies in B-plant and non-B-control at diakinesis.

CATEGORY	No. of cells analysed	Bivalents		Univalents Average/Cell	B-Chromosome Average/Cell	Average chiasmata	't' value
		Rings Average/Cell	Rods Average/Cell				
	145 (Flower buds with 'B')	3.08	3.84	0.13	0.19	10.01 ± 0.0684 (0.6803)	2.0757*
B-PLANT	106 (Flower buds without 'B')	3.10	3.77	0.22		9.99 ± 0.0776 0.6378	2.1244*
CONTROL	200	3.22	3.75	0.05		10.19 ± 0.0533 (0.5698)	

Numbers in parenthesis indicate variance value ; * Significant at 5% level

In the present study, the B-chromosome was not distinctly heteropycnotic. Differential staining of extensive segments proximal to the centromere and lack of heterochromatin has been reported in *Petunia* by Padmaja & Reddi (1981). The same feature of differential staining is also reported in *P hybrida* (Abirached-Darmency et al. 1992). B-chromosomes either bring about an increase in A-chiasma frequency as in *Festuca marei* (Malik & Tripathi 1970) and *Impatiens balsamina* (Raghuvanshi & Mahajan 1982) or decrease as in *Lolium perenne* (Cameron & Rees 1968),*Aegilops speltoides* (Zarchi et al. 1972) and *Trigonella foenum-graecum* (Pant & Raghuvanshi 1982). In the present study, mean A-chromosome chiasma frequency per cell was slightly decreased while variance for mean for the same parameter showed an increase.

Regarding the origin of B-chromosome, there is some renewed interest. Several views have been expressed about the origin of B-chromosome. Patton (1977) suggested that centric fusions are presumably responsible for the genesis of the large metacentric chromosomes in *Perognathus baileyi* which could have produced a centric fragment, evolving into supernumeraries. The phenomenon of origination of B-chromosome involves a polysomic element, usually a trisomic, generated by a misdivision, asymmetrical translocation, fission, non-disjunction or other rare event (Jones & Rees 1982, Jones 1985). The present study also supports this hypothesis, since the B-chromosome was observed in the progeny of trisomics of *Petunia* and the sibs do not include chromosomally aberrant types. Sapre & Deshpande (1987) proposed that supernumerary chromosomes may have arisen in species of the genus *Coix* through natural interspecific hybridizations. Puertas et al. (1985) introduced supernumeraries from *Secale cereale* into the related *S.vavilovi* through controlled hybridization experiments. This body of evidence should not be interpreted to discount the concept of 'trisomic-origin-of-Bs', even though in the said instance 'hybridization' is the source of duplicated chromatin which in turn affords the opportunity for B-chromosome formation. Had the present material been analysed for DNA sequences, etc. the evidence would have been at the molecular cytogenetic level. We have the opinion that data at the molecular level would support the concept of 'B-origin-via trisomy' and such an approach does not rule out alternate pathways for origin of B-chromosomes.

One of us (PCP) is grateful to the University Grants Commission for awarding Fellowship in Special Assistance Programme.

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ERRATA

In "A line x tester analysis for combining ability and genetic components in tasar silkworm" (A.A.Siddiqui, *J.Cytol.Genet.*1995, **30** : 135-141), the name of the second author, D.P.Das Mahapatra was inadvertently omitted. We regret the error.

J.Cytol. Genet. **31** (1) : 109 (1996)

BOOK REVIEW

Winslow, R.M., Vandergriff, K & Intaglietta M (eds) 1995. **Blood Substitutes: Physiological Basis of Efficacy,** Birkhauser, Boston. pp 205, ISBN 3-7643-3804-0.

The Book "Blood Substitutes: Physiological Basis of Efficacy" is published by Birkhauser, Boston and it is a Proceeding of the meeting "Current Issues and Blood Substitute Research and Development - 1995 sponsored by the Departments of Medicine and Bioengineering, University of California, the National Institutes of Health and the US Army.

It contains 13 chapters covering the wide area of transfusion alternatives to synthetic blood and its delivery by lyposomes. Each chapter is written by the expert, however, the information on this technology is not much and the authors have tried their best to collect the literature and given their critical views on the subject.

Chapters by Dr. Peter Tomasulo and Dr. Joseph Fratantoni are aimed at issues of efficacy demonstration. Dr. Fratantoni distinguishes between efficacy, the capacity for a product to do something physiologically useful for a patient and activity in case of synthetic blood or blood substitutes. Dr.Robert M.Winslow describes the broad physiological basis of transfusion trigger. Dr. Herman Suit describes the existing applications of cell free extent carriers in the treatment of cancer. All the three editors have contributed chapters in this book which are also worth reading.

This book has its importance in the context where there is a possibility of transmitting agents such as HIV which causes the deadly shyndrome AIDS through blood transfusion. The technology for development of a substitute for transfusion red blood cells is a highly sought area however, the physiology of the applications of the blood substitutes have to be worked out in detail so that we can eliminate the blood transfusion taken from one person and give to a patient.

This book is a good reference for all those who are interested in physiology of circulatory system and the researchers and technologists who are interested in synthetic substitutes for biomaterials.

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