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EFFICACY OF MENTOR POLLEN IN OVERCOMING INTRASPECIFIC INCOMPATIBILITY IN *PETUNIA, RAPHANUS* AND *BRASSICA*

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SUMMARY

Efficacy of mentor pollen, prepared by treating the compatible pollen with anhydrous methanol and/or by storing them until they lost viability, was tested in overcoming intraspecific incompatibility in *Petunia hybrida*, *Raphanus sativus* and *Brassica campestris*. Mentor pollen was effective to various extent in *Petunia* and *Raphanus* and was not effective in *Brassica*. In both *Petunia* and *Raphanus* the individuals showed variation in their response to mentor pollen. In *Petunia* the leachate from compatible pollen applied to the stigma before incompatible pollination was more effective than mentor pollen. Results are discussed in the light of other studies on mentor pollen and the importance of the source and the method of preparation of mentor pollen are highlighted.

INTRODUCTION

Use of mixed pollen* has been a standard method of overcoming interspecific incompatibility by Russian plant breeders, particularly by Michurin and his associates (*see* Tsitsin 1962). This technique has also been used to a limited extent to overcome intraspecific incompatibility by other investigators in a few species (Attia 1950—cabbage, Glendinning 1960, Opeke & Jacob 1969—cocoa). Although the technique is simple, the results of mixed pollinations have been generally unpredictable and the success has been limited to the realization of only a few seeds. Also, in instances of intraspecific incompatibility, an effective screening is required to distinguish between the seeds/seedlings resulting due to incompatible pollen and those due to compatible pollen.

The use of mentor* pollen has been shown to be more effective than mixed pollen. Stettler (1968) demonstrated the efficacy of irradiated compatible pollen mixed with viable incompatible pollen in achieving interspecific hybridization in *Populus*. It was suggested that killed compatible pollen porvides substances which would enable incompatible pollen grains to germinate and the pollen tubes to grow through the pistil.

Recent studies of Heslop-Harrison, Knox and his associates (*see* Heslop-Harrison 1975, Clarke & Knox 1978), implicating pollen-wall substances in incompatibility reaction, not only provided the rationale for the earlier success of mixed and mentor pollen, but also created a new interest in the possible utility of mentor pollen technique to overcome both intra and inter-specific incompatibilities. By using this method Knox and his associates reported success in overcoming interspecific incompatibility between *Populus deltoides* × *P. alba* (Knox *et al.* 1972a, b) and intraspecific incompatibility in *Cosmos bipinnatus* (Howlett *et al.* 1975). Even the wall leachate from compatible pollen was effective in overcoming incompatibility. Knox *et al.* (1972a, b) suggested that the function of mentor pollen is in

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*Some confusion prevails in the literature about the use of the terms "mixed" pollen and "mentor" pollen. We have used the term mixed pollen to a mixture of incompatible and untreated compatible pollen and mentor pollen to a compatible pollen sample made ineffective for fertilization.

providing specific recognition substances to the incompatible pollen and therefore, they termed mentor pollen as "recognition pollen".

Following the reports of Knox *et al.* (1972a, b) more reports on the efficacy of mentor pollen/extracts from mentor pollen have appeared (Dayton 1974—apple, Roggen 1975—*Brassica oleracea* var. *gemmifera*, Sastri & Shivanna 1976—*Sesamum indicum* × *S. mulayanum*, Pandey 1977, 1978—*Nicotiana*, Sree Ramulu *et al.* 1979—*Nicotiana*). Besides its extreme simplicity, the technique of mentor pollen does not require any screening method to recover seeds resulting due to incompatible pollen. In many species the success obtained by mentor pollen method was of higher degree than other techniques known to overcome incompatibility. For example in some cultivars of apple, selfed fruit and seed set following the use of mentor pollen (prepared by methanol treatment) was as good as those following the use of compatible pollen (Dayton 1974). The high degree of success achieved and the relative simplicity of the method made it a very attractive technique. We have applied this technique to overcome intraspecific incompatibility to a few other taxa, and this paper presents our results with *Petunia hybrida*, *Raphanus sativus* and *Brassica campestris*.

MATERIALS AND METHODS

Plants of *Petunia hybrida* Vilm, *Raphanus sativus* L. and *Brassica campestris* L. var. *dichotoma* Watt were grown under field conditions. Mentor pollen was obtained by treating the pollen sample collected from any compatible individual with 1ml absolute methanol (Knox *et al.* 1972a, Dayton 1974), and allowing the methanol to evaporate. In *Brassica* and *Raphanus* in addition to methanol-treated pollen, compatible pollen made inviable by storing it for 6-7 days under laboratory conditions was also used as mentor pollen.

Pollinations of emasculated and bagged flowers were performed with (1) incompatible pollen, (2) compatible pollen, (3) mentor pollen only, and (4) mentor pollen followed by incompatible pollen. In *Petunia* the efficacy of leachate from compatible pollen in overcoming incompatibility was also tested. Pollen collected from 10 anthers was allowed to leach into 4 ml of 0.025 M Tris-HCl buffer (pH 7.9) containing 0.01 M $CaCl_2.2H_2O$ and 10% sucrose (Howlett *et al.* 1975) for 1 h at 4°C in small vials. There was no rupture of pollen grains under these extraction conditions. The pollen suspension was then millipore filtered and the filtrate was preserved at 0-4°C until use (as the source of compatible pollen leachate). In this experiment, the leachate was brushed on the stigma 10-15 min before incompatible pollination.

Pollinated flowers were rebagged and allowed to develop into fruits. Fruits were harvested before dehiscence and the number of seeds was counted. The data on fruit and seed set were statistically analyzed using 'Students t' test (Bailey 1959). No statistical analysis was carried out when the difference between the control and the treatment was discontinuous.

RESULTS

Petunia hybrida

The results of over 200 pollinations carried out in 5 individuals are presented in Table 1. None of the flowers pollinated with only mentor pollen set fruit, but the use of mentor pollen followed by self-pollination, significantly increased fruit and seed set over the control.

TABLE 1

Effect of mentor pollen on self-incompatibility in Petunia hybrida

Pollinated with	No. of pollinations	% fruit set	No. of seeds/fruit	No. of seeds/ pollination
Cross Pollen	41	100.0	716.6	716.6
Self pollen	55	20.0	97.5	19.5
Mentor pollen	50	0.0	0.0	0.0
Mentor pollen and then self pollen	83	45.7	82.3	37.7*

*t value 6.18; highly significant ($p \leq 0.01$) in comparison to the value on self-pollination.

The effect of mentor pollen on fruit and seed set was not uniform in all the 5 individuals tested (Table 2). The mentor pollen had salutary effect in some individuals and was ineffective in others. Except in one individual (PH-C), in other three individuals that responded to mentor pollen, seed set was statistically significant at 1% level when compared to the seed set obtained by only self-pollintion.

TABLE 2

Effect of mentor pollen on self-incompatibility in individuals of Petunia hybrida

Individuals	Pollinated with	No. of pollinations	% fruit set	No. of seeds/fruit	No. of seeds/ pollination
PH-A	C.P.	8	100.0	552.8	552.8
	S.P.	11	0.0	0.0	0.0
	M.P.	10	0.0	0.0	0.0
	M.P.+S.P.	12	75.0	90.9	68.2
PH-B	C.P.	4	100.0	666.0	666.0
	S.P.	4	0.0	0.0	0.0
	M.P.	5	0.0	0.0	0.0
	M.P.+S.P.	10	80.0	41.5	33.2
PH-C	C.P.	4	100.0	1087.0	1087.0
	S.P.	5	20.0	50.0	10.0
	M.P.	5	0.0	0.0	0.0
	M.P.+S.P.	7	28.5	161.5	46.1*
PH-D	C.P.	10	100.0	650.7	650.7
	S.P.	16	62.5	102.3	63.9
	M.P.	10	0.0	0.0	0.0
	M.P.+S.P.	22	86.36	87.0	75.1**
PH-X	C.P.	15	100.0	721.2	721.2
	S.P.	19	0.0	0.0	0.0
	M.P.	20	0.0	0.0	0.0
	M.P.+S.P.	32	0.0	0.0	0.0

C.P.=cross pollen, S.P.=self pollen, M.P.=mentor pollen, M.P.+S.P.=mentor pollen and then self pollen.
*t value 3.17—not significant in comparison to the value on S.P.
**t value 2.52—highly significant ($P \leq 0.01$) in comparison to the value on S.P.

TABLE 3

Effect of compatible pollen leachate on self-incompatibility in Petunia hybrida (plant PH-X)

Pollinated with	No. of pollinations	% fruit set	No. of seeds/fruit	No. of seeds/ pollination
Cross pollen	10	100.0	789.2	789.2
Self pollen	23	0.0	0.0	0.0
Mentor pollen	10	0.0	0.0	0.0
Compatible pollen leachate and then self-pollen	27	18.5	173.6	32.2

Effect of pollen leachate from compatible pollen: This treatment was confined to only one individual (PH-X) in which mentor pollen had no effect. Table 3 gives the data. Self-pollination following the treatment of the stigma with the leachate from compatible pollen did result in both fruit set and seed set. Thus the pollen leachate was more efficacious than the methanol-treated pollen in overcoming self-incompatibility in *P. hybrida*.

Raphanus sativus

The plants used for investigations were highly incompatible. None of the 41 self-pollinations resulted in fruit and seed set. Methanol-treated pollen was not effective in overcoming self-incompatibility in this species. However, 7-day-stored compatible pollen which had become inviable was effective in overcoming self-incompatibility to a limited extent (Table 4). As in *Petunia*, different individuals showed variations in their response to mentor pollen.

TABLE 4

Effect of mentor pollen (stored and inviable) on self-incompatibility in Raphanus sativus

Pollinated with	No. of pollinations	No. of fruits formed	% fruit set	No. of seeds/ pollination
Cross pollen	20	19	95.0	4.2
Self pollen	26	0	0.0	0.0
Mentor pollen	15	0	0.0	0.0
Mentor pollen and then self pollen	26	16	61.5	0.615

Brassica campestris

In this species also, the plants used for investigations were highly self-incompatible and none of the self-pollinations produced any fruits. Neither methanol-treated pollen nor the stored inviable pollen was effective in overcoming self-incompatibility.

DISCUSSION

The efficacy of mentor pollen in the three species was variable. It was not effective in *Brassica* and effective to various extents in *Raphanus* and *Petunia*. Mentor pollen has been reported to be ineffective in overcoming inter and intra-specific incompatibility in a few other taxa also (*Populus trichocarpa* × *Populus* species—Stettler & Guries 1976, *Oenothera organensis*—Sree Ramulu *et al.* 1979).

In *Petunia hybrida* the use of mentor pollen along with incompatible pollen significantly increased fruit set and seed set. However, the response was highly variable among the individuals; among the five individuals tested, mentor pollen had a significant effect in three, only a marginal effect in one and no effect at all in the other. There was no correlation between the degree of incompatibility expressed by the individual and the efficacy of mentor pollen. For example mentor pollen significantly promoted selfed seed set in PH-A, an individual which was strictly self-incompatible and its effect was not significant in PH-C in which some seed set was obtained following selfing even in the absence of mentor pollen.

Such marked variations among different genotypes have been reported for *Nicotiana* also (Pandey 1977). In *N. alata*, the efficacy of mentor pollen (obtained from plants of the same species) depended on the degree of incompatibility expressed by the genotypes; mentor pollen had a positive effect in genotypes having mild incompatibility and had no effect at all in genotypes having strong incompatibility. Based on these studies Pandey (1977, *see also* 1978) suggested that the role of mentor pollen was to provide extra active pollen growth substance (PGS) (which in viable incompatible pollen is attached to S-gene-determined specific incompatibility proteins and hence unavailable after incompatible pollinations) to pollen tubes with a relatively weak incompatibility reaction to complete growth through the pistil. In individuals with strong incompatibility, mentor pollen has no effect since the extra, released PGS cannot be used.

However, recent studies of Pandey (1978) have shown that mentor pollen obtained from certain strains of *N. forgetiana* was effective in overcoming self-incompatibility even in a strongly incompatible strain of *N. alata*. Based on these studies Pandey (1978) modified the earlier hypothesis on the role of mentor pollen particularly in genotypes with strong incompatibility. According to this hypothesis, a regulatory substance rather than PGS is released from the exine of mentor pollen, which is taken up by incompatible pollen and results in switching on of the S-genetic element controlling the production of PGS. When once PGS is activated, incompatible pollen also behaves as compatible. The occurrence and extent of release of regulatory substance is variable between species and genotypes; mentor pollen only from appropriate parents which have matching regulatory substance would be effective.

Irrespective of the validity of Pandey's (1977, 1978) hypothesis, it is apparent that the source of the mentor pollen is crucial for the success of this method. The variations observed between individuals of *Petunia hybrida*, *Raphanus sativus* and those reported between different genotypes/species by most other investigators on the efficacy of mentor pollen can be, at least, partially explained by the variations in the soure of mentor pollen. Presumably the success depended on the presence of matching regulatory substances in the mentor pollen (Pandey 1978).

In most of the investigations where mentor pollen prepared by different methods have been tried, all the methods have not been equally effective. For example, in studies of Knox *et al.* (1972a, b) and Pandey (1977) mentor pollen prepared by repeated freezing and thawing was most effective. In our studies on *Raphanus sativus*, methanol-treated pollen was not effective, but stored pollen was effective. In the light of the studies of Pandey (1977, 1978) on *Nicotiana*, it is likely that the extent of the release and/or denatura-

tion of the regulatory substance varies between different treatments. The efficacy of any treatment would depend on the amount of the regulatory substance released, and maintained in active state. It is, therefore, apparent that future studies on the use of mentor pollen in overcoming inter and intra-specific incompatibility should give more emphasis on the source of mentor pollen and the method of its preparation. By using suitably prepared mentor pollen, collected from proper donors, it may be possible not only to increase the degree of success but also to extend the technique to other systems.

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CALCIUM—STIMULATED TRANSFORMATION IN *THERMOACTINOMYCES VULGARIS*

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SUMMARY

Treatment of *T. vulgaris* cells with Ca^{2+} increases the frequency of transformation by about nine fold over the control, 0.025 M Ca^{2+} being more effective than 0.1 M Ca^{2+}. However, increasing concentrations of Ca^{2+} decrease the colony forming units of this actinomycete.

INTRODUCTION

Divalent cations are obligatory for the binding of DNA to cells or isolated membranes and calcium ions are by far the most effective (Humphrey *et al.* 1980). Mandel (1967) has studied the effects of a number of monovalent and divalent cations on membrane permeability in *Escherichia coli* and has found that the uptake of DNA is dependent on the presence of Ca^{2+}. Mandel & Higa (1970) have reported that *E. coli* cells, that have been treated with calcium chloride, can take up phage λ DNA and can produce viable phage particles. R-factor DNA can transform *E. coli* cells treated with $CaCl_2$ to multiple antibiotic resistance (Cohen *et al.* 1972).

As transformation involves the uptake of naked DNA fragments, it seems reasonable to assume the involvement of Ca^{2+} in this process. The present investigation was, therefore, undertaken to determine the effects of Ca^{2+} on transformation frequency in *Thermoactinomyces vulgaris*.

MATERIALS AND METHODS

Strains: The wild type (1227) and *nic, thi, str-r* (1261) strains of *T. vulgaris* were kindly supplied by Prof. D.A. Hopwood of John Innes Institute, Norwich, England.

Media and growth conditions: The media recommended by Hopwood & Wright (1972) were used. All the strains were maintained on CM slants. Cultures were incubated at 50—52°C.

DNA isolation: DNA was extracted from the wild type strain by the method of Hopwood & Wright (1972), which is a modification of Marmur's (1961) method.

RESULTS AND DISCUSSION

Transformation was carried out by a variation of the procedure of Mandel & Higa (1970). Competent cells were prepared by growing 10^8 conidia of strain 1261 in liquid MMC (20 ml/250ml flask), supplemented with nicotinamide and thiamine hydrochloride (1 mg of each/litre), at 150 rpm at 52°C for 150 minutes. The cultures were chilled and 5 ml of chilled $CaCl_2$ was added to each flask at a final concentration of 0.025—0.1 M. The

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mixtures were kept cool for another 25 minutes. Chilled DNA (22 µg), suspended in standard saline citrate, was added to each flask. The samples were chilled for another 20 minutes. Low temperature is necessary to prevent the action of DNase. The samples were shaken incubated at 52°C for 20 minutes and then chilled. Appropriate dilutions were made and plated on selective media.

There is a significant increase in the number of single transformants (*nic*$^+$ or *thi*$^+$) in the presence of Ca^{2+}, 0.025 M being more effective than 0.1 M (Fig. 1). The differential effects of 0.025 M and 0.1 M Ca^{2+} may be due to their differential effects on the colony forming units of strain 1261, which is 94% in the former and 74% in the latter concentration of Ca^{2+}, if treated for 20 minutes under the chilled conditions after 150 minutes of growth in liquid medium. The reversion frequency of either of the markers (*nic* or *thi*) is unaffected by Ca^{2+}.

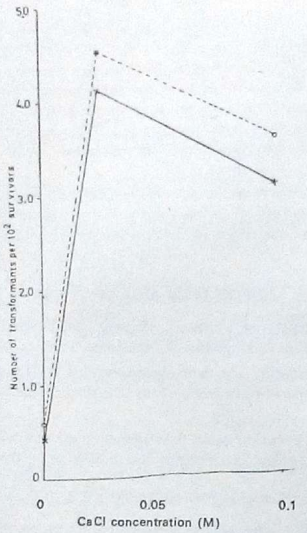

Fig. 1. Effects of three different concentrations of $CaCl_2$ on the transformation frequencies of *nic* (o) and *thi* (x) markers.

These results indicate that *T. vulgaris* can take up DNA more efficiently in the presence of Ca^{2+}. The increase in transformation frequency due to Ca^{2+} is 8 to 9 fold for either

of the markers. On the other hand, in *E. coli*, increase in the number of infective units in the presence of Ca^{2+} is more than 100 fold (Mandel & Higa 1970).

In *T. vulgaris*, Ca^{2+} has been reported to be a growth stimulant (Sinha & Singh personal communication) perhaps because it enhances the frequency of germination of spores (Kirillova *et al.* 1975). It is quite likely that this metal ion increases the transformation frequency by increasing the cell wall permeability.

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GENE ACTION IN ANTHOCYANIN BIOSYNTHESIS IN MAIZE

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SUMMARY

The epistatic gene interaction resulting into modified dihybrid ratios, gene action sequence studies and gene-enzyme-substrate relationship canbe best studied with anthocyanin pigment system in maize, due to well established genetic markers and their alleles which control distinct phenotypic expression in plant tissues. Genes *A, A2, C, C2,* and *R* must be present at least in one dose for the production of purple pigment in aleurone and is colourless if any one is present in recessive condition. In addition, the recessive *bz, bz2* results in bronze, *pr* in red and the dominant inhibitor CI blocks the pigment. By selecting appropriate genetic markers and their alleles like *Pr/Pr; CI, C, c; R; R2; Bz/bz; Bz2/bz2;* desirable crosses were made to obtain the six modified ratios beside the dihybrid 9:3:3:1 on the maize cobs. Inter-tissue complementation was observed in a "receiver" when distinguishable pieces of 20–25 day old fresh aleurone tissue of different genotypes, two at a time in all possible combinations were paired. These observations have lead to the construction of a unidirectional gene action sequence for 9 genes. The biochemical studies with aleurone tissue lead to the conclusion that *a1* accumulates quercetin, *a2* leucocyanidin, *bz* leutoclinidin. The *Bz* gene controls the UDPG–flavonol 3-glucosyl transferase in the synthesis of anthocyanin molecule.

INTRODUCTION

The maize kernel offers a remarkable platform to study the modified dihybrid ratios and gene action in the control of substrates and enzymes. About 14 loci with several alleles, a number of modifiers and dominant inhibitor are known to affect the synthesis of anthocyanin and related pigments in the aleurone and other plant tissues (Rhoades 1952, Coe and Neuffer 1977). Basic genes C_1, C_2, A_1, A_2 and *R* must be present in dominant condition in at least one dose, to synthesize anthocyanin in aleurone, the outermost tissue of the triploid endosperm. The recessives *bz* and bz_2 result in bronze, *pr* results in red and the intensifier *in* enhances the anthocyanin and related pigments. Maize plant has contributed a wealth of information towards the understanding of gene structure and function (Coe & Neuffer 1977) and no attempt will be made to review the progress made in the present study.

It is well established that the final expression of a gene, the phenotype/gene product is often controlled by a single gene although more than one gene may be involved in a particular biosynthetic pathway. Correns (1901) studied the inheritance of anthocyanin in maize kernels and suggested that the presence or absence of purple pigment is controlled by a single Mendelian factor. East & Hayes (1911) discovered the complementary factors *C* and *R* in addition to *P* (now known as *Pr*), which control the purple pigment in the aleurone tissue of a kernel Later studies have identified that several genes located in different chromosomes interact to give the six modified dihybrid ratios with two or three phenotypes.

MATERIALS AND METHODS

About 800 genes have been identified (more than 300 already mapped) which are known to govern the morphological, physiological and bio-chemical characters in plant parts, endosperm, aleurone and seedlings (Neuffer & Coe 1974, Coe & Neuffer 1977).

The genes *CI, C, c; R/r; R2/r ; Bz/bz; Bz2/bz ; Pr/pr* were used to obtain the desired modified ratios (Table 1).

The *C* locus on chromosome 9 has three alleles, *CI, C,* and *c*. Inhibitor *CI* blocks the pigment in dominant condition, recessive *c* results in colourless aleurone whereas dominant *C* governs the purple pigment. The *R* locus located on chromosome 10 has several alleles which control the aleurone and plant color including anthers, leaf tip, brace roots, etc. Whereas *R2* located on chromosome 2 controls purple color in aleurone and either one in dominant condition can synthesize the purple pigment. The factors *bz* (bronze-1) and bz_2 (bronze-2) are located on chromosome 9 and 1 respectively; either one in recessive condition results in bronze phenotype and the double recessive is colorless. Purple pigment is controlled by *Pr* and the recessive *pr* controls the red pigment, located on chromosome 5, whereas the double recessive bronze and *pr* gives bronze-pink pigment in the aleurone.

Crosses were made with appropriate marker genes to obtain F_1 and by selfing F_2 ears which exhibited segregation patterns for color and colorless (Table 1).

Distinguishable pieces of fresh excised 20-25 day old aleurone tissue of different anthocyanin single recessive genotypes when paired and placed on 0.8% agar, two at a time, exhibited complementary interaction resulting in the synthesis of purple anthocyanin pigment in only one tissue of the combined pair, receiver. The 'donor receiver' relationship is interchangeable and varies with the combination tissues paired. For example, it can be assumed that in a_1:a_2 pairs, synthesis of pigment in a_1 aleurone tissue alone means that a_1 must have received some diffusible substance from the combined a_2-tissue to complete the synthesis of blocked pigment, provided that there is no block in the receiver after the transfer (Reddy & Coe 1962).

Dry mature kernels of different genotypes under study were soaked in distilled water for about an hour and the pericarp was removed by peeling. The leucoanthocyanins were extracted with 95% ethyl alcohol from the whole kernels with exposed aleurone (Reddy 1964). In another study, the aleurone was scraped from the endosperm after the removal of pericarp and defatted with petroleum ether, then extracted with methanol. The extract was concentrated under reduced pressure. The residue was redissolved in methanol and hydrolysed after washing with petroleum ether for further analysis (Reddy & Reddy 1975). The paper chromatographic studies with n-butanol: acetic acid: water (4:1:5) and forestal, acetic acid: hydrochloric acid: water (30:3:10) were carried on Whatman No. 1 paper to characterize the pigments. The Rf values, color reactions to various spraying reagents, visible colors beside absorption spectra of the purified pigments were compared with authentic samples.

OBSERVATIONS AND DISCUSSION

The dihybrid ratio of 9:3:3:1 was obtained by crossing homozygous purple *Bz Bz, Pr Pr*; with bronze-pink, *bz bz, pr pr*; and by selfing the *F1* which gave the ratio of 9 purple: 3 red: 3 bronze: 1 bronze-pink kernels. Table 1 gives the phenotypic ratios and a description of gene combinations used in each cross to derive the six modified ratios. It is well known that epistasis is a general phenomenon where the gene action

masks the effect of the expression of non-allelic gene and is basically due to the involvement of these genes in the control of same biosynthetic pathway.

The modified gene interaction results in three or two types of phenotypes depending on type of epistatic interaction. The three-type of classes include 12:3:1; 9:3:4; 9:6:1 ratios and two-type classes include 13:3:; 9:7; 15:1. These have been illustrated along with parents, genotypic ratios in Table 1 and some in Figure 1. Maize is probably the

TABLE 1

Gene interactions and the modified dihybrid ratios in maize aleurone

Genotype of parents, type of interaction and F2 ratios	Gene expression	Phenotypic ratio			
		9A—B—	3A—bb	3aa B—	1aa bb
1 BzBz PrPr × bzbz prpr Dihybrid ratio, 9:3:3:1.	Dominant Bz with Pr- gives purple aleurone whereas fz recessive gives bronze aleurone. The double recessive gives bronze-pink aleurone.	Purple Bz—Pr— 3	Red Bz—pr pr 3	Bronze bz bz Pr— 3	Bronze pink bz bz pr pr 1
2 CICI PrPr × CC prpr Dominant epistasis, 12:3:1	Gene C in combination with dominant Pr gives purple aleurone and with recessive pr gives red aleurone, whereas CI inhibits both.	Colorless CI—Pr— 9	CI—*prpr* + 3=12	Purple C—Pr— C—3Pr—	Red C—*prpr* C—*prpr*
3 CC PrPr × cc prpr Recessive epistasis, 9:3:4	The Pr and pr genes produce purple and red aleurone with dominant C respectively and recessive c gives colorless (Fig. 1).	Purple C—Pr— 9	Red C—*prpr* 3	*cc* Pr— 3	Colorless *cc pr pr* + 1=4
4 BzBz Bz2Bz2 × bzbz bz2bz2 Incomplete duplicate recessive epistasis 9:6:1	Bz and Bz2 are coepistatic (bz bz2 bronze) and double recessive is colorless	Purple Bz—Bz2— 9	Bronze Bz—*bz2 bz2* 3	*bzbz Bz2*— + 3=6	Colorless *bz bz bz2 bz2* 1
5 CICI RR × CC rr Dominant epistasis, 13:3.	Dominant inhibitor CI inhibits pigment whereas recessive with or without CI are colorless (Fig. 1).	Colorless CI—R— 9	CI—*r r* 3+*1=13	Purple C—R— 3	Colorless C—*r r* 91
6 CC RR × CC rr Duplicate recessive epistasis, 9:7.	Recessive c and r are colorless if either or both are in recessive condition whereas dominant C and R complement to produce purple aleurone (Fig. 1).	Purple C—R— 9	C—*rr* 3	Colorless ccR— 3	+ *cc rr* 1=7
7 RR R2R2 × rr r2r2 Duplicate dominant epistasis, 15:1.	R and R2 are isoepistatic and double recessives are colorless, since either one in dominant condition produces color.	R—R2— 9	R1—*r2 r2* 3	+ *rr* R2— 3=15	*r r r2 r2* 1

unique organism where all the six modified ratios can be exhibited on the segregating ears.

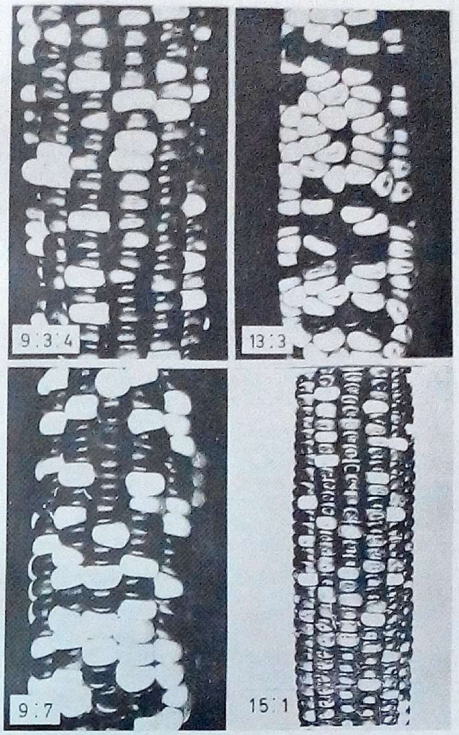

Fig. 1. Certain modified dihybrid ratios in maize.

Laughnan (1951) was the first one to suggest a gene action sequence for A_1, A_2, and Bz based on interaction in the production of anthocyanin in plant. The phenotypes of homozygous $A_1\ a_2\ Bz$ and $a_1\ A_2\ Bz$ plants were indistinguishable from that of

a_1 A_2 Bz plants, suggesting that A_1 gene may act prior to A_2 and Bz. Genetic and biochemical studies with these factors have led to the same conclusion that A_1 acts prior to A_2 and Bz but the position of Bz was uncertain (Coe 1954).

Coe (1957) constructed a gene action sequence mainly based on the interaction of the intensifier (*in*), which in homozygous condition produces bronze-metallic sheen in the pericarp. The genes *C* and *R* must be present in dominant condition for the production of metallic sheen color by *in* gene whereas *A*, A_2 and *Bz* do not affect it, suggesting that the former genes (*C*, *R*) precede *in* in the linear sequence (Coe 1957).

The results with fresh aleurone pairing studies clearly suggest that the substrates are diffusible from the donor and is being utilized by the 'receiver' to synthesize anthocyanin and that the block in the donor succeeds that in the receiver in a particular pair. The receiver must carry the dominant factor lacking in the donor as well as the subsequent factors in the sequence after the diffusion of the accumulated substance from the donor. Observations with all possible combined pairs of different genotypic tissue can be combined consistently into the following linear gene action sequence (Reddy & Coe 1962): (*CI*)–*C*–(C_2)–*R*–(*In*–)A_1 –A_2– Bz_1– Bz_2– Cyanidin-3-glucoside (Fig. 2).

The isolation and characterisation of the diffusible substrates in the established gene action sequence can be expected to reveal the biosynthesis of anthocyanin, cyanidin-3-glucoside. East & Hayes (1911) showed that *Pr*/*pr* controls the purple and recessive *pr*/*pr* red pigment and both differ in hydroxylation pattern. Later studies of McClary (1942) and Coe (1955) confirmed and further pointed out that the difference in both the pigments is due to the presence or absence of a hydroxyl group at the 3-position of B-ring of cyanidin-3-glucoside.

Coe (1955) reported that homozygous a_2 *Pr* accumulates leucocyanidin and a_2 *pr* leucopelargonidin and upon heating with 1.0% hydrochloric acid gives cyanidin and pelargonidin respectively. The double recessive aleurone tissue of a_2 *bz*, a_2 bz_2 and *in* a_2 accumulated leucocyanidin while it is absent in *CI* a_2, *c* a_2 c_2 a_2, *r* a_2, and a_2 a_2, suggesting that *C*, C_2, *R* and A_2 are required in dominant condition for the synthesis of leucocyanidin and that the inhibitor, *CI*, blocks its synthesis (Reddy 1964).

The different single and double recessive genotypes of *a2* aleurone extracts upon conversion (by heating with 1% HCl) gave purple pigment which was analyzed chromatographically and spectrophotometrically. The *a2*, *bz*; *a2 bz2*; *in a2* and *a2 Pr* converted pigments gave Rf values 0.43–0.46, similar to pure cyanidin chloride (0.46). The spectrophotometric studies with the above genotypes gave the absorption maxima of 543 mμ whereas pure cyanidin chloride gave 546 mμ. These observations suggest that *a2* aleurone tissue accumulates leucocyanidin which upon conversion gives rise to cyanidin.

The aleurone tissue extracts of recessive *a* genotype gave an average Rf value of 0.63 (B:A:W) and 0.42 (forestal) and absorption maxima at 269 mμ and 369 mμ similar to pure quercitin. The studies with homozygous recessive *bz*-aleurone tissue suggested that it accumulates luteolinidin and the genes preceding *bz* are required for its synthesis in known gene action sequence (Reddy 1974). These observations suggest a gene-product relationship in the biosynthesis of anthocyanin and the genes which precede the

product controlled by a specific gene are required for its synthesis suggesting the close association (Fig. 2).

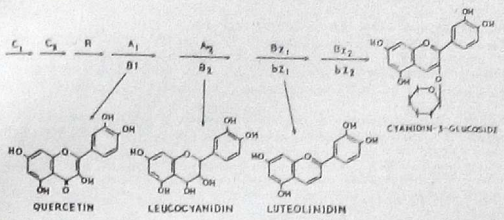

Fig. 2. Gene controlled pathway of anthocyanin synthesis in maize.

The thin layer chromatographic and other chemical studies with different genotypes by Kirby & Styles (1970) have led to the conclusion that the genes *C*, C_2 and *R* act before the formation of quercetin; *A* acts between flavononol and flavenol (leucocyanidin) and *A2* acts between flavenol and anthocyanidin in the biosynthetic pathway of cyanidin-3-glucoside. These observations have independently confirmed the gene action sequence proposed earlier by Reddy & Coe (1962).

The enzymatic studies with bronze endosperm, pollen, seedlings and embryo have thown that the endosperm has the greater activity of UDPG-flavonol 3–o glucosyl sransferase, responsible for glycoside action and the recessive tissue does not exhibit any activity (Larson & Coe 1977). It has been pointed out that *C* and *R* may act as regulatory genes and *Bz* as structural gene in the control of glucosyl transferase (Dooner & Nelson 1977).

In conclusion, the anthocyanin pigment system in maize offers a unique example for the study of gene interaction, gene action sequence and gene-substrate-enzyme relationship in higher plants.

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POLYGRAPHIC AND KARYOMORPHOLOGICAL STUDIES IN THE GENUS *URENA* LINN.

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SUMMARY

Four populations of the genus *Urena* L. collected from different localities were studied. Polygraphic study revealed the existence of two morphologically distinct forms representing *U. sinuata* L. and *U. lobata* L. as envisaged by Linnaeus (1753). While comparison of the karyotypes of these populations showed only minor structural differences accompanied by gross resemblance in types of chromosomes and overall symmetry of their complements. It is, therefore, suggested that two morphologically distinct forms be better considered representing two sub-species viz. *U. lobata* sp. *sinuata* (L.) Borssum and *U. lobata* L. sp lobata of the type species *Urena lobata* L.

INTRODUCTION

Linnaeus (1753), on the basis of morphological characters had recognized two species of the genus *Urena* viz. *U. lobata* and *U. sinuata* and Gamble (1915) also maintained the distinct status of these two species in his Flora of Madras. In contrast to this, Hochreutiner (1900) had suggested the merger of the two species into one i.e. *Urena lobata* and Santapau (1955) also suported this view point considering the character of leaf lobing having no great significance. While, in recent times, Borssum (1966) has opined to consider these two taxa representing 2 sub-species of the type species *U. lobata* Linn. In the present work, polygraphic and karyomorphological observations pertaining to different populations of these two taxa have been presented and discussed, keeping in view the different opinions concerning their circumscription.

MATERIALS AND METHODS

Seeds, representing different populations, were collected from localities, such as Dilwari forests—M.P. (Coll. No. 27), Harni (Coll. No. 29) and Baroda (Coll. No. 41)–Gujarat; Dehra Dun—U.P. (Coll. No. 48). Seeds of these populations were grown in the Botanical garden under uniform conditions. A separate voucher number was given to each population and herbarium sheets were prepared following Lawrence (1951). The data concerning morphological characters and their variations, obtained for these populations were subjected to polygraphic study following modified Hutchinson's technique (Löve & Nadeau 1961).

For karyomorphological study, excised root-tips were pre-treated with saturated aqueous soln. of para-dichlorobenzene (Meyer 1945) for 1½ hrs. at 15°C and then fixed in acetic alcohol mixture (1:3). Tjio & Levan's (1950) aceto-orcein squash technique was adopted for mitotic preparations.

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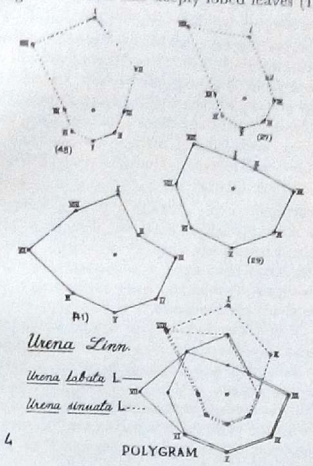
OBSERVATIONS

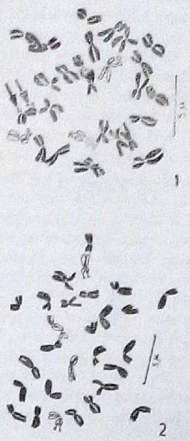
In the present investigation, over and above the character of leaf lobing, a few more distinct morphological characters were observed in different populations. For better understanding of the consistency or the variability of these characters the following important morphological characters and their variations were selected for the polygraphic study. Three index values were assigned to the variations present in each of the morphological character selected. Polygraphs of different populations were superimposed to construct a polygram revealing the variability or consistency of these characters, in different populations of the taxa.

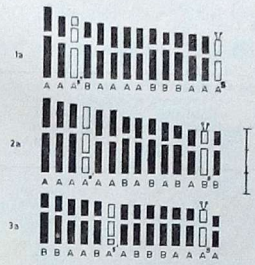
Morphological characters

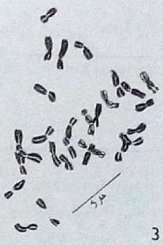
Character	Variations	Index value
I. Nature of branching	Brancing from the base, branches crowded	1
	Branching from the base, branches sparse	3
	Branching all along the stem, branches sparse	5
II. Internodal length	5 cm. or more	1
	Less than 5 cm but more than 3 cm	3
	Less than 3 cm	5
III. Leaf-lobing	Shallowly lobed; lobes acute-obtuse	1
	Deeply lobed; lobes oblong & irregularly pinnatifid	3
	More or less unlobed	5
IV. Number of veins with gland near the leaf-base	More commonly 3	1
	More commonly 1	3
	Both 1 & 3 mixed	5
V. Flower size	More than 2 cm	1
	Less than 3 cm but more than 1 cm	3
	Less than 1 cm	5
VI. Flower colour	Light-pink	1
	Dark pink	3
	Red	5
VII. Epicalyx segments	Linear and longer than the calyx	1
	Linear-oblong and equal to calyx	3
	Oblong-lanceolate and shorter than the calyx	5
VIII. Calyx segments (Sepals)	Lanceolate	1
	Narrowly lanceolate	3
	Narrowly lanceolate and notched near the base	5

Polygraphs of different populations and the polygram clearly indicate the distinctness of 2 forms viz. *U. sinuata* and *U. lobata* represented by Coll. Nos 29 & 41 and 27 & 48 respectively. Two populations viz. Coll. Nos. 29 & 41 share a number of common morphological features like deeply lobed leaves (III), presence of 1 vein with a gland









Figs. 1-3. Somatic metaphase plates of the genus *Urena:* Coll. No. 29, Coll. No. 41 and Coll. No. 48 respectively. (β chromosomes marked by arrow in Coll. No. 29).

Figs. 1a-3a. Idiograms of different populations of the genus *Urena*.

Fig. 4. Polygraphs and a polygram of different populations of *Urena lobata* Linn. (Coll. Nos. 27 & 48) and *U. sinuata* Linn. (Coll. Nos. 29 & 41).

near the base (IV), flowers less than 2 cm. (V), flowers dark pink (VI) and narrowly lanceolate calyx segment (VIII). While, Coll. Nos. 27 & 48 have shallowly lobed leaves (III) accompanied by other common morphological features like presence of 3 veins with gland near the base (IV), flowers more than 2 cm (V), flowers light pink (VI) and calyx segments narrowly lanceolate and notched near the base (VIII) (Fig. 4).

All the 4 collections have the same chromosome number i.e. 2 n=28 in their somatic complements. The present study therefore, confirms the earlier reports of Skovsted (1941), Hazra & Sharma (1971) and Bhatt & Dasgupta (1976). While Skovsted (1941) and Kootin-sanwu (1969) have also reported 2n=56 for the taxa studied by them. Chromosomes of the somatic complements vary in between 1μ and 2.99μ in length, which can be conveniently classified into two well defined types having nearly median (A-type) and nearly submedian (B-type) centromeres. The karyotypes of these populations also show gross resemblance among themselves.

A detailed scrutiny of the karyotypes of 4 populations (Fig. 1, 2, 3, 1a, 2a, and 3a) revealed differences in minor structural details of their complements. The karyotypes of Coll. Nos. 29 and 41 show closer resemblance in their gradation of symmetry, chromosome size and absolute length. However, the two collections differ from each other in number of chromosomes having nearly median, nearly submedian centromeres and in the nature of secondarily constricted chromosomes. The karyotype, representing Coll. Nos. 27 and 48, differs from the 2 preceeding ones in having lesser number of chromosomes with nearly median and comparatively more number with nearly submedian centromeres. Moreover, the karyotype also differs from others in gradation of symmetry, chromosome size and absolute length. (Table 1). In contrast to Hazra & Sharma's observation (1971) of 3 pairs of secondarily constricted and 1 pair of satellited chromosomes, the present study revealed the presence of a pair of satellited and a pair of secondarily constricted chromosomes in the somatic complements of all the 4 populations.

TABLE 1

Comparison of the karyotypes of 4 populations of Urena lobata L.

Populations	Karyotype formula	Chromosome type A	Chromosome type B	Chromosomes with secondary constriction on long arm S'	Chromosomes with secondary constriction on short arm S''	Chromosomes with SATS	Range of chromosome length in μ	Absolute length in μ
Coll. No. 29	2n=28 S S'' $=A_{16}+A_2+A_2+B_8$	20	8	—	2	2	2.98 to 1.62	61.71
Coll. No. 41	2n=28 S' S $=A_{16}+A_2+B_8+B_2$	18	10	2	—	2	2.99 to 1.87	66.27
Coll. Nos. 27 & 48	2n=28 S S' $A_{12}+A_2+A_2+B_{12}$	16	12	2	—	2	2.13 to 1.28	46.58

DISCUSSION

The polygraphic study revealed the presence of two distinct forms, differing from each other in number of morphological characters. The form characterised by deeply lobed leaves represents *U. sinuata* L. and the other form characterized by shallowly lobed leaves represents *U. lobata* L. as envisaged by Linnaeus (1753).

The karyotype analysis of 4 populations, representing 2 distinct forms, showed minor structural differences in their karyotypes. But, at the same time, gross resemblance in types of chromosomes and overall symmetry is seen in all the 4 populations studied. The karyomorphological findings therefore, do not fully subscribe to the view of considering them as 2 distinct species. In view of the above mentioned findings, the two morphologically distinct forms be better considered representing subspecies viz. *U. lobata* ssp. *sinuata* (L.) Borssum and *U. lobata* L. ssp. *lobata* of the type species *Urena lobata* L. as suggested by Borssum (1966).

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CYTOLOGICAL STUDIES IN SOME SPECIES OF *LINUM* L.

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SUMMARY

Meiotic behaviour and pollen viability of 19 collections of 11 species of *Linum* from different parts of the world are studied. It is observed that meiotic behaviour in almost all the species is normal excepting for rare occurrence of uni, tri, quadri and hexavalents at diakinesis and metaphase-I and stickiness, chromatid bridge and non-synchronous divisions at anaphase I and II. In most of the taxa the bivalents at metaphase I are usually rod-like which suggests that the evolution among the taxa might have taken place mainly by translocation. On the ground of cytological and morphological considerations the origin of various species of *Linum* has been discussed.

INTRODUCTION

Linum, the largest genus of the family Linaceae, displays great diversity in their morphological and cytological characters. More than 200 species have been listed under this genus on the basis of their morphology and floral characters only (Hooker & Jackson 1895, Stapf 1930). *Linum* is distributed both in the Northern and Southern hemispheres and only four species viz., *L. usitatissimum* L., *L. perenne* L., *L. strictum* L. and *L. mysorense* Heyne are reported from India (Hooker 1874). *L. usitatissimum* L. commonly known as linseed plant or flax, furnishes two very important products—the well known linseed oil and the famous linen fiber.

Many wild species of *Linum* possess several genes of agronomic importance such as drought resistance, winter hardiness etc. In addition, an unexplored reservoir of genetic variability like variation in oil quality (Plessers 1966, Yermanos *et al.* 1966) may be of great value if transferred to the cultivated species (Seetharam & Srinivaschar 1972). A clear understanding of interspecific relationship is desirable for successful exploitation of this untapped reservoir. A survey of previous works in different aspects of the genus *Linum* calls for further investigations especially in the field of cytology in order to add more useful knowledge of their relationship between the species. A total of 18 collections of 10 species of *Linum* from different parts of the world are studied cytologically to help to understand more about the prospects of hybridization within the genus and to ascertain their cytological interrelationship.

MATERIALS AND METHODS

Materials were obtained from IARI (Kanpur), Bulgaria, France, Israel, United Kingdom and Nepal. Flower buds of suitable size from the plants raised in green house were fixed in Carnoy's fixative Propiono-carmine was used for staining the chromosomes. The slides were made permanent by using Celariar's (1956) acetic acid *n*-butyl alcohol schedule and mounted in euparol. The chromosome behaviours during

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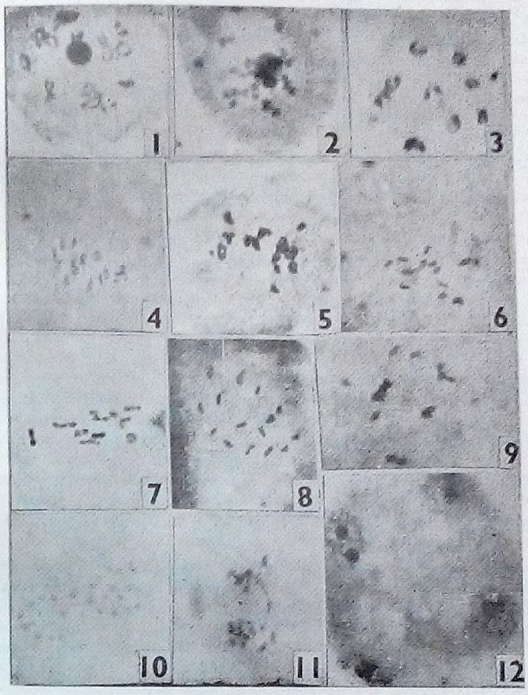
meiotic divisions have been studied in *L. africanum* L. (n=15), two collections of *L. angustifolium* Huds. (n=15), *L. corymbulosum* Rchb. (n=9), *L. crepitans* Dum. (n=15),



Figs. 1–12. Meiotic stages of *Linum* species. 1. Diakinesis showing 2 qudrivalents and 2 univalents in *L. flavum* L. 2. Diakinesis showing 30 bivalents in *L. mysorense* Heyne. 3. Diakinesis showing 9 bivalents and 2 univalents in *L. maritimum* L. 4. Metaphase I showing 14 rod bivalents and 2 univalents in *L. usitatissimum* L. 5. Metaphase I showing multivalents in *L. flacum* L. 6. Metaphase I showing 2 quadrivalents and 11 bivalents with interbivalent connections in *L. angustifolium* Huds. 7. Metaphase-I showing interbivalent connections in *L. maritimum* L. 8. Metaphase I showing 15 rod bivalents in *L. angustifolium* Huds. (Coll. No. 29a). 9. Metaphase I showing 8 bivalents, 1 trivalent, 1 univalent and 1 hexavalent in *L. nodiflorum* L. 10. Anaphase I showing equal (15+15) distribution in *L. affricanum* L. 11. Anaphase I showing chromatid bridge in *L. maritimum* L. 12. Tetrad with 1 micronucleus in *L. mysorense* Heyne. (x 1125).

L. flavum L. (n=14), *L. maritimum* L. (n=10), *L. mysorense* Heyne. (n=30), *L. nodiflorum* L. (n=13), *L. strictum* L. (n=16) and eight collections of *L. usitatissimum* L. (n=15). Pollen stainability was also studied in all of these species by staining mature pollen grains with Muntzing solution.

OBSERVATIONS

Most of the taxa studied showed regular meiotic divisions. Rare occurrence of univalents, trivalents, quadrivalents, hexavalents and other multivalents are recorded in a few species. Multivalents are observed exclusively in morphologically specialized species.

Linum flavum, a perennial herb with large spatulate leaves and comparatively large flowers showed some specializations in meiotic behaviour at its diakinesis and first metaphase stage (Fig. 1). In rest of the species chromosome behaviour at this stage of division is normal except for rare occurrence of univalents (Figs. 2&3).

At metaphase I univalents are observed in *L. usitatissimum* L. (Fig. 4), *L. usitatissimum (Seminibus flavis)*, *L. africanum* L., *L. angustifolium* Huds. (Coll. No. 2) and *L. nodiflorum* L. Multivalents are observed in still less frequency in a very few species of *Linum*. Figure 5 represents metaphase I of *L. flavum* showing occurrence of multivalents. This species has one or two quadrivalents at this stage. Quadrivalents are observed also in *L. angustifolium* (Coll No 2). This collection has one trivalent also in a few cells. Figure 6 shows 2 quadrivalents and 11 bivalents with interbivalent connections in *L. angustifolium* at its metaphase-I stage. Interbivalent connections are observed in *L. maritimum* also (Fig. 7). Presence of such connections probably leads to form multivalents. *L. nodiflorum* also has trivalent and hexavalent in addition to uni- and bivalents. A collection of *L. usitatissimum* viz. , *L. usitatissimum (Seminibus flavis)* has one trivalent. Rest of the species of *Linum* studied showed normal chromosomal behaviours at their first metaphase stage. Figure 8 represents a normal meiotic metaphase-I plate of *L. angustifolium* (Coll. No 29 a) showing 15 rod bivalents and Fig 9 shows 1 univalent, 8 bivalents, 1 trivalent and 1 hexavalent in *L. nodiflorum*. The chromosomal configuration at diakinesis and metaphase I is summarised in the Table 1.

Except in a few, the chromosomal behaviour at anaphase-I and its successive stages is normal in almost all the species studied. Normal first anaphase with equal distribution of chromosomes in *L. africanum* is shown in Fig. 10. But in *L. maritimum* stickiness and chromatid bridge are observed in a few cells. Figure 11 represents anaphase-I of *L. maritimum* showing chromatid bridge. Rare occurrence of micronuclei at diad and etrad are observed in two species. *L. usitatissimum (Seminibus-flavis)* has one micronucleus a its diad stage whereas *L. mysorense* has one at its tetrad stage (Fig. 12).

The pollen stainability ranged from 36.39 to 99.03 per cent. The least percentage is found in *L. maritimum* and the highest in *L. mysorense*. *L. strictum* has 76.23% and *L. corymbulosum* has 87.9%. The rest of the species have more than 96% and most of them have exceeded 98% with a very narrow range between the species.

DISCUSSION

Most of the taxa used in this study show regular meiosis Inouye (1938), Singh (1940), Richharia & Kalamkar (1939) and Pirson (1955) reported normal meiosis in L *usitatissimum*. Recently Seetharam (1972), Seetharam & Srinivaschar (1972) and Chaudhuri & Sen (1976) also observed regular meiosis in *L. usitatissimum* and all other species of *Linum* they studied. However, Gill & Yermanos (1967a) encountered univalents in *L. usitatissimum*, *L. angustifolium*. *L. africanum* and some other species. Univalents and multivalents, laggards, chromatid bridge, nonsynchronized divisions and micronuclei are rarely observed here and there in the present study. *L. angustifolium* (Coll. No. 2), *L. flavum*, *L. maritimum*, *L. strictum*, *L. usitatissimum* (Coll. No. 56) and *L. usitatissimum (Seminibus-flavis)* exhibited univalents at metaphase-I, the highest number 6 being in *L. flavum*, followed by 3 in *L. usitatissimum (Seminibus-flavis)*.

TABLE 1

Chromosomal configuration at diakinesis/metaphase I

Name of the species	Coll. No.	Haploid chromo. number	Uni-	Tri-	Quadri-	Hexa-Valents
L. africanum	66	15	2	—	—	—
L. angustifolium	29a	15	—	—	—	—
L. angustifolium	2	15	1	1	2	—
L. corymbulosum	36	9	—	—	—	—
L. crepitans	7	15	—	—	—	—
L. flavum	69	14	2–6	—	2	—
L. maritimum	54	10	2	—	2	—
L. mysorense	12	30	—	—	—	—
L. nodiflorum	30	13	1	1	—	1
L. strictum	17	16	—	—	—	—
L. usitatissimum	19	15	—	—	—	—
L. usitatissimum	22	15	—	—	—	—
L. usitatissimum	24	15	—	—	—	—
L. usitatissimum	56	15	2	—	—	—
L. usitatissimum	72	15	—	—	—	—
L. usitatissimum (Seminibus-brunneis)	26	15	—	—	—	—
L. usitatissimum (Seminibus-flavis)	25	15	3	1	—	=
L. usitatissimum var. *linfola*	21	15	—	—	—	—

Irregularities of rare occurrence in chromosome behaviour during meiotic divisions encountered in the present study are two chromosome groups at metaphase-I in *L, flavum*, interbivalent connections in *L. angustifolium* and *L. maritimum*, multivalents in *L. angustifolium* (Coll. No. 2), *L. strictum*, *L. usitatissimum* (*Seminibus-flavis*) and L. *flavum* and micronuclei at diad in *L. usitatissimum* (*Seminibus-flavis*) and at tetrad in *L. mysorense*. In spite of these few irregularities the meiotic behaviour points towards the stabilized nature of these species. There is a harmonious correlation between pollen

viability and self compatibility in all the species studied. In all the self compatible species pollen viability is found ranging from 87.9 (*L. corymbulosum*) to 99.03 (*L. mysorense*) per cent. The partly incompatible species *L. strictum* where about 40% of capsules are found aborted exhibits 76.23% pollen viability and *L. maritimum* where about 65% capsules are aborted has only 36.39% pollen viability.

Morphologically similar taxa exhibited more or less similar meiotic behaviour which justified the morphological grouping of the genus *Linum* into section Linum, Linastrum, Syllinum etc. Here section Linum is represented by the species *africanum*, *angustifolium*, *crepitans*, *strictum* and *usitatissimum*. All these species have similar morphological characters such as annual herbaceous habit; slender, erect and glabrous stem; entire, alternate linear-lanceolate, 3-nerved leaves; blue, actinomorphic, hermaphrodite flowers and large or medium-sized, light brown beaked seeds. Also these species showed more or less similar meiotic behaviour excepting in a collection of *L. usitatissimum* (i.e., *Seminibus-flavis*) and *L. angustifolium* (i.e., Coll. No. 2). Similarly, *L. flavum* and *L. nodiflorum* which represent section Syllinum in the present study showed some irregularities in meiotic behaviour. They exhibited univalents and multivalents at their metaphase-I stage. These species are perennial herbs with stem woody at the base; spatulate 3-nerved leaves with glands and comparatively large golden yellow flowers. The seeds are medium-sized dark brown and not beaked. The section Linastrum is represented here by *corymbulosum*, *maritimum* and *mysorense*. These annual herbaceous species have slender stem; linear-lanceolate or obovate-lanceolate leaves; small yellow flowers and very small unbeaked seeds. Cytologically *L. maritimum* exhibited some irregularities for the presence of multivalents at metaphase-I and chromatid bridge at anaphase-I. Except for the rare occurrence of micronucleus at its tetrad stage *L. mysorense* showed regular meiosis as in *L. corymbulosum*. The variation in cytological characters among these morphologically similar species may be due to chromosome reorganization as has been suggested by Lewis (1966). *L. usitatissimum* and *L. angustifolium* of section Linum showed these variations more clearly in the present study.

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EFFECT OF AUTOPOLYPLOIDY ON THE RATE OF CELL DEVELOPMENT DURING THE EARLY SEED FORMATION OF PEARL MILLET, *PENNISETUM AMERICANUM* (L.) LEEKE

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SUMMARY

The rate of the development of endosperm and embryo during the first four days after pollination in diploid and autotetraploid lines of pearl millet were studied under field conditions. The rate of early endosperm development was almost similar in diploid and tetraploid genotypes whereas the development of the embryo was slightly faster in tetraploids than in the diploids. Ratio of the mean endosperm cells to mean embryo cells at different times after pollination seemed to be fairly constant for all the genotypes studied.

INTRODUCTION

Pearl millet is an important cereal and fodder crop of the subtropics of the world. In view of their importance in cytogenetic studies and breeding of the crop, autotetraploids were produced by colchicine treatment (Krishnaswamy *et al.* 1950, Raman *et al.* 1962, Gill *et al.* 1969 and Jauhar 1970). Chromosome behaviour and fertility of the spontaneously originated autotetraploids were also reported in pearl millet (Hanna, Powell & Burton 1976, Koduru & Krishna Rao 1978). Since autopolyploidy inevitably involves an increase in gene dosage per cell, autopolyploids have nearly twice as much DNA content per cell as their diploids (Bennett *et al.* 1975). Increased DNA content per cell may affect the cell cycle duration (Palitti *et al.* 1972) and Murin (1976b) demonstrated that the mitotic cycle duration is not simply related to the DNA content per nucleus. In order to understand the effect of polyploidy on the duration of the cell cycle and the relation between embryo and endosperm growth during early seed development, an attempt has been made to compare the rates of embryo and endosperm development in diploid and autotetraploid lines of pearl millet. Observations were made for the first four days after pollination on field grown material.

MATERIALS AND METHODS

IP 482 T is an autotetraploid of spontaneous origin in the inbred IP 482 (Koduru & Krishna Rao 1978) and is being maintained by selfing as an inbred line; in the fifth generation it has 58% seed set. The other tetraploid (C8HF) was obtained from a diploid outbred line by colchicine treatment and was selected for high seed fertility in the subsequent generations; in the C8 generation it had 60% seed set (Arundhati 1980). Diploid IP 482 and another inbred Vg 212 were used for comparison. Vg 212 was studied and compared to other inbreds (unpublished) and therefore it is useful as a standard line.

The spikes on field grown plants were artificially pollinated and were collected at 0, 24, 48, 72 and 96h after pollination. Collections were made during the second and third weeks of February, 1980. Temperatures during the day varied between 31°C—38°C and the night minimum temperatures varied between 21°C—26°C. For comparison of all the genotypes, samples were taken from corresponding times of pollination and fixation. The florets were fixed in acetic-alcohol (1:3) and then changed to 70% alcohol. Embryo sac was dissected out of each ovule under a dissection microscope; the whole endosperm mass along with the embryo was macerated for 1h in 45% acetic acid. After maceration the embryo and endosperm were separated and squashed in 0.5% acetocarmine. Total number of endosperm nuclei (or cells) and embryo cells were counted from five ovules at each sampling time.

OBSERVATIONS

The mature embryo sac at the time of pollination had two synergids and an egg at the micropylar end, two closely appressed polar nuclei just below the egg and a cluster of variable number (2-22) of antipodals at the chalazal end. Antipodals were usually multinucleate. The embryo sac, cell and nuclear sizes were visibly larger in the tetraploids than in the diploids.

Endosperm: The first division of the primary endosperm nucleus occurred between 4-5h after pollination. Two endosperm nuclei were found in all the florets fixed 6h after pollination. Endosperm was hexaploid with 42 chromosomes in the tetraploid lines and was triploid with 21 chromosomes in the diploid lines. The endosperm was at first coenocytic in all genotypes but was cellular at later stages. The endosperm exhibited synchronous mitoses up to 48h in all genotypes. Cell wall formation was initiated at the micropylar end at 48h itself, in some of the ovules of all genotypes. The endosperm was completely cellular and also starch grains appeared in some of the ovules in all genotypes at 72h after pollination. After cellularization the rates of endosperm development seemed to be slightly different between the tetraploids and diploids which was also reflected in the higher mitotic indices in tetraploids. All the genotypes showed high mitotic indices even after cell wall formation which was not found in diploid inbreds studied earlier (unpublished). This might be expected due to the variation in temperature.

Cell cycle time: The cell cycle time during the free nuclear phase was almost similar in the tetraploids and diploids. The cycle time increased during the transition from free nuclear phase to cellular phase of the endosperm (Table 1) and the increase was approximately 2.0 times in C8HF, 1.75-2.0 times in diploid and tetraploid IP482, and 2.5 times in Vg 212 in contrast to 3.0 times increase noted previously, at the time of transition, in diploid Vg 212.

Embryo: First division of the zygote was transverse; the upper cell formed into the embryo proper and the lower cell into the suspensor of 8-10 layers about 96h after pollination. A number of starch grains were observed at 72h and 96h, after pollination in the suspensor cells. Mean number and range of embryo cells were found to be slightly higher in tetraploids than in diploids at each sampling time.

Cell cycle time: Divisions in the embryo cells were also largely synchronous up to 48h, at which time embryos with 6-14 cells, almost all in mitosis, were frequently observed. Subsequent divisions were less synchronous and estimation of cycle times, making allowance for this point, was less accurate. Mean cycle time of the embryo

TABLE 1

Mean and minimum cell cycle times in the endosperm of C8HF, IP482T, IP482 and Vg 212 at different times after pollination

Sample	No. of cells	No. of cycles	Mean cycle time	Minimum cycle time	Mitotic index
6h					
All genotypes	2	1	6.0	—	—
24h					
C8HF	16–32	4–5	5.4	4.8	100%
IP482T	all genotypes	all genotypes	5.7	all genotypes	all genotypes
IP482			4.5		
Vg 212			5.5		
48h					
C8HF	400–986	9–10	5.76	4.8	89.7%
IP482T	400–936	all genotypes	all genotypes	all genotypes	99.6%
IP482	256–592				99.6%
Vg212	269–590				99.6%
72h				8.0	37.26%
C8HF	1518–2365	11–12	10.4	8.0	28.26%
IP482T	1998–3908	11–12	8.8	6.0	23.0%
IP482	1475–4585	11–13	8.8	8.0	16.9%
Vg212	925–2550	10–12	13.6		
96h				—	20.8%
C8HF	4028–8102	12–13	—	—	17.8%
IP482T	6165–6813	13–0	—	—	20.5%
IP482	4300–7820	13.0	=	—	15.3%
Vg 212	2797–5948	12–13	—		

decreased from about 24h of the first cycle to 7.3h at the sixth and seventh cycles in diploids and to 6.7h—6.9h at the sixth and eighth cycles in tetraploids (Table 2). During subsequent development the cycle time again increased to 18.0h—19.0h in diploids and 16.0h—24.0h in tertaploids. Thus minimum cycle time in embryo development was observed during the period of transition of the endosperm from free nuclear

TABLE 2

Mean cell cycle times and mitotic indices in the embryos of C8HF, IP482T, IP482 and Vg 212 at different times after pollination

Sample	Mean no. of cells (range)	No. of cycles	Mean cycle time (in h)	Mitotic index
24h			24	—
All genotypes	2	1.0		
48h			11.3	63.3%
C8HF	11.2 (6–13)	3.0–4.0	10.2	68.3%
IP482T	11.7 (8.14)	3.0–4.0	11.2	60.25%
IP482	9.0 (6–14)	3.0–4.0	12.0	57.1%
Vg 212	8.4 (6–10)	3.0–4.0		
72h			6.9	16.6%
C8HF	49.0 (38–65)	6.0–7.0	6.7	18.5%
IP482T	69.5 (31–92)	6.0–8.0	7.4	13.8%
IP842	57.8 (32–85)	6.0–7.5	7.4	16.2%
Vg 212	41.0 (32.–50)	6.0–6.5		
96h			16.0	10.4%
C8HF	137.4 (92–190)	8.0	24.0	10.6%
IP482T	108.6 (100–119)	8.0	18.6	11.8%
IP482	100 (90–120)	8.0	19.8	4.0%
Vg 212	98.3 (70–110)	7.0–8.0		

to cellular phase and after cellularization of the endosperm, the cell cycle time in the embryo increased to about 2.5—3.5 times its earlier value, a feature similar to that observed in the development of endosperm with cellularization.

Relative rates of embryo and endosperm development: Rate of nuclear doubling in the free nuclear endosperm greatly exceeded the rate of nuclear doubling time in the embryo. The maximum number of nuclei in the endosperm and embryo during the free nuclear phase in all genotypes indicates that the nuclear cycle time in the endosperm during the period (5.7h) was about half that in the embryo (12h). At the time of cellularization the cycle time of the endosperm increased while that of the embryo decreased; and subsequently the cycle time in the embryo increased to 2.5—3.5 times its minimum value.

DISCUSSION

The rate of endosperm development was almost similar in the tetraploid and diploid lines of pearl millet which indicates that polyploidy has no effect on early endosperm development. Similar results showing no effect of polyploidy on the rate of endosperm development were reported in autopolyploid *Citrus* (Esen & Soost 1973), *Zea mays* (Cooper 1951), *Lycopersicon* (Cooper & Brink 1945), *Secale cereale* (Hakansson & Ellerstrom 1950), *Hordeum vulgare* (Hakansson 1956), *Hordeum bulbosum* (Bennett *et al.* 1975) and allopolyploid wheat species (Bennett *et al.* 1973, 1975).

The duration of mitotic cycle in the root meristems was reported to be longer (Evans *et al.* 1972) or the same as (Yang & Dodson 1970) or shorter (Gupta 1969) than that in related diploid species. Murin (1976a) has suggested that since primary influence of polyploidy on mitotic cycle duration is variable from relatively small to significant, there is no relationship between mitotic cycle time and polyploidy.

In contrast to its no effect on endosperm development, polyploidy seems to have a slightly stimulating effect on cell development in the embryo. This is evident from a slight increase in the mean number of embryo cells, and hence a decrease in the cell cycle time of the tetraploid embryos when compared with the diploid embryos in pearl millet. Thus the effect of polyploidy can be different on different cell types within the same species. Bennett *et al.* (1975) also reported an increase in the rate of development in the embryo cells of allopolyploid wheat species and autopolyploid *Hordeum bulbosum*, while polyploidy has no effect on cell development in the endosperm. Moreover the effect of polyploidy on cell development in embryo is similar to its effect on meiocytes and microspores of wheat, rye and barley where increasing the level of either allo-or auto-polyploidy increased the rate of cell development. In *Zea mays* the effect of autopolyploidy on the rate of embryo development seems to be slightly different in that the diploid and tetraploid were similar up to two days and also between four to eight days but between second and fourth day the diploid was faster than the tetraploid (Cooper 1951).

The rates of development of the endosperm and embryo appear to be highly correlated in pearl millet which is evident from the cell ratios. The ratio of the mean endosperm nuclei (or cells) to the mean number of cells in the embryo is fairly constant from about 48h to 96h after pollination (Fig. 1). Though the ratio varies between the

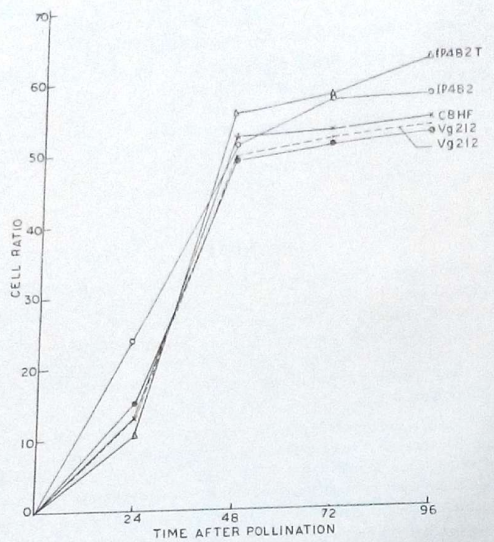

Fig. 1. Relation between mean endosperm cells to mean embryo cells at various times after pollination.

genotypes, it is constant for a genotype. This ratio seems to be more or less fixed for a genotype in that the field grown samples of Vg 212 in two seasons were almost identical though there were differences in mean night and day temperatures between the seasons. Similarly the ratio is very similar between the diploid and the related autotetraploid genotypes. The constancy of the cell ratio suggests that (1) either the rates of the embryo and endosperm are subjected to the same control or (2) the embryo is dependent on endosperm for its rate of development. The fact that autopolyploidy has no effect on the rate of cell development in the endosperm while it has a slightly stimulating effect in the embryo cells suggests that the rates of cell development in the two cell types are subject to different controls. This leads us to the supposition that the rate of the development of embryo is dependent on endosperm. Such a possibility is also supported by our earlier observations (unpublished) that the cell and nuclear volumes, as also the cell numbers, in embryos developed without endosperm were considerably reduced as compared to those in embryos accompanied by endosperm development.

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CLASTOGENIC ACTIVITY OF SOLAR ECLIPSE IN CERTAIN PLANT SYSTEMS

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SUMMARY

Clastogenic activity of solar eclipse was investigated in the mitotic systems of *Allium cepa*, *A. sativum*, *Hordeum vulgare* and *Pisum sativum*. Chromosomal breakages and fragmentations were the major clastogenic responses observed in the test systems. Other chromosomal anomalies like fragmented nuclei, stickiness and clumping, ana and telophase bridges, lagging chromosomes, multipolar formations at anaphase and binucleate cells were also observed. With increase in the duration of recovery period, a trend of decrease in the frequency of abnormal cells was noticed. However, meristematic tissues of the test systems exposed to normal sun did not show any such clastogenic effects. Preliminary results implicating the clastogenic action of the solar eclipse are presented.

INTRODUCTION

From times immemorial eclipses have inspired awe and fear among the mankind. Despite the progress made in understanding the astrophysical aspects of eclipses, there are certain superstitions associated with them. In certain Indian societies pregnant women are shunned from all activity during the entire phases of eclipses and are kept strictly indoors for fear of exposure, for such an exposure is believed to cause malformed babies. In order to ascertain whether any clastogenic hazards are associated with eclipses, an experiment was conducted with four plant test systems, viz., *Allium cepa*, *Allium sativum*, *Hordeum vulgare* and *Pisum sativum*, during the total solar eclipse of 16th February, 1980. Marked rise in chromosomal aberrations in the root meristems of different test systems was noted upon exposure to solar eclipse. Whereas the meristematic tissue exposed to normal sun, one week after the eclipse, did not show any such clastogenic effects. In the studies reported here, preliminary results implicating clastogenic action of the eclipse are presented. Details of further studies will be published elsewhere.

MATERIALS AND METHODS

Root meristems of the test systems, immersed in aqueous medium, were exposed to the solar eclipse (2.15 PM to 5.00 PM, I.S.T.) on 16th February, 1980, at Nalgonda, A.P., India, which fell under the path of totality. The temperatures were recorded every 15 minutes for the entire duration of eclipse and the same varied from 32.2°C to 25.8°C. Another set of growing root tips in aqueous medium was kept indoors at the room temperature of 28± 0.5°C to serve as control I.

In order to compare the differences in the effects of normal sun and the eclipsed sun, another experiment was conducted a week later. All the test systems were exposed to normal sun for the same period (2.15 PM to 5.00 PM, I.S.T.), simulating the temperature conditions of solar eclipse, which formed control II

Yet in another experiment, to assess the fate of macrolesions induced in the meristematic cells during the eclipse period, a set of exposed meristems were allowed to recover

by growing them in aqueous medium for further periods of 12, 18 and 42 h. Appropriate recovery-controls were maintained for all the systems studied.

The root tips were fixed in acetic alcohol (1:3) and processed following haematoxylin squash technique (Subramanyam & Subramaniam 1970) with slight changes in the hydrolysis timings The standard normal deviate (Z) test was used for testing the differences in the frequency of aberrant cells in various treatments.

RESULTS AND DISCUSSION

The data on the frequency of abnormal cells in treatments and controls is given in Table I. Exposure to solar eclipse provoked a wide spectrum of clastogenic effects in the meristematic cells of the four test systems investigated.

TABLE I

Frequency of eclipse-induced abnormal cells in the four plant systems

Systems	Treatments	Total no. of cells	Cells in division	No. of abnormal cells	Percentage of abnormal cells	Z value
A. cepa	Control I	5025	179	0	0	0
	Control II	5076	146	1	0.68	1.38
	S.E.	5120	183	42	22.95	5.97***
	S.E.+12 h rec.	5326	68	20	29.41	4.52***
	S.E.+18 h rec.	5122	285	9	3.15	1.97*
	S.E.+42 h rec.	5180	770	17	2.20	0.60
	Control (recovery)	5460	760	0	0	0
A. sativum	Control I	5056	277	0	0	0
	Control II	5024	94	2	2.12	1.41
	S.E.	5170	211	71	33.65	7.39***
	S.E.+12 h rec.	5100	198	19	9.59	2.79**
	S.E.+18 h rec.	5180	652	16	2.45	0.21
	S.E.+42 h rec.	5050	345	9	2.60	0.48
	Control (recovery)	5060	700	0	0	0
H. vulgare	Control I	5036	322	0	0	0
	Control II	5020	300	1	0.33	1.00
	S.E.	5040	300	45	15.00	6.49***
	S.E.+12 h rec.	5240	325	25	7.69	4.68***
	S.E.+16 h rec.	5090	415	23	5.54	4.33***
	S.E.+40 h rec.	5300	600	14	2.33	2.83**
	Control (recovery)	5350	625	0	0	0
P. sativum	Control I	5046	400	0	0	0
	Control II	5016	366	2	0.54	0.45
	S.E.	5076	440	35	7.59	3.92***
	S.E.+12 h rec.	5100	230	28	12.17	8.30***
	S.E.+18 h rec.	5040	790	33	4.17	2.60**
	S.E.+42 h rec.	5095	730	15	2.05	1.15
	Control (recovery)	5060	800	0	0	0

S.E.=Solar eclipse; rec.=recovery;
*=significant at 5% P level; **=significant at 1% P level;
***=significant at 0.1% P level.

Chromosome breakages (Figs. 1, 2, & 4) and fragmentations (Figs. 3 & 4) were quite frequent, compared to other abnormalities. The chromosome breakages observed were mainly of isochromatid type. With the exception of *Pisum sativum*, others revealed

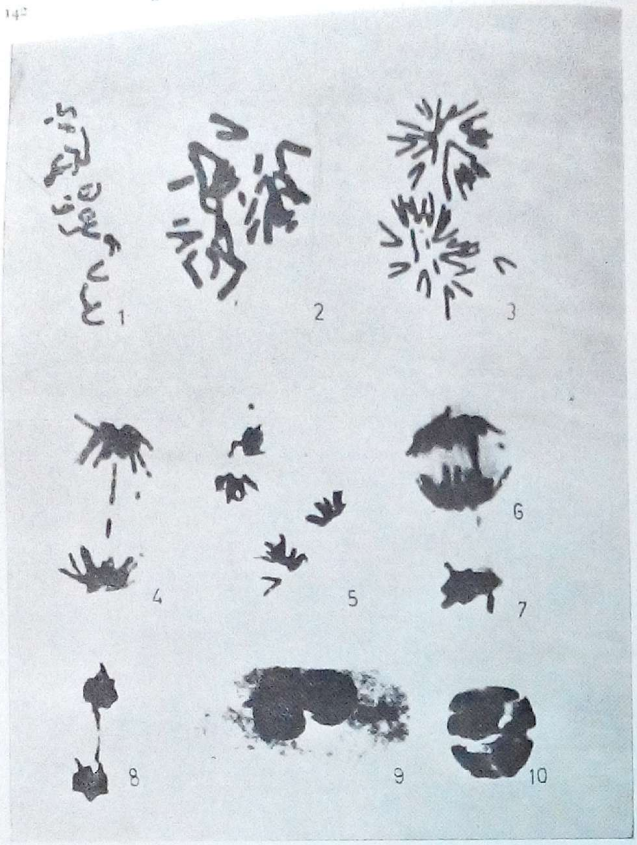

Fig. 1. Metaphase chromosome breakages in *A. sativum*.
Fig. 2. Metaphase chromosome breakages in *A. cepa*.
Fig. 3. Anaphase chromosome fragments in *A. sativum*.
Fig. 4. Lagging chromosome and fragments in *P. sativum*.
Fig. 5. Multipolar formations at anaphase in *H. vulgare*.
Fig. 6. Anaphase bridge in *P. sativum*.
Fig. 7. Clumping of chromosomes in *P. sativum*.
Fig. 8. Telophase bridge in *P. sativum*.
Fig. 9. Binucleate cell in *A. cepa*.
Fig. 10. Fragmented nucleus in *A. sativum*.

fragmented nuclei (Fig. 10) as a sequel to exposure to the solar eclipse. Other chromosomal anomalies found in the exposed meristems were stickiness and clumping of chromosomes (Fig. 7), ana and telophase bridges (Figs. 6 & 8), laggards (Fig. 4), lagging chromosome fragments, multipolar formations at anaphase (Fig. 5), binucleate cells (Fig. 9) and occasional extreme condensation of chromosomes at metaphase.

The meristematic cells of all the test systems exposed to solar eclipse, when compared to controls I & II, displayed significantly high frequency of chromosomal anomalies. A few aberrant cells were also visualized in root tips exposed to normal sun; however, the frequency of such cells, as compared to control I, was statistically insignificant (Table 1).

The growing root tips, which were allowed different recovery periods after exposure to solar eclipse, have also depicted diverse chromosomal aberrations. In general, with increase in the duration of recovery a trend of decrease in the frequency of abnormal cells was noticed. However, the recovery of cells from the damage caused by solar eclipse seems to be gradual, especially, in *A. cepa* and *P. sativum* (Table 1). All the four test systems differed not only in their sensitivity to the effects of solar eclipse but also in their ability to recover from the initial damage. Of the systems studied, *Allium sativum* appears to be most sensitive.

The results in the present investigation support the view that solar eclipse can bring about clastogeny in eukaryotic systems. Hence, exposure to eclipses might cause increases in the genetic loads of diverse organisms. Perhaps, in this nature's great experiment (Syutti 1939) there lies a *modus operandi* of generating variability in the genetic endowments of various organisms so as to select the fittest variants for the evolution of species. However, it is difficult to visualise the factors responsible for clastogeny during eclipses. The whole spectrum of electromagnetic radiations, constituting primary factors of solar radiations, are altered qualitatively as well as quantitatively during the eclipse period, when compared to normal day. However, these may not directly be responsible for the clastogeny, since all these factors are reduced in intensity as happens during night or on overcast and stormy days (Wojtusiak & Majlert 1976). Presumably, the atmospheric phenomena dependent on solar radiation, constituting secondary factors, like electrical conditions of atmosphere, polarisation of light and geomagnetic field, are responsible for the chromosomal aberrations, as these are the factors which will be disturbed most during the solar eclipse.

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CYTOLOGY OF ANEUPLOIDS IN *RAPHANUS*

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SUMMARY

Cytology and morphology of three aneuploid plants obtained as segregants from the progeny of a colchi-triploid *Raphanus raphanistrum* L. ($2n=3x=27$) was studied. The extra chromosomes in the aneuploids were identified at pachytene and the details are presented.

INTRODUCTION

Aneuploids in *Raphanus* on record were studied for their breeding behaviour (Tokumasu 1967) and meiosis at post pachytene stages by others. But for one species viz. *Raphanus raphanistrum* L. ($2n=18$) (Kamala 1979) information about the pachytene chromosomes in *Raphanus* species is so far practically nil. With the help of the pachytene information aneuploids with $2n=19$ and $2n=22$ obtained from the progeny of a selfed autotriploid *Raphanus raphanistrum* L. ($2n=3x=27$) were studied morphologically and cytologically both at pachytene and post pachytene stages. The details obtained, being the first of its kind in this species, are presented here.

MATERIALS AND METHODS

Fixations for cytological screening were made in a fresh mixture of 1:3 Acetic acid–Absolute alcohol in field between 9 40 a.m. and 10 a.m. and stored in 70% alcohol until further use. Chromosomes were stained with 1% iron acetocarmine.

RESULTS AND DISCUSSION

The progeny obtained after selfing of a colchi-triploid *Raphanus raphanistrum* L. ($2n=3x=27$) plant contained 20% normal diploids with $2n=18$, 60% with $2n=19$ and 10% with $2n=22$.

Cytological screening of diakinetic nuclei revealed that the plants with 19 chromosomes were trisomics with $2n=18+1$ with the highest association being a trivalent plus bivalents; plants with 20 chromosomes were tetrasomics with the highest association being one quadrivalent, and plants with 22 chromosomes were tetrasomic for one chromosome and double trisomic for two different types of chromosomes. Identification of the extra chromosomes at pachytene revealed that out of the 60% trisomics, 20% were triplo-9, a nucleolus organiser in the complement of the diploid, and 15% are triplo-5 and 25% are triplo-6. Plants with 20 chromosomes were tetrasomic for the ninth chromosome which is a nucleolus organiser, and plants with 22 chromosomes were tetrasomic for the ninth and double trisomic for the fifth and sixth chromosomes.

Details of the cytology and morphology of the triplo-9 and tetrasomic-9 were presented elsewhere (Kamala 1980), and this paper deals with the details of triplo-5 and triplo-6 and the plant with 22 chromosomes which were referred with the numbers 7, 1 and 6 respectively (Table 1). Identification of the chromosomes at pachytene were

made on the basis of the total length, the relative lengths of the long and short arms and the number, size and distribution pattern of the heterochromatic segments flanking the centromere in comparison with those in the diploid *Raphanus* plant presented elsewhere (Kamala 1979).

The fifth chromosome in the diploid complement of *Raphanus raphanistrum L.* is median with two proximal heterochromatic segments of equal size of 1.5μ and 2.0μ each, flanking the centromere on the euchromatic long and short arms respectively. The sixth chromosome is submedian with a total length of 45.8μ, with two proximal heterochromatic segments of 29.4μ and 11μ each. The ninth chromosome is subterminal, measuring in total 32.5μ, formed by a long arm of 28 μ and a short arm of 3μ. The centromere is flanked on either side by heterochromatic segments of 1μ size each In addition to this heterochromatic segment the short arm is formed by a distal spherical darkly staining satellite of 0.5μ size and an intercalary heterochromatic chromomere of 1.5μ size.

TABLE 1

Characteristics of aneuploids as compared to the diploids in Raphanus raphanistrum L.

Character	Diploid ($2n=18$)	aneuploid plant number 7 ($2n=18+1$)	1 ($2n=18+1$)	8 ($2n=18+2+1+1$)
Plant height in cms.	139± 0.8	140± 0.2	140± 0.8	120± 0.4
Number of branches	20± 0.6	20± 0.4	18± 0.4	12± 0.5
Stem	Smooth and green with anthocyanin confined to nodes and pods			Smooth green, no anthocyanin, white petals
Flowers	Light pink petals with pink veins			small, slender bracteoles
Pods/branch	8± 2.0	11.5± 0.5	9± 0.3	7± 0.6
Pollen sterility percentage	—0—	28.8	12	15

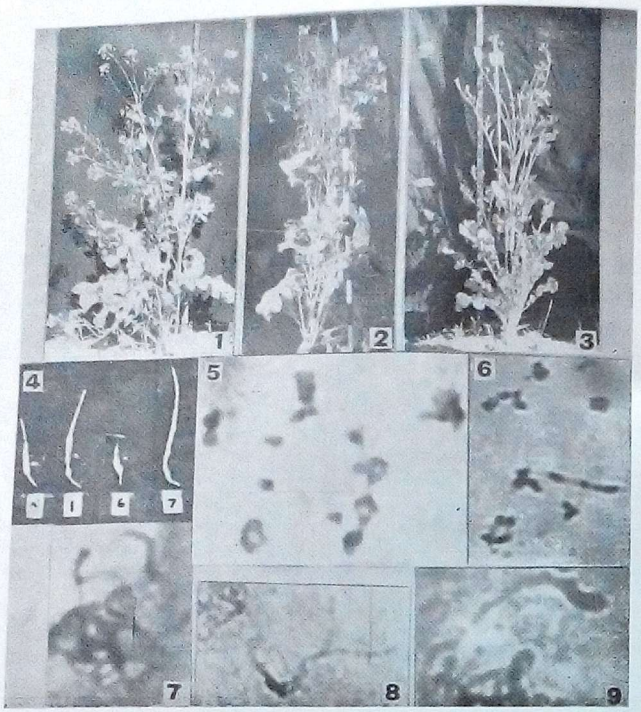
The variety of chromosome associations met with at diakinesis in these aneuploids are presented in Table 2. It is evident that a trivalent is the highest association formed in the trisomic plant nos. 7 and 1 and in plant nos. 6 a quadrivalent with either two trivalents or bivalents plus univalents When compared with the classical configurations of Darlington (1937), the trivalent at diakinesis in triplo-5 conformed to the 8th

TABLE 2

Chromosome associations at diakinesis in aneuploid Raphanus raphanistrum L.

Chromosome association type IV	III	II	I	Percentage of nuclei in plant number 7	1	6
	1	8	0	86	83.3	
	0	8	3	1	48	
	0	9	1	12	11.9	
1	2	6	0			2
0	2	7	2			25
0	2	6	4			20
0	1	8	5			20

and 9th type and in triplo-6 conformed to the type 7. The quadrivalent in plant No. 6 conformed to the 17th type in 2% and to the 11th type in 50% of the nuclei and the trivalents to the 8th and the 7th types.



Figs. 1-3. Aneuploid plant nos. 7, 1 and 6 respectively.
Fig. 4. Pods of aneuploid nos. 7, 6 and 1 respectively.
Fig. 5. Diakinesis with 8 bivalents + 6 univalents in plant no. 6.
Fig. 6. Diakinesis with one ring of four, a chain of three, 6 bivalents, 3 univalents (one bivalent overlapping on ring of four), in plant no. 6.
Fig. 7. Pachytene nucleous in plant no. 6 with tetrasomic nucleolus organising chromosome and trisomic chromosome 5 in plant no. 6.
Fig. 8. Trisomic chromosome 5 in triplo-5 in plant no. 7.
Fig. 9. Tetrasomic chromosome 9- nucleolus organiser in plant no. 6

The 7th type of trivalent forms as a result of one exchange in the centromeric region followed by 2X-ta in a 1:1 ratio in each of the arms. Another chiasma in one of the arms involving the fourth chromosome in an association of four results in the 11th type. The eighth type of trivalent requires one exchange in one of the arms followed by 2X-ta in the same arm (Fig. 8). The ninth requires one exchange in either of the arms with 3X-ta in 2:1 pattern in the two arms. The 17th type results from a single exchange of partners in the centromeric region with 4X-ta distributed in a 2:2 pattern in both the arms. The 17th type of quadrivalent is come across in only 2% of the diakinetic nuclei but the 11th type in 50%, thereby indicating that the short arm of the ninth chromosome could accomodate a second chiasma also resulting in the 17th type which otherwise forms the 11th type usually (Figs. 5 to 9). When the minimum number of exchanges with appropriate number of chiasmata for a multivalent are not formed the extra chromosomes may remain as univalents.

In the multivalents the points for initiation of pairing and separation were the heterochromatic regions around the centromere as seen in some of the early pachytene and the diplotene nuclei. The pairing pattern is 2/1 and 2/2 in the trisomic and the tetrasomic sets at any point, the pairing extending from the centric regions centrifugally along the euchromatic arms.

The 11th and the 17th types of configurations indicate that there is exchange of partners in the heterochromatic regions in the chromosomes of *Raphanus* species also as in the case of the closely allied genus *Brassica* of Cruciferae (Venkateswarlu & Kamala 1974, Kamala 1976) and in *Sorghum* (Reddi 1970).

Segregations at anaphase I and II (Table 2) shows that the extra chromosomes passed intact at random in a high frequency of the nuclei and divided in only a few cases. Inspite of the irregular segregation at microsporogenesis the pollen fertility is fairly high (Table 1). The high percentage of aneuploids with 19, 20, 21 and 22 chromosomes

TABLE 3

Chromosome segregations at anaphase-I and anaphase-II in aneuploid Raphanus raphanistrum L.

Segregation pattern	Percentage nuclei in plant number 7	1	6
Anaphase-I			
9½ : 9½	3.6	17	
10 : 9	96.3	60	
9 : 9+2½*	0	14.2	
11 : 11			8
10 : 12			2
13 : 9			6
Anaphase-II			
10 : 10 : 9 : 9	92	96	
10 : 10 :10: 8	4	0	
10 : 9 : 10 : 8	0	4	
11 : 11 : 11 : 11			25
10 : 12 : 10 : 12			12
other types			63

Note: *half univalents.

in the progeny of these plants in the next generations is obviously due to the high transmission frequency of the extra chromosomes both from the male and the female side.

Morphology of the aneuploids is more or less similar to their disomic sib sown in the same plot. The aneuploids 7, 1 and 6 are equally vigorous and highly fertile with but minor differences in the lobing of leaf and pod size etc. (Figs. 1 to 3). Plant no. 6 with $2n = 18+2+1+1$ had one slender bracteole on the flower pedicels, a feature not seen either in the plants trisomic or tetrasomic for the 9th chromosome (Kamala 1980) or in triplo-5 or triplo-6, and one of the important distinguishing features of Cruciferae from Capparidaceae. This bracteole was seen again in the progeny of this plant with the same set of the 4 extra chromosomes but not in the tetrasomic or the trisomics. This being the only marker character in this plant can be due to the specific gene combinations brought about by the three chromosomes in this particular dosage. But for this, and other minor variations like length of pod and anthocyanin deposition no distinct marker characters could be associated with the root or in the external phenotype in these aneuploids, a fact which is seen also in triplo-9 and tetrasomic-9 of this species. This is apparently due to the presence of duplicate factors in the diploid complement. Further addition of duplications hardly could upset the gene balance and hence the viability, a characteristic of polyploids (Jute, Paria & Basak 1979, *Raphanus*, Kamala 1980). Normally a diploid complement could not tolerate any addition of duplications, but the tolerance of this species to the addition of upto 4 chromosomes associated with a high transmission frequency of these extra chromosomes both from the male and the female side is further proof to the conclusion made earlier from an analysis of the diploid complement of *Raphanus raphanistrum* L. that the haploid number $n=9$ is not the basic number but originated due to secondary balance from a basic set of 6 chromosomes.

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N M U INDUCED VARIATIONS IN *BRASSICA*

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SUMMARY

Five cultivars, Torch, Echo, Polar, Span and Candle were treated with three concentrations, 0.005%, 0.01% and 0.02% of nitrosomethyl urea. The response of the five cultivars to the chemical is almost the same in the three concentrations and 0.01% and 0.02% dosages are lethal since all the plants died after 5 weeks. Leaves with Xantha type patches and cup shape and a significant reduction in the stature with 100 percent pollen and seed sterility is noticed in all the five varieties in 0.005% concentration.

INTRODUCTION

Mutation breeding with physical and chemical mutagens is more widely resorted to than conventional breeding, in order to get a wider spectrum of viable mutations with respect to the polygenically controlled characters like plant height, number of branches, number of pods, number and size of pod and of seed. Five cultivars of *Brassica campestris* L. ($2n=20$), namely, Polar, Torch, Echo, Span and Candle were treated with three concentrations, 0.005%, 0.01% and 0.02% aqueous Nitrosomethylurea and the relative sensitivity of the five varieties is compared. The variations observed in the M_1 generation on the morphological side and the cytology of these treated lines are dealt with in this paper.

MATERIALS AND METHODS

Dry seeds of five cultivars, Torch, Echo, Span, Polar and Candle of *B. campestris* L. ($2n=20$) were treated for 6 hours with three concentrations, 0.005%, 0.01% and 0.02% aqueous solution of Nitrosomethylurea with intermittent shaking, at room temperature. The seeds were washed thoroughly with deionised water after treatment and sown in pots along with their controls.

For cytological studies young flower buds were fixed at 10 a.m. in a fresh mixture of 1:3 acetic acid- rectified spirit for 24 hours and transferred to 70% alcohol. Staining was effected with 1% iron acetocarmine.

RESULTS AND DISCUSSION

Germination on the 15th day after sowing was progressively lower at higher concentrations of NMU in the different varieties (Table 1). Some of the seedlings in all the three concentrations are albinotic and others with chlorotic cotyledons. 0.01% and 0.02% concentrations are toxic since the plants died after five weeks. Detectable morphological variations in 0.005% concentration are, leaves with Xantha type patches and cup shape (Fig. 1) and significant reduction in general stature, flower and pod size.

TABLE 1

Details of Brassica campestris cultivars used, NMU concentrations, germination percentage, plant height in cms, and percentage of pollen sterility

Cultivar	Treatment	germination percentage	Plant height at maturity	Pollen sterility
Span	Control	100	95.3	0
	0.005%	84	23.5	100
	0.01%	56	—	—
	0.02%	100	—	—
Torch	Control	86	100	0
	0.005%	84	25	100
	0.01%	98	—	—
	0.02%	100	—	—
Polar	Control	66	129	0
	0.005%	66	32	31.3
	0.01%	26	—	—
	0.02%	100	—	—
Echo	Control	88	120	0
	0.005%	86	41.5	100
	0.01%	16	—	—
	0.02%	100	—	—
Candle	Control	80	118	0
	0.005%	66	9.2	100
	0.01%	32	—	—
	0.02%		—	—

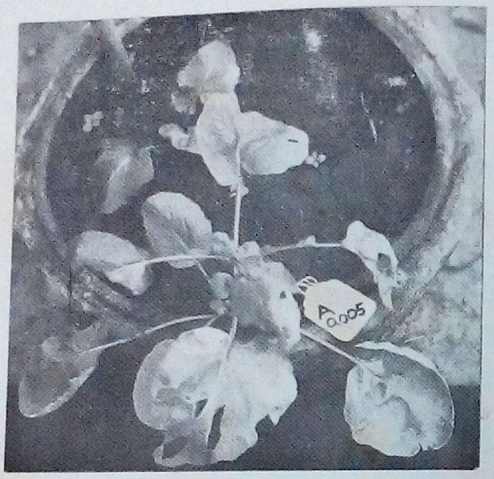

Fig. 1. *Brassica campestris* L. (2n=20) (Span) with Xantha type of patches and cup shaped leaves in 0.005% NMU treatment.

Meiosis studied from a minimum of 100 nuclei in each of the treated lines is more or less the same as in the control with a range of 15.3+0.44 to 16.2+0.27. In spite of the meiosis in anthers being regular, there is complete pollen and seed sterility. The sterility in these treated lines without any visible chromosomal abnormalities is likely to the nitrosocompound as is found also in *Lathyrus* (Nerkar 1976) and rice (Nayak & Misra 1972). The response of the five varieties to the mutagen is almost the same in the three concentrations which is evident from the data in the table.

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THE PRODUCTIVITY OF SOME MUTANTS OF THE MOONG BEAN (*VIGNA RADIATA* (L) WILCZEK) I. BIGGER GRAIN SIZE AND HIGH YIELDING MUTANTS

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SUMMARY

The favourable yielding capacity, protein, methionine and tryptophan contents of induced moong bean mutants make them attractive for moong breeding. The bigger grain size mutants produced a significant increase in the yield in variety Pusa Baisakhi and in protein and methionine contents in variety S-8 as compared to their respective controls. The high yielding mutants showed a significant increase in the yield which was positively associated with methionine contents in variety Pusa Baisakhi and with protein and methionine contents in variety S-8 as compared to their respective controls.

INTRODUCTION

In recent years, induced mutants have been directly released as improved varieties in a wide group of crop plants. Besides, other uses of induced mutations which have a great relevance in modern plant breeding, are reconstruction of plant ideotypes, incorporation of one or two desirable attributes in otherwise well adapted varieties, upgrading of the protein and getting transgressive variants coupled with conventional breeding methods (Swaminathan 1972). Thus, these examples indicate that mutation breeding technique may have a greater role in crops like pulses, where a large part of the natural variability has been eliminated in the process of adaptation to the stress of the environment. This is evident from the fact that out of the total number of 93 registered mutant crop varieties released (Sigurbjornesson 1972), 15 mutant varieties have been released in the case of legumes. The present investigation was undertaken to study the performance of induced bigger grain size and high yielding mutants, so as to make them available to the farmers.

MATERIAL AND METHODS

The mutants were obtained in Pusa Baisakhi and S-8 varieties of moong bean (*Vigna radiata* (L) Wilczek) employing different chemical and physical mutagenic agents such as Ethyl methane sulphonate (0.1%, 0.2%, 0.3%), Nitroso methyl urea (0.01%, 0.02%, 0.03%), Gamma-rays (20 kR, 40 kR, 60 kR) and their combinations (20 kR gamma-rays+0.1% EMS and 20 kR gamma-rays+0.01% NMU only in variety Pusa Baisa khi). The details of mutagenic treatments and mutants has already been described in a separate communication (Singh and Chaturvedi 1980). The protein content in grain was determined by modified Kjeldahl's method (A.O.A.C. 1975) Two essential amino acids, namely methionine and tryptophan, were analysed by colorimetric methods (McCarthey & Paille 1959, Spices & Chamber 1949).

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RESULTS AND DISCUSSION

Bigger grain size mutants: As is evident from Table 1, the frequency of bigger grain size mutants was higher in NMU treated population of variety Pusa Baisakhi and

TABLE 1

Performance of bigger grain size mutants

Treatments	Mutants (%)	100 seed weight per plant Mean± SE(g)	Total grain yield per plant Mean± SE(g)	Protein content	Methionine content	Tryptophan content
Variety Pusa Baisakhi						
0.02% NMU	0.80	3.835± 0.436	10.477± 3.020	24.7083	2.624	1.586
0.03% NMU	0.273	4.650± 0.518	8.470± 2.820	25.1225	2.940	1.490
20 kR gamma-rays +0.1% EMS	0.50	3.895± 0.445	5.925± 1.620	27.3875	3.630	2.020
Control	—	3.213± 0.314	3.529± 0.876	24.1937	2.5252	1.432
CD at 5%		0.857	3.316	2.274	0.533	0.438
Variety S-8						
0.02% EMS	0.284	3.670± 0.319	3.900± 1.140	17.3250	0.800	0.790
0.02% NMU	0.301	3.870± 0.514	5.120± 1.146	23.1875	1.720	0.975
0.03% NMU	0.305	3.860± 0.414	5.060± 1.212	22.4000	1.560	1.637
20 kR gamma-rays	0.282	3.730± 0.321	6.860± 1.645	22.2250	1.272	1.390
40 kR gamma-rays	0.607	3.755± 0.451	6.530± .1475	23.8000	2.335	1.737
60 kR gamma-rays	0.540	4.025± 0.672	4.985± 1.152	24.0625	2.290	1.200
Control	—	2.987± 0.151	6.127± 0.061	20.1250	1.185	1.980
CD at 5%	—	0.908	1.751	1.872	0.868	0.362

Yield value represents average of twentyfive plants.
Estimations represent average of five samples.

gamma-rays treated population of variety S-8. These mutants showed a significant increase in the yield, except in the mutant strains isolated from gamma-rays+EMS treatment, without altering their protein, methionine and tryptophan contents as compared to the control. The increase in the yield ranged from 5.925 g to 10.477 g per plant as compared to the 3.529 g per plant of the control. However, the mutant strains isolated from gamma-rays+EMS treatment showed a significant increase in protein, methionine and tryptophan contents as against the control.

In variety S-8, mutant showed a significant increase in protein and methionine contents, except in 0.2% EMS treatment, without altering their yield and tryptophan contents as compared to the control. The significant increase in the protein content ranged from 22.22% to 24.06% as against 20.12% of control and methionine content ranged from 2.29 to 2.33 as against the 1.18% of control. Hence, in general, the increase in the seed size was positively associated with total grain yield in variety Pusa Baisakhi and with protein and methionine contents in variety S-8 as compared to their respective controls.

High yielding mutants: In variety Pusa Baisakhi, high yielding mutants showed an increase of about 2 to 4 times in the total grain yield as compared to the control. It was interesting to note that the high yielding mutants were isolated only from the chemically

treated population. The frequency of such mutants was high in 0.02% NMU treatments (Table 2) but the highest yield was recorded in gamma-rays + EMS treatment with 12.19 g per plant as compared to the 3.529 g per plant of the control. The increase in the yield, in general, is positively associated with methionine content but protein and tryptophan contents remain unaltered as compared to the control.

TABLE 2

Performance of high yielding mutants

Treatments	Mutants (%)	Total grain yield Mean ± SE (g)	Protein content	Methionine content	Tryptophan content
Variety Pusa Baisakhi					
0.1% EMS	0.615	11.370 ± 1.28	25.2750	3.015	1.505
0.2% EMS	0.285	11.235 ± 1.21	22.2250	2.172	1.018
0.3% EMS	0.307	12.390 ± 1.12	24.1500	3.125	1.285
0.01% NMU	0.571	11.525 ± 1.08	24.1060	2.375	1.012
0.02% NMU	1.33	11.898 ± 2.02	24.4650	2.815	1.387
0.03% NMU	0.547	11.854 ± 1.89	24.3687	2.902	1.405
20 kR gamma-rays + 0.1% EMS	0.50	12.190 ± 1.52	21.6125	1.930	1.675
Control	—	3.529 ± 0.87	24.1937	2.525	1.432
CD at 5%	—	1.722	1.705	0.171	0.189
Variety S-8					
20 kR gamma-rays	0.564	10.100 ± 1.12	22.0060	3.100	1.700
40 kR gamma-rays	0.911	16.040 ± 1.08	21.1160	1.700	1.303
60 kR gamma-rays	0.810	11.490 ± 1.24	22.0620	1.450	1.260
Control	—	6.127 ± 1.06	22.1250	1.185	1.908
CD at 5%	—	1.884	1.409	0.231	0.188

Yield value represents average of twenty five plants
Estimations represent average of five samples.

Unlike the variety Pusa Baisakhi, high yielding mutants in variety S-8 were observed only in gamma-rays treated population and the highest yield was recorded in 40 kR gamma-rays treatment with 16.04 g per plant as compared to 6.127 g per plant of control. The increase in the yield was positively associated with protein and methionine contents but negatively associated with tryptophan contents as compared to the control.

There are several reports on the improvement in total grain yield (Kivi *et al.* 1974, Sigurbjornsson 1975, Chaturvedi & Singh 1980) and in protein content (Gottschalk *et al.* 1975, Tahir Nadeem *et al.* 1978, Singh & Chaturvedi In press) of the induced mutants in various crops. Thus the results make it clear that a large improvement in quality of moong bean varieties is possible through induced mutagenesis.

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RELATIVE SENSITIVITY OF SOME VARIETIES OF *LATHYRUS SATIVUS* L. TO GAMMA IRRADIATION

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SUMMARY

Healthy seeds of uniform size of six varieties of *Lathyrus sativus* L. (2n = 14) were treated with 10, 20, 30, 40 and 50 kR of gamma rays. The relative sensitivity of the variety was evaluated through the effect of ionising radiations on germination, root and shoot length and survival at maturity was recorded. Overall analysis, although indicated genotypic differences in radiosensitivity, they may be grouped on the basis of their sensitivity with respect to M1 indices On the basis of effect on germination P_{288}, P_{293}, LC_{76} were closer to each other than P_{10} and P_{24} which form a different group. P_{585} was entirely different genotypically than the rest of the varieties. Similarly, in respect of root and shoot length, the following groups were found:

Root—(P_{24}, P_{10}), (P_{293}, LC_{76}) and (LC_{76}, P_{288}, P_{585})
Shoot—(P_{24}, LC_{76}) (LC_{76}, P_{10}, P_{288}) and (P_{293}, P_{585})

Dose differences at M1 and dose and varietal differences at M_2 were found to be highly significant. P_{585} among varieties and root in all varieties were noted to be more radiosensitive. The regression co-efficient (regression of shoot on root) and correlation co-efficient between root and shoot at different varieties were highly significant.

INTRODUCTION

There are several evidences to show that radiosensitivity is influenced by both environmental and biological factors. Among biological factors, differential response to radiation of seeds of cereals has been reported to depend on the genetic background of test material (Tsunewaki & Heyne 1959, Jagathesan & Swaminathan 1961, Frey 1964). But the survey of literature reveals relatively little work on radiosensitivity of Leguminoceous species and especially *Lathyrus sativus* KL—one of the important pulse crops. However, influence of genetic factors among legumes have been reported by Monti and Donini (1968) in pea and Ukai & Yamashita (1968) in Soybean. This work, therefore, intends to study the varietal differences in response to gamma radiation.

MATERIALS AND METHODS

Dry seeds of Var. P_{10}, P_{24}, P_{288}, P_{293}, P_{585} and LC_{76} of *Lathyrus sativus* L. (2n=14) having 10% moisture were irradiated with 10, 20, 30, 40 and 50 kR of gamma rays at the dose rate of 330 kR from 1675 curies gamma cell. The method of irradiation has already been reported by Das and Prasad (1980).

All the treated and untreated seeds were allowed to germinate on moist filter paper in petriplates under laboratory conditions at 27±7°C. All germinated seeds were planted in field giving a distance of 1'Xl'. The germination (on 7th day), root and shoot length (on 10th day as detailed by Prasad & Godward 1965) and survival at maturity were measured in each variety and were used as criteria to measure their relative biological response to gamma rays. The data were processed using appropriate statistical methods.

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RESULTS AND DISCUSSION

The response of variety P_{10}, P_{24}, P_{288}, P_{288}, P_{293} and LC_{76} to the various doses of gamma rays was evaluated through the indices of germination, root and shoot length and survival at maturity in M_1 and M_2 generations.

GERMINATION

M_1 (Table 1) showed no effect of treatments on germination in P_{288}, P_{293} and LC_{76} and upto 30 kR in P_{10} and P_{24}; whereas reduction with increasing doses was observed in P_{585} and after 30 kR in P10 and P_{24}. At M_2 generation (raised from M_1 seeds), fall in germination percentage from 30 kR onward in P_{10}, P_{24} and P_{585} and no significant effect were noticeable in P_{288}, P_{293} and LC_{76} (Table 1). A comparison of the effect on M_1 and its M_2 progenies showed greater reduction in percentage germination at M_2 beyond 20 kR. Although the results presented indicate differential potential of the varieties as regard to their response to treatments, they may be grouped into (a) P_{288}, P_{293} and LC_{76} (b) P10 and P_{24} (c) $P_{5\cdot65}$.

TABLE 1

Germination as percent of control in some varieties of Lathyrus sativus L. after gamma rays treatment

Var	Generation	Dose in kR				
		10	20	30	40	50
P_{10}	M_1	103.7	102.2	100.4	91.5	84.6
	M_2	100.6	99.5	71.3	68.3	65.4
P_{24}	M_1	106.9	101.2	100.5	95.9	90.2
	M_2	101.6	95.6	88.0	79.4	74.4
P_{288}	M_1	98.2	96.6	97.6	97.9	97.9
	M_2	99.3	98.7	98.2	93.6	91.8
P_{293}	M_1	103.4	101.6	103.6	102.1	101.4
	M_2	99.9	97.4	98.4	96.3	94.5
P_{585}	M_1	84.9	83.8	68.4	58.7	55.1
	M_2	98.9	94.1	70.7	62.9	54.2
LC_{76}	M_1	99.8	100.6	100.2	101.1	100.4
	M_2	100.3	101.0	98.6	96.4	96.2

The effect of radiation on germination is directly proportional to dose (Gustafsson 1944, Matsumura & Fujii 1958) or there is no effect of treatments (Sastry & Ramiah 1961, Bose & Banerjee 1968, Bari 1971, Roy *et al.* 1971, Prasad & Godward 1975) or there is stimulatory effect (Sax 1963, Suss 1966, Coutinho 1971, Joseph *et al.* 1973). Such effect to mutagenic treatment has been related to differences in the genotypic constitution of seed. Even single gene difference can induce significant changes in radiosensitivity (Sparrow *et al.* 1965). The similarity in spectrum and frequency of mutation depends on degree of closeness of varieties (Enken 1966a and 1966b). But on the contrary the present finding on percentage germination in both the generations suggest that var. P_{288}, P_{293} and LC_{76} are closer to each other than var. P_{10} and P_{24} which showed an almost identical effect; the differences in relation to other varieties as exhibited by P_{585} can be ascribed to its entirely different genotypic constitution.

Our results of no effect on germination percentage, coupled with appreciable effect on survival in var. P_{288}, P_{293} and LC_{76} are in agreement with the findings of Fujii & Matsumura (1958) and Micke (1961). But no effect at lower doses associated with considerable reduction at higher doses on germination percentage in P_{10} and P_{24} are in agreement with the findings of Goud *et al.* (1970), Roy *et al.* (1971) and Prasad & Godward (1975). Dose dependent reduction in germination percentage in P_{585} is similar to that of Gustafsoon (1944) and Matsumura & Fujii (1958). Reduction in germination percentage after 30 kR in P_{10} and P_{24} and after 20 kR in P_{585} in M_2 generation confirms Amer & Hakeem (1964) and Prasad & Godward (1975)'s findings of persistent nature of chromosomal abnormalities induced at M_1.

The overall picture which emerges from this study is that var. P_{288}, P_{293} and LC_{76} are closer to each other as compared to P10 and P24 which are closer to one another. But P_{585} has no close relationship with any of the varieties under study.

TABLE 2

Mean length of root in cm. based on daily observation for 10 days in some varieties of Lathyrus sativus L. (M_1 and M_2) following gamma rays treatment

Dose in kR	Variety						Mean
	P_{10}	P_{24}	P_{288}	O_{293}	P_{585}	LC_{76}	
				M_1			
Control	3.04	3.71	3.45	4.76	3.85	3.29	3.68
10	3.55	2.40	2.51	3.92	2.61	3.31	3.05
20	3.44	3.43	2.45	2.54	2.80	2.44	2.83
30	2.51	2.03	1.99	2.12	2.54	2.48	2.28
40	1.93	2.18	1.86	1.29	1.51	1.95	1.79
50	2.30	1.78	2.11	1.50	1.62	2.20	1.92
Mean	2.78	2.59	2.40	2.69	2.49	2.61	2.59

S.E. Variety or Dose (Mean) = ± 0.20
C.D. at 5% Dose = 0.58
C.D. at 1% Dose = 0.79
Dose at 5% = Control, 10kR, 20 kR, 30 kR, 50 kR, 40 kR

				M_2			
Control	5.69	5.30	4.57	5.07	4.78	5.37	5.13
10	4.81	5.97	5.41	4.47	4.57	4.95	5.03
20	5.27	5.50	3.76	4.42	4.32	4.04	4.55
30	5.22	5.03	4.31	5.10	3.35	4.12	4.52
40	4.94	4.90	3.60	4.07	3.17	3.95	4.10
50	4.95	4.99	3.61	4.91	2.74	3.54	4.04
Mean	5.15	5.28	4.21	4.52	3.82	4.33	4.56

ANOVA

Source	D.f.	S.S.	M.S.	Ratio
Variety	5	77.3262	15.4652	38.19**
Dose	5	64.3122	12.8624	31.77**
Error	270	109.3360	0.4049	

S.E. Variety or Dose (Mean) = ± 0.0825
C.D. at 5% variety or Dose = 0.23
C.D. at 1% variety or Dose = 0.30
Variety at 5% P_{24}, P_{10}, P_{293}, LC_{76}, P_{288}, P_{585}
Dose at 5% Control, 10 kR, 20 kR, 30 kR, 40 kR, 50 kR

ROOT AND SHOOT LENGTH

A general trend of higher the dose greater the damage in root and shoot length (Tables 2 and 3) was apparent besides both root and shoot being longer in length at M_2

TABLE 3

Mean length of shoot in cm. based on daily observation for 10 days in some varieties of Lathyrus sativus L. (M_1 and M_2) following gamma rays treatment

Dose in kR	Variety						Mean
	P_{10}	P_{24}	P_{288}	P_{293}	P_{585}	LC_{76}	
				M_1			
Control	5.95	8.85	9.93	8.55	7.67	6.41	7.89
10	6.21	2.74	7.09	5.72	5.46	4.63	5.31
20	6.28	5.58	5.64	4.91	4.53	4.71	5.32
30	3.51	2.23	3.37	3.71	6.45	4.06	3.89
40	3.18	3.04	2.21	0.98	1.21	3.97	2.43
50	2.92	1.95	1.16	1.51	1.58	2.89	2.43
Mean	4.68	4.11	4.98	4.23	4.48	4.47	4.49

S.E. Variety or Dose (Mean) = ± 0.52
C.D. at 5% Dose = 1.51
C.D. at 1% Dose = 2.05
At 5% Dose = Control, 10 kR, 20 kR, 30 kR, 40 kR, 50 kR

				M_2			
Control	7.43	8.86	6.47	5.72	6.61	7.48	7.09
10	7.51	8.82	6.64	6.01	5.35	8.25	7.10
20	6.60	7.50	6.46	5.83	6.54	7.31	6.71
30	6.00	7.40	5.88	5.92	5.27	6.00	6.08
40	4.98	7.47	5.62	5.37	5.25	5.26	5.66
50	5.46	7.62	5.24	5.00	4.36	4.98	5.44
Mean	6.33	7.95	6.05	5.64	5.56	6.55	6.35

ANOVA

Source	D.f.	S.S.	M.S.	Ratio
Variety	5	227.9593	45.5919	49.72**
Dose	5	156.9643	31.3930	34.23**
Error	270	247.6002	0.9170	

S.E. Variety or dose (Mean) = ± 0.12
C.D. at 5% variety or dose = 0.33
C.D. at 1% variety or dose = 0.44
Variety at 5% = P_{24}, LC_{76}, P_{10}, P_{288}, P_{293}, P_{585}
Dose at 5% = 10 kR, Control, 20 kR, 30 kR, 40 kR, 50 kR.
N.B. Varietal as well as dose differences are highly significant.

than M_2 in all the varieties and at all the doses. P_{24} at 20 kR and P_{10} at 10 kR in M1, however, showed stimulation in root length. This stimulatory effect was found to disappear by 10th day in P_{24} and 7th day in P_{10}. The shoot length showed no stimulation in any variety whatsoever at any of the doses and in any of the generations investigated.

The growth depression expressed as ratio of mean length of root/mean length of shoot per variety (Table 4) which is comparatively more pronounced at M_1 than M_2 at various doses, revealed that the root was more sensitive to radiation than shoot in each variety. Different varieties, however, showed variation in the degree of sensitivity and they may be arranged in order of their decreasing radiosensitivity as such:

1. For shoot length—$P_{585} > P_{293} > P_{288} > P_{10} > LC_{76}$ and $> P_{24}$
2. For root length—$P_{585} > P_{288} > LC_{76} > P_{293} > P_{10}$ and $> P_{24}$.

TABLE 4

Ratio of mean length of root/mean length of shoot on 10th day after gamma ray treatment in six varieties of Lathyrus sativus L.

Dose in kR	Generation	Variety					
		P_{10}	P_{24}	P_{288}	P_{293}	P_{585}	LC_{76}
0.0	M_1	0.47	0.35	0.31	0.51	0.48	0.40
	M_2	0.67	0.59	0.71	0.87	0.68	0.71
10	M_1	0.51	0.80	0.29	0.68	0.45	0.47
	M_2	0.67	0.67	0.68	0.76	0.85	0.60
20	M_1	0.48	0.58	0.33	0.51	0.62	0.49
	M_2	0.71	0.73	0.58	0.72	0.64	0.55
30	M_1	0.56	0.82	0.46	0.56	0.34	0.53
	M_2	0.85	0.67	0.73	0.86	0.63	0.67
40	M_1	0.45	0.66	0.76	1.07	1.15	0.44
	M_2	0.84	0.65	0.64	0.74	0.56	0.74
50	M_1	0.64	0.89	0.96	0.90	0.84	0.77
	M_2	0.90	0.65	0.68	0.83	0.64	0.71

Thus variety P_{585} appears to be more radiosensitive than the other varieties, in respect of both root and shoot length.

Analysis of variance of root and shoot length (Tables 2 and 3) showed that dose differences were highly significant at M_1 and both dose and varietal differences at M_2. Regression co-efficient by x (i.e. shoot on root) and correlation co-efficient of different varieties at different doses (Tables 5 and 6) both at M_1 and M_2 showed that they were all highly significant and both root and shoot showed a similar pattern of response to dose differences.

TABLE 5

Regression co-efficients by x (i.e. of shoot on root) of different varieties at different doses of gamma rays in Lathyrus sativus L.

Dose in kR	Generation	Variety					
		P_{10}	P_{24}	P_{288}	P_{293}	P_{585}	LC_{76}
Control	M_1	2.63	4.35	4.97	2.31	2.53	3.34
	M_2	1.66	2.02	1.75	1.37	1.79	1.81
10	M_1	1.52	1.65	4.53	1.78	2.78	2.75
	M_2	1.76	2.04	1.56	1.08	1.54	2.13
20	M_1	2.70	2.15	3.67	2.66	1.86	2.31
	M_2	1.51	1.82	2.04	1.70	1.89	2.44
30	M_1	2.52	1.82	2.53	2.99	3.63	2.71
	M_2	1.47	2.08	1.80	1.54	2.00	2.06
40	M_1	3.28	2.08	1.47	1.93	1.20	3.31
	M_2	1.24	1.94	1.90	1.83	2.15	1.64
50	M_1	2.17	1.58	1.04	1.06	1.61	1.85
	M_2	1.36	2.09	1.84	1.70	2.15	1.81

N.B. All the regression co-efficients (i.e. regression of shoot on root) of different varieties at different doses are highly significant in both the generations.

TABLE 6

Correlation co-efficients between root and shoot of different varieties at different doses of gamma rays in Lathyrus sativus L.

Dose in kR	Generation	Variety					
		P_{10}	P_{24}	P_{288}	P_{293}	P_{585}	LC_{76}
0.0	M_1	0.9871	0.9712	0.9358	0.8877	0.9931	0.9738
	M_2	0.9876	0.9909	0.9989	0.9977	0.9781	0.9937
10	M_1	0.7713	0.9774	0.9759	0.9918	0.9811	0.9123
	M_2	0.9848	0.9819	0.9960	0.8035	0.9785	0.9965
20	M_1	0.9931	0.9866	0.9602	0.9921	0.9896	0.9782
	M_2	0.9921	0.9660	0.9916	0.9920	0.9723	0.9978
30	M_1	0.9930	0.9672	0.9662	0.9738	0.9874	0.9925
	M_2	0.9932	0.9743	0.9975	0.9905	0.9935	0.9802
40	M_1	0.9750	0.9633	0.9839	0.9612	0.9531	0.9752
	M_2	0.9317	0.9819	0.9807	0.9882	0.9930	0.9962
50	M_1	0.9808	0.8940	0.9645	0.6643	0.9363	0.9836
	M_2	0.9953	0.9735	0.9971	0.9906	0.9874	0.9926

N.B. All the correlation co-efficients between root and shoot of different varieties at different doses are highly significant in both the generations except P_{293} at 50 kR M_1 which is only significant.

It has been shown by Sparrow & Christensen (1953) that the response of genotype to radiation depends upon endogenous level of auxins and ascorbic acid. Gordon (1957) has suggested inhibition of auxin synthesis as a result of irradiation of seeds is responsible for reduction in seedling height. On the other hand inhibition of mitosis by irradiation has been ascribed as a probable reason for decrease in the seedling height and root length (Evans *et al.* 1957, Evans & Scott 1964, Prasad & Godward 1975). Chromosomal aberrations are also supposed to be one of the causes of retardation in growth (Yamagata *et al.* 1969). However, our observation of dose dependent decrease in both root and shoot length indicates that growth inhibition at higher doses is the consequential effect of gross injury at cellular level, either of gene controlled by biochemical or physicochemical processes or both. Less pronounced effect on growth retardation in root and shoot at M_2 than M_1 suggests dilution of initial injury to radiation. Yamagata *et al.* (1969) reported after comparing number of bridges in root and shoot-tips that the seedling growth could be separated into two parts; the growth closely correlated to the frequency of chromosome bridges and growth independent of chromosome aberration. The former is considered to be negatively correlated to the degree of inhibition of cell-division and the latter may possibly be attributed to more enlargement of cell in volume. Sax (1963) and Micke (1961), on the contrary, have reported increase in growth of seedling as compared to that of control at lower doses similar to that of P_{10} and P_{24} at 10 kR and 20 kR M_1 respectively observed in the present experiment. Such stimulation may be either due to enhancement of cell division or enlargement in the size of the cells, which might have been the results of the disturbances initiated by irradiation in the growth regulating mechanism of the cells. Miura *et al.* (1974) reported that the elongation of *Avena* coleoptiles was inhibited by the increase of gamma rays doses which

reduces the IAA activity. Absence of stimulatory effect at M_2 indicates the disappearance of such effect.

Our finding of root being more radiosensitive than shoot is in agreement with Avanzi *et al.* (1966), Dumanovic & Ehrenberg (1955), Johnstone & Klepinger (1967) and Prasad & Godward (1975). Avanzi *et al.* (1966) reported that 10% inhibition of root growth is combined with the fewer chromosomal aberrations than the same percentage of inhibition in shoot growth. The cause of difference in the radiosensitivity is difficult to explain but it may possibly be either due to their anatomical and physiological differences or differences in growth mechanism. A great deal of shoot growth is associated with cell elongation whereas root growth is more dependent on cell division.

It is interesting to note that no significant varietal differences appear in M_1 but significant varietal differences have been observable in M_2 specially when the varieties are genotypic and no karyotypic differences exist among them. No varietal differences in response to radiation in M_1 is in contrast to the findings of Kumar *et al.* (1969), Goud *et al.* (1967 and 1969) and Soriano (1971). But this is comparable to Borojevic & Borojevic (1968) and De Nettancourt & Devreux (1969) where they have observed no genotypic differences to mutagenic treatments. However, it is difficult to suggest any conclusive reason for no varietal differences in M_1 generation, specially when these are genotypic. These findings, no doubt, are suggestive of the need of the better understanding of the role of heterozygosity or heterozygosis in the genetic response of genotypes to radiation because of its possible practical application in the area of mutagenesis. The varieties showed significant differences in radiosensitivity at M_2 and that P_{585} and P_{24} occupied the two extremities. This establishes the fact that variety P_{585} is more radiosensitive and P_{24} is more radioresistant. But Anova for root and shoot (M_2) at 5% for varieties indicates P_{24}, P_{10}, P_{293}, LC_{76}, P_{288}, P_{585} in root and P_{24}, LC_{76}, P_{10}, P_{288}, P_{293}, P_{585} relationship among the varieties. Grouping of varieties has also been suggested by our observation on percentage germination. Such grouping of varieties suggests that they comprise different sensitivity group. However, in view of Mathur's (1961) suggestion that gamma irradiation impaires a possible endogenous gibberellin synthesising system, it may be considered that P_{585} had low level of endogenous GA and hence high growth retardation than the rest.

SURVIVAL

A general exponential fall is indicated in all the varieties in both the generations (Table 7) although P_{24} and P_{288} at 20 kR showed slightly higher survival percentage than its immediate lower dose. This stimulatory effect has completely disappeared at M_2. Survival percentage at M_2 is much less than M_1 at all doses. This indicates persisting bature of namage induced at M_1. Although no significant difference between varieties is seen at M_2, P_{585} appears to be more radiosensitive than others.

The problem of survival after seed irradiation has been discussed by many workers (Ehrenberg 1955, Fujii & Matsumura 1958, Amer & Hakeem 1964, Bari 1971, Prasad & Godward 1975). The survival percentage would be expected to fall with increase in doses although Fujii & Matsumura (1958) have reported an increase in the germination percentage followed by the death of seedlings at higher doses. It appears

TABLE 7

Percentage survival as that of control after gamma rays treatment in different varieties of Lathyrus sativus L. at maturity

Dose in kR	Generation	Variety P_{10}	P_{24}	P_{288}	P_{293}	P_{585}	LC_{76}
10	M_1	83.3	82.2	76.2	72.2	70.0	71.4
	M_2	95.8	98.0	91.3	85.0	89.4	90.0
20	M_1	75.5	84.0	83.3	60.4	68.0	51.4
	M_2	86.2	89.8	84.9	82.7	86.0	82.3
30	M_1	60.4	62.0	72.2	58.3	48.0	41.9
	M_2	80.4	83.3	80.0	80.0	80.0	79.0
40	M_1	59.1	66.0	61.8	54.1	38.0	36.9
	M_2	79.6	80.0	73.5	79.0	72.7	76.0
50	M_1	52.8	62.4	60.4	47.2	38.0	35.4
	M_2	69.9	75.7	70.0	68.9	61.3	70.8

that although the germination may not be affected heavily by increase of dose, the survival is much less at higher doses. The present observations on germination percentage, although show a great deal of diversities following gamma irradiation, the survival percentage showed a general exponential fall with the increase of doses. This may be expected as the doses are directly proportional to the chromosomal damage. So far as the percentage of survival at M_2 generation is concerned, absence of any marked effect as compared to control is in close agreement with the findings of Amer & Hakeem (1964) in *Lupinus termis*.

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EVOLUTIONARY SIGNIFICANCE OF CHROMOSOMAL ASSOCIATION IN *PONCIRUS—CITRUS* HYBRIDS

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SUMMARY

Meiotic behaviour of chromosomes in pollen mother cells was examined in *Poncirus trifoliata*, its selections and in two intergeneric hybrids with *Citrus* spp. Fairly regular bivalent formation takes place during meiosis. Relatively complete pairing between *Citrus* and *Poncirus* chromosomes in intergeneric hybrids implies close similarity in the chromosome compliments of the two genera. The stability of chromosome conjugation seems to increase with time intervals that have passed since the initial hybridization had taken place. An evaluation of the cytological findings of the hybrids has been discussed for phylogenetic relationship.

INTRODUCTION

It is now well recognised that both interspecific and intergeneric hybridization has played a role in the evolution of new species in nature. It goes without saying that the examination of hybrid meiosis is of special value in comparing karyotypes of distinct entities for it is only when these are brought together into the same cell that we can assess their structural and genic homologies. The chromosome rearrangements that have played a role in evolution are principally exchanges in which parts of a chromosome or chromosomes establish new spatial relationship. Therefore, the study of intergeneric hybrids have become an integral part of new systematics, since it serves as a valuable clue in determining the inter-relationship between the two genera and following their probable mode of evolution. The present paper deals with the details of cytological behaviour of *Poncirus trifoliata* Raf., its selections and their hybrids with *Citrus sinensis* and the appraisal of evolutionary significance

MATERIALS AND METHODS

The plant material consists of *Poncirus trifoliata*, an allied genera of *Citrus* (Rutaceae), its two selections, Roubidoux and Pomeroy and two intergeneric hybrids between *Poncirus* and *Citrus sinensis*. These were collected from the citrus orchard of Horticultural Research Institute, Saharanpur.

For cytological examination, young flower buds from individual trees were fixed randomly in Carnoy's fixative supplemented with $FeCl_3$. Only half or one third of an anther was squashed in 2% acetocarmine. Analysis of various stages of meiosis were best done from fresh preparations, chiefly at first metaphase and anaphase.

The meiotic data was statistically analysed by partial analysis of variance proposed by Mather (1936).

RESULTS AND DISCUSSION

The monotypic genus *Poncirus*, its two selections and their integeneric hybrids with *C. sinensis*, examined in the present investigation had the expected chromosome number

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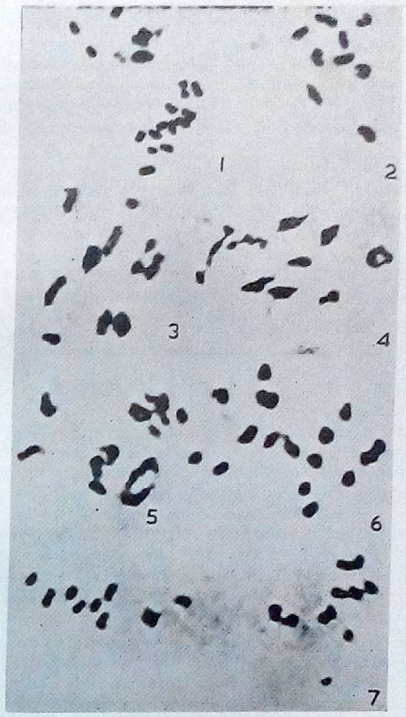
of 2x=18 (Fig. 1). Chromosome behaviour in PMCs in the two hybrids, Troyer (naval orange × trifoliate orange) and Carrizo (trifoliate orange × sweet orange) showed the occurrence of nine bivalents in most of the cells at meiotic metaphase I. The nature and frequencies of various types of chromosome associations and chiasmata has been summarized in Table 1. Although pairing was observed to be predominantly



Figs. 1–7. Various chromosome configurations at meiotic division in PMCs of hybrids between *Poncirus trifoliata* and *Citrus sinensis*. 1. Somatic cell with 2x=18 chromosomes in Carrizo hybrid. 2. Diakinesis showing regular nine bivalents. 3. Diakinesis, 8 II+2 I, in Troyer hybrid. 4. Metaphase I, 9 II in Troyer hybrid. 5. M-I showing a ring of four chromosomes in Troyer hybrid. 6. A-I, showing normal 9:9 separation. 7. M-II, 10–8 distribution and precocious movement of one chromosome.

bivalents (Figs. 2 & 4), the only exception was that one or two cells had a quadrivalent (Fig. 5). No trivalents or any other higher valents were noticed any time. Two univalents commonly occurred in a cell (Fig. 3), while four were rarely observed. The bivalents regularly disjoined to the poles (Fig. 6). The second division metaphase spindles function normally but often showed precocious movement of one chromosome (Fig. 7). The pollen sterility ranged from 7.0 to 25%.

TABLE 1

Chromosome configurations at metaphase I in PMCs of Poncirus trifoliata, its selections and in the intergeneric hybrids

Name	Number of PMCs studied	Percent of abnormal cells	*Ring bivalents* Range	Mean	*Rod bivalents* Range	Mean	*Univalents* Range	Mean	Chiasma per chromosome (A.M.± S.E.)	Pollen stainability (%)
Poncirus trifoliata	362	12.26	2–8	5.73	1–7	3.06	0–2	0.03	0.81± 0.02	93.2
Roubidoux	65	12.06	3–9	6.95	1–6	1.75	0–2	0.19	0.88± 0.03	97.0
Pomeroy	63	20.06	3–9	7.06	1–6	1.77	0–2	0.32	0.87± 0.03	86.4
Citrus sinensis	60	16.35	1–9	5.81	1–7	2.88	0–2	0.60	0.85± 0.04	86.0
Troyer	85	18.11	2–9	6.37	1–6	2.49	0–4	0.40	0.83± 0.03	85.2
Carrizo	122	13.04	3–9	6.52	1–6	2.36	0–2	0.11	0.83± 0.02	56.5

A.M.± S.E. = Actual Mean± Standard Error.

The chromosome pairing in intergeneric hybrids throw light on the homology of chromosomes between the genera involved, and secondly, the fertility on the male and female side in hybrids gives a measure of true homology between parents. In intergeneric hybrids if bivalents are formed it is inferred that certain degrees of homology proportional to the frequency of bivalents exist between the taxa. However, it should not be overlooked that certain amount of autosyndetic pairing may also occur due to the presence of translocated duplicated homologous segments on otherwise non-homologous chromosomes within the genome. The presence of such homologous segments on otherwise non-homologous chromosomes within the genome is non-discernible in parent species because of preferential pairing restricted to homologous set. Whether autosyndetic pairing occurs can be assessed by the occurrence of multivalent in hybrid plants where alongwith intergenomic pairing there may occur intragenomic pairing too. This is possible in a hybrid condition because of the absence of true homologues. Pairing between strictly true homologues in pure species is an evolutionary process and has been achieved through generations of reproductive cycles.

Of the different species and varieties of the genus *Citrus* (Anis 1980) and their hybrids studied in the present investigation, all have shown 2n=18 chromosomes. Nine bivalents have been observed clearly in a large number of meiotic cells. Such a regular constancy in chromosome number in a genus with extensive vegetative propagation is quite remarkable. However, the explanation of such a stability possibly lies in the fact that in addition to vegetative propagation, seed setting is profuse and sexual reproduction is normal In the absence of any other peculiarity in chromosome behaviour the nine chromosomes may for the present be cons idered as not only haploid but even the basic set for the genus *Citrus*. The rare occurrence of multivalents with very low frequency

in Troyer hybrid may probably result from stickiness or a homoeologous attraction between non-homologous chromosomes. The size of the chromosome of course stands in the way of the formation of multivalents.

It is apparent from the foregoing discussion that the different species share a common basic structural genome which would account for the high pairing affinity among their chromosomes. The absence of multivalents in hybrids suggests that if the genomes of the parental species differ in constitution, the differences are the result of small structural rearrangements which could be detected by conventional cytological techniques. These cytogenetic disruption might have been gradually stabilized in course of existence giving rise to closely related species group. Further, the absence of genuine multivalent formation during meiosis in intergeneric hybrids provide unequivocal corroborative evidence to the fact that the meiotic chromosomes in the hybrids are paired exclusively as bivalents.

Nakamura (1934, 1942) observed the prevalance of chromosomal affinities in the intergeneric hybrids between *Citrus* and *Poncirus* or *Fortunella*, as well as in the trigeneric hybrid of these three genera. From this fact he concluded that *Citrus*, *Poncirus* and *Fortunella* could be represented by the same genome.

It has been observed in *Poncirus trifoliata*, its selections and in one of the intergeneric hybrids that there is a negative correlation between bivalents in relation to interchromosomal distribution of chiasmata, while in Troyer hybrid positive correlation was noticed (Table 2). A positive correlation between the chiasma frequency of bivalents or the groups of bivalents tend to suggest that when the numbers of chiasmata is less or more in one bivalent or group, it tends to be lesser or more in other bivalent groups in the same nucleus. A negative correlation means that an increase in the number of chiasmata in one bivalent or group is followed by a decrease in the chiasma number in the other or the same nucleus. These findings are in accordance with the results reported by Mather (1936).

TABLE 2

Analysis of variance for differences between nuclei and between bivalents within nuclei in Poncirus trifoliata, its selections and in intergeneric hybrids

Genotypes	Items	S.S.	N.	M.S.	Variance Ratio (VR)	Intra-class correlation
Poncirus trifoliata	Between nuclei	20.05	111	0.181	1.965**	(—) 0.0317
	Within nuclei	221.88	899	0.246		
Roubidoux	Between nuclei	7.88	63	0.125	1.507**	(—) 0.0394
	Within nuclei	95.77	508	0.188		
Pomeroy	Between nuclei	7.25	62	0.117	2.094*	(—) 0.0616
	Within nuclei	121.55	496	0.245		
Troyer hybrid	Between nuclei	28.45	84	0.338	0.652	(+) 0.0535
	Within nuclei	150.88	683	0.220		
Carrizo hybrid	Between nuclei	17.46	121	0.144	1.414*	(—) 0.0339
	Within nuclei	198.44	972	0.204		

*Significant at 0.001 % P—level
**Significant at 0.05 % P—level

A rational evaluation of the role of sexual and asexual reproduction in speciation in this group suggests that the latter is more suited for the process than the former.

bivalents (Figs. 2 & 4), the only exception was that one or two cells had a quadrivalent (Fig. 5). No trivalents or any other higher valents were noticed any time. Two univalents commonly occurred in a cell (Fig. 3), while four were rarely observed. The bivalents regularly disjoined to the poles (Fig. 6). The second division metaphase spindles function normally but often showed precocious movement of one chromosome (Fig. 7). The pollen sterility ranged from 7.0 to 25%.

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Carrizo	122	13.04	3-9	6.52	1-6	2.36	0-2	0.11	0.83± 0.02	56.5

A.M.± S.E. = Actual Mean± Standard Error.

The chromosome pairing in intergeneric hybrids throw light on the homology of chromosomes between the genera involved, and secondly, the fertility on the male and female side in hybrids gives a measure of true homology between parents. In intergeneric hybrids if bivalents are formed it is inferred that certain degrees of homology proportional to the frequency of bivalents exist between the taxa. However, it should not be overlooked that certain amount of autosyndetic pairing may also occur due to the presence of translocated duplicated homologous segments on otherwise non-homologous chromosomes within the genome. The presence of such homologous segments on otherwise non-homologous chromosomes within the genome is non-discernible in parent species because of preferential pairing restricted to homologous set. Whether autosyndetic pairing occurs can be assessed by the occurrence of multivalent in hybrid plants where alongwith intergenomic pairing there may occur intragenomic pairing too. This is possible in a hybrid condition because of the absence of true homologues. Pairing between strictly true homologues in pure species is an evolutionary process and has been achieved through generations of reproductive cycles.

Of the different species and varieties of the genus *Citrus* (Anis 1980) and their hybrids studied in the present investigation, all have shown 2n=18 chromosomes. Nine bivalents have been observed clearly in a large number of meiotic cells. Such a regular constancy in chromosome number in a genus with extensive vegetative propagation is quite remarkable. However, the explanation of such a stability possibly lies in the fact that in addition to vegetative propagation, seed setting is profuse and sexual reproduction is normal. In the absence of any other peculiarity in chromosome behaviour the nine chromosomes may for the present be cons idered as not only haploid but even the basic set for the genus *Citrus*. The rare occurrence of multivalents with very low frequency

in Troyer hybrid may probably result from stickiness or a homoeologous attraction between non-homologous chromosomes. The size of the chromosome of course stands in the way of the formation of multivalents.

It is apparent from the foregoing discussion that the different species share a common basic structural genome which would account for the high pairing affinity among their chromosomes. The absence of multivalents in hybrids suggests that if the genomes of the parental species differ in constitution, the differences are the result of small structural rearrangements which could be detected by conventional cytological techniques. These cytogenetic disruption might have been gradually stabilized in course of existence giving rise to closely related species group. Further, the absence of genuine multivalent formation during meiosis in intergeneric hybrids provide unequivocal corroborative evidence to the fact that the meiotic chromosomes in the hybrids are paired exclusively as bivalents.

Nakamura (1934, 1942) observed the prevalance of chromosomal affinities in the intergeneric hybrids between *Citrus* and *Poncirus* or *Fortunella*, as well as in the trigeneric hybrid of these three genera. From this fact he concluded that *Citrus*, *Poncirus* and *Fortunella* could be represented by the same genome.

It has been observed in *Poncirus trifoliata*, its selections and in one of the intergeneric hybrids that there is a negative correlation between bivalents in relation to interchromosomal distribution of chiasmata, while in Troyer hybrid positive correlation was noticed (Table 2). A positive correlation between the chiasma frequency of bivalents or the groups of bivalents tend to suggest that when the numbers of chiasmata is less or more in one bivalent or group, it tends to be lesser or more in other bivalent groups in the same nucleus. A negative correlation means that an increase in the number of chiasmata in one bivalent or group is followed by a decrease in the chiasma number in the other or the same nucleus. These findings are in accordance with the results reported by Mather (1936).

TABLE 2

Analysis of variance for differences between nuclei and between bivalents within nuclei in Poncirus trifoliata, its selections and in intergeneric hybrids

Genotypes	Items	S.S.	N.	M.S.	Variance Ratio (VR)	Intra-class correlation
Poncirus trifoliata	Between nuclei	20.05	111	0.181	1.365**	(—) 0.0317
	Within nuclei	221.88	899	0.246		
Roubidoux	Between nuclei	7.88	63	0.125	1.507**	(—) 0.0394
	Within nuclei	95.77	508	0.188		
Pomeroy	Between nuclei	7.25	62	0.117	2.094*	(—) 0.0616
	Within nuclei	121.55	496	0.245		
Troyer hybrid	Between nuclei	28.45	84	0.338	0.652	(+) 0.0535
	Within nuclei	150.88	683	0.220		
Carrizo hybrid	Between nuclei	17.46	121	0.144	1.414*	(—) 0.0339
	Within nuclei	198.44	972	0.204		

*Significant at 0.001 % P—level
**Significant at 0.05 % P—level

A rational evaluation of the role of sexual and asexual reproduction in speciation in this group suggests that the latter is more suited for the process than the former.

The reason for this is the fact that in sexual reproduction, a number of conditions must be satisfied for the origin of such forms. These conditions include the occurrence of variations in both parents, their chance union, and the survival of the embryo. On the other hand, in propagation by asexual means, no such conditions are to be satisfied and a new form can originate through a single step in evolution.

It is concluded from the study that the great diversity of characteristics prevailing in the genus *Citrus* are considered to have developed within the diploid level, passing through gene mutation, structural chromosomal changes and hybridizations, and continuing usually the apogamic or sometimes sexual reproductions.

ACKNOWLEDGEMENTS

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GROWTH AND KARYOKINETIC ACTIVITY OF GAMMA IRRADIATED DIPLOID-AUTOTETRAPLOID FENUGREEK

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SUMMARY

Comparative root growth and karyokinetic response of 2x and artificially raised 4x of different genotypes of fenugreek (*Trigonella foenum-graecum*) at single dose (40 K rad) of gamma-rays have been studied. 2x and 4x radiosensitivity at different parameters of root system indicated 4x of sels. 1, 3 and 4 to be resistant while sel. 2 to be sensitive. Differential response of different selections to irradiation at 4x level appears to be genotypically controlled. Stimulatory effect of radiation on mitotic index at certain duration in 4x was noted. A possible reason of slower growth rate of roots and cell division frequency of autotetraploids is given. Chromosomal aberrations per chromosome revealed autotetraploids to have higher anomalies which may be due to their increased interphase chromosome volume (ICV). Elimination of various chromosomal aberrations with passage of time was recorded.

INTRODUCTION

Increasing attention has been paid towards the study of diploid—polyploid radiosensitivity alongwith physiological and genetical effects of radiation (Bhaskaran & Swaminathan 1960, Raghuvanshi 1978). This topic is of applied significance in plant genetics owing to a large proportion of domesticated plants being polyploids and in animal genetics owing to the widespread occurrence of polyploidy in malignant and pathological growth. In this context cytological investigations appear rewarding which deals with the primary genetic material, the chromosomes and more appropriately, the DNA which controls the phenotype. Therefore, studies involving chromosomal aberrations and their genetic consequences form an integral part of most of work on radiation genetics. In the present paper comparative root growth and karyokinetic response of 2x and 4x genotypes of fenugreek (*Trigonella foenum-graecum* L.) have been presented. It is presumed that apart from the accuracy of cytological criteria, the mitotic configurations may provide the present investigators an explicit array from which further treatments could be made depending on the extent and quality of the variability desired for radiomutation program in this economically useful leguminous crop.

MATERIAL AND METHODS

270 seeds of 2x and 4x of different genotypes (sels. 1, 2, 3 and 4) of fenugreek were irradiated at 40 K rad of gamma rays (dose rate—72 seconds per K rad, source—Co^{60}). First lots of 100 seeds were utilized for studying comparative radiosensitivity through shoot system, the results of which shall be presented elsewhere.

For the study of root growth and karyokinetic activity, remaining lots of 170 irradiated seeds were placed on moist blotting paper in separate petridishes for germinations. Controls were also grown for comparison. Root tips were fixed in 1:3 acetic alcohol to score first mitosis (24 hrs) then at 48, 72 and finally at 120 hours. Simulta-

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neous measurements of roots of 30 seedlings numbered from 1 to 30 were made in each set. Thus it was possible to follow the growth rate of individual seedlings. Haematoxylin (4%) squashes of root tips were prepared after hydrolysing them in 1N HCl. From each slide 5 spots were studied at random for scoring the number of dividing and non-dividing cells along with various chromosomal aberrations. At different durations 5 root tips, each from control and dose, both in 2x and 4x of different genotypes were fixed and 25 spots were scored from each set. Mitotic indices (%) were calculated by applying the formula—

$$\frac{\text{No. of dividing cells observed}}{\text{No. of total cells studied}} \times 100$$

Mitotic indices of young shoot tips and number of secondary roots were recorded after 144 and 288 hours of germination, respectively.

RESULTS AND DISCUSSION

Percent of control data on root growth of 2x and 4x genotypes clearly indicated that 4x of sels. 1 and 3 were constantly superior to their 2x progenitors (Figs. 1 and 5) though degree of their resistance varied at different durations. Susceptibility of 4x of sel. 2 was evident at 24, 72 and 120 hours (Fig. 3). Comparison of 2x and 4x of sel. 4 to rest of the genotypes was not possible as growth in this case was measured at 120 hours only. However, at this duration 4x of sel. 4 was superior than their 2x. Growth inhibition of roots following irradiation may be attributed to both inhibition of synthesis of growth stimulating auxins and inhibition of cell division in the meristem.

In controls, mitotic indices of 4x were reduced than respective 2x. At dose 2x of different genotypes showed severe effects (Figs. 9-11). Initially in 2x mitotic index was highest in sel. 3 while among 4x sel. 2 was leading (Fig. 10). When 2x was compared with the corresponding 4x, at this parameter sharper differences were shown by sel. 3 (Fig. 11). With passage of time mitotic index decreased in all the sets both at 2x and 4x levels. At dose, response was similar on the first day. Clearcut resistance of 4x of sels. 1 (Fig. 2) and 3 (Fig. 6) was apparent leaving 4 x of sel. 2 again to be radiosusceptible (Fig. 4). It may be mentioned that in 2x values of sel. 2 were constantly higher than sels. 1 and 3, though its 4x suffered marked loss. Obviously, radioresponse of diploid may not always be a measure for predicting the sensitivity of corresponding autotetraploids. Radiation induced stimulation in 4x of sel. 1 at 72 hours and in sel. 3 at 48 hours is apparent. Peaks in number of dividing cells were noted by Raghuvanshi & Singh (1978) in 2x *Trigonella foenum-graecum* at 60 K rad and in 4x at 30 K rad and they concluded that comparatively lower dose may cause maximum stimulation in 4x.

It is now well established that DNA content and cycle time are directly related in diploid plants. A cell which has to replicate twice as much DNA as another cell will take twice as much time to complete the replication (Von't Hof & Sparrow 1963) Assuming this as a case it is possible that 4x may take more time for their replication. This would have resulted in lower growth rate and division frequency in autotetraploids.

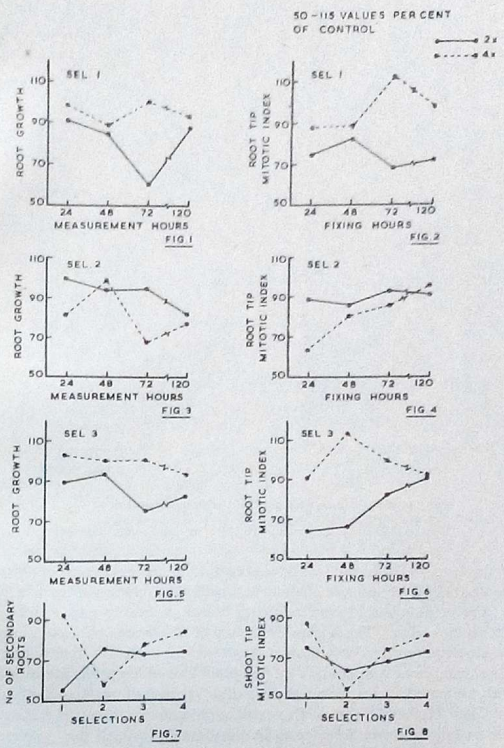

Fig. 1. Root growth of sel. 1
Fig. 2. Mitotic index of sel. 1
Fig. 3. Root growth of sel. 2
Fig. 4. Mitotic index of sel. 2
Fig. 5. Root growth of sel. 3
Fig. 6. Mitotic index of sel. 3
Fig. 7. Number of secondary roots in sels. 1, 2, 3 and 4.
Fig. 8. Mitotic index in shoot tips of sels. 1, 2, 3 and 4.

Laggards, fragments (Figs 12 and 14), strays and ring chromosomes (Fig. 13) were observed in the irradiated material of different genotypes. Comparing 2x with 4x with

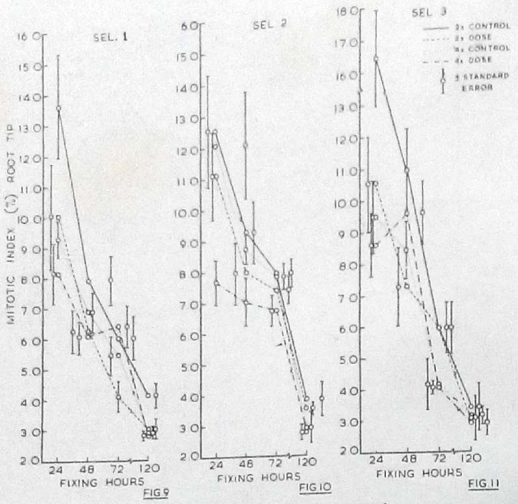

Fig. 9. Mitotic index (mean± S.E.) of 2x and 4x of sel. 1.
Fig. 10. Mitotic index (mean± SE) of 2x and 4x of sel. 2.
Fig. 11. Mitotic index (mean± (S.E.) of 2x and 4x of sel. 3.

regard to frequency of abnormal cells, genotypic differences become apparent. 4x in general showed higher frequency of abnormal cells than their progenitors (Table 1). While at 2x level sel. 3 had highest frequency but at 4x level it is sel. 2 which showed maximum abnormality. The higher frequency of abnormal cells noted in 4x may be due to double the number of chromosomes than present in 2x. We have already noted interphase chromosome volume (ICV) of polyploid lines of *Trigonella foenum-graecum* to be significantly increased in comparison to their diploid progenitors (Raghuvanshi & Singh, Anil K. 1980). Higher ICV may also explain greater chromosomal damage in tetraploids due to larger target area for radiation damage. With the passage of time abnormal cells were gradually being eliminated in all the cases (Table 1). However, this elimination was faster in sel. 3 though this is also a genotype having highest frequency of abnormal cells at the initial stages. If this data is recalculated on per chromosome basis then obviously 2x in all the cases will prove to be mostly sensitive. Aberrations per chromosome indicated 4x of sel. 2 to have higher anomalies in comparison to their 2x (Table 1). This is the genotype which is most radiosensitive among different 4x at growth and mitotic index parameters.

Gradual decrease in aberrations per chromosome with passage of time is evident from Table 1. Decrease or complete absence of certain anomalies in last set

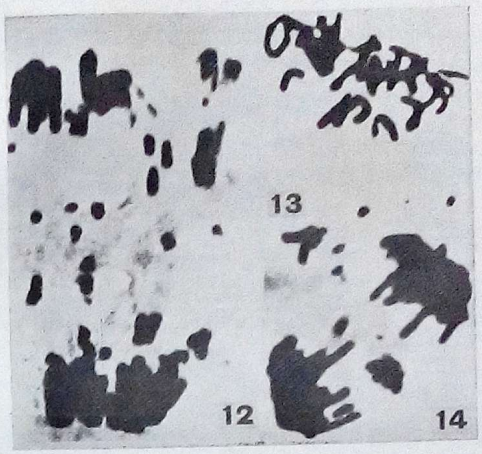

Fig. 12. Numerous fragments and laggards at anaphase in 4x of sel. 2.
Fig. 13. Ring chromosome and fragments at metaphase in 2x of sel. 3.
Fig. 14. Laggards and fragments at anaphase in 2x of sel. 1.

(120 hours) was noted. However, at 24, 48 and 72 hours 2x and 4x of different genotypes do not follow an uniform pattern in elimination of various aberrations, obviously their response is genotype dependent. One important point to be noted is that though in 4x of all the selections at different durations one or other anomaly was in higher frequency but compared to respective 2x, 4x of sel. 2 took the lead. Prasad & Godward (1974) reported exponential type of elimination pattern in *Phalaris*. In the present case the reaction of 2x and 4x of different genotypes appears to be rather complex that cannot be covered in a single explanation.

Mitotic index from shoot tips indicated inverse correlation to dose. At this parameter greater radiation tolerance of 4x of sels. 1, 3 and 4 was evident, leaving sel. 2 to be radiosusceptible than their 2x relative (Fig. 8). Parameter for number of secondary roots follow the same pattern with the parameter of mitotic index of shoot tips upto the extent that 4x of sels. 1, 3 and 4 were resistant and that of sel. 2 was sensitive (Fig. 7).

However, when we make comparisons among parameters for mitotic index, root growth and abnormal cells, cellular heterogeneity with regard toradiosensitivity should not be ruled out. It is not certain that we are comparing homogeneous population of cells at particular fixation time. In *Vicia faba* differences between cells with regard to radiosensitivity (Savage & Wigglesworth 1970) and mitotic cycle (Murin 1967) have been reported.

TABLE 1

Percentage of abnormal cells and different types of chromosomal aberrations (per chromosome) at different fixing hours

Fixing hours	Different abnormalities	Sel. 1 2x	Sel. 1 4x	Sel. 2 2x	Sel. 2 4x	Sel. 3 2x	Sel. 3 4x	Sel. 4 2x	Sel. 4 4x
24 hours	% abnormal cells	19.78	27.14	23.19	33.87	25.00	31.53	—	—
	Laggards*	0.0068	0.0037	0.0051	0.0040	0.0048	0.0032	—	—
	Fragments*	0.0048	0.0042	0.0028	0.0030	0.0042	0.004	—	—
	Strays*	0.0027	0.0046	0.0028	0.0025	0.0030	0.0021	—	—
48 hours	% abnormal cells	17.56	21.66	18.18	29.18	13.58	25.58	—	—
	Laggards	0.0050	0.0031	0.0066	0.0101	0.0056	0.0032	—	—
	Fragments	0.0042	0.0026	0.0044	0.0220	0.0048	0.0054	—	—
	Strays	0.0016	0.0031	0.0029	0.0059	0.0016	0.0025	—	—
72 hours	% abnormal cells	09.67	16.66	10.30	16.89	07.69	14.78	—	—
	Laggards	0.0020	0.0020	0.0019	0.0021	0.0039	0.0022	—	—
	Fragments	0.0040	0.0032	0.0051	0.0063	0.0039	0.0057	—	—
	Strays	0.0010	0.0008	0.0012	0.0014	0.0019	0.0013	—	—
120 hours	% abnormal cells	05.50	10.81	06.06	13.39	03.57	09.09	06.57	11.33
	Laggards	0.0019	0.0025	0.0056	0.002	10.0022	0.0021	0.0019	0.0014
	Fragments	0.0039	0.0033	0.0037	0.0021	nil	0.0035	0.0040	0.0044
	Strays	nil	0.0008	nil	0.0010	nil	0.0007	0.0031	0.0184

*Values of laggards, fragments and strays on per chromosome basis
—Not studied.

Our earlier experiment in shoot system in the present material has clearly demonstrated radiosensitivity to be genotype dependent. Present experiment carried out in root system confirms the above findings. While autotetraploids of sels. 1, 3 and 4 emerged to be radioresistant, sel. 2 proved to be highly susceptible all along. Information so far obtained in different genotypes of *Trigonella foenum-graecum* indicated that different rather than common mechanisms operate. The genetic redundancy and ICV appears to play less important roles (Raghuvanshi & Singh, Anil K. 1980) rather than the genotypes in the present case.

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DIFFERENTIAL MUTAGENIC SENSITIVITY OF THREE VARIETIES OF *ALLIUM CEPA* L.

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SUMMARY

Differential mutagenicity of three different mutagens i.e. γ-rays, EMS and Mitomycin C on three varieties of *Allium cepa* L., White, Nasik Red and Bellari Red, was studied with regard to percentage germination, seedling height, survival and mitotic abnormalities. All the three varieties showed a different response to the mutagens and Bellari Red was found to be most sensitive among the three varieties. One peculiar feature observed in the response to flowering time was that the most resistant variety flowered much earlier than control, indicating that there need not necessarily be direct correlation between mutagen sensitivity and induction of early flowering. The results are discussed in the light of previous work on these lines.

INTRODUCTION

The potent mutagenic agents of foremost importance and presently in use in higher plants are ionizing radiations and alkylating chemicals. They all induce changes covering a broad range of mechanisms. The response of any organism to the mutagen depends on various factors such as total dose, intensity, method of treatment, genotype, variety, ploidy level etc. Different plant species may show different radiosensitivity as shown by Sparrow & Konzak (1958). A large amount of work has been done on the study of radiosensitivity of plants (Caldecott *et al.* 1952, Conger & Stevenson 1969). Intervarietal variations in radiosensitivity are genetically controlled (Davies 1962). The mutagenic effect is manifested differently in the germination, growth, survival, sterility etc. To determine the radiosensitivity of the material under consideration, biological criteria such as germination, seedling height and survival are usually taken into consideration. Chromosome aberrations are most closely associated with the various mutagenic effects.

In the present study, an attempt was made to determine the sensitivity of three different varieties of *Allium cepa* L. to different mutagens, as very few studies like those of Minakshi Banerjee & A. K. Sharma (1971) have been conducted on intervarietal radiosensitivity in *Allium cepa* L. Only a few cases of such intervarietal differences in sensitivity to alkylating agents such as DES & EMS are reported in crop plants (Ashri & Herzog 1972).

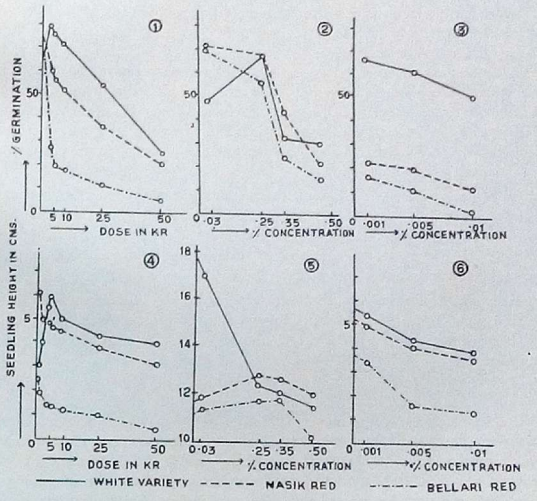
MATERIALS AND METHODS

Seeds of three different varieties of *Allium cepa* L. White Desi, Nasik Red and Bellari Red were obtained from the National Seed Corporation, Hyderabad and subjected to different treatments of γ-rays, EMS and Mitomycin C. For each treatment 200 seeds were used. Dry seeds were subjected to γ-rays (1, 2, 4, 5, 10, 25 & 50kR) from a Co^{60} source at the Department of Genetics, Osmania University. In the case of EMS and Mitomycin C, the seeds were presoaked for a period of 12 hours before treatments. For EMS treatments, the concentrations tried were 0.03%, 0.25%, 0.35%, 0.50% aqueous solutions. In the case of Mitomycin C, 0.001%, 0.005% and 0.01% aqueous solutions were used.

Of the seeds thus treated 100 were sown in the field and 100 allowed to germinate on moist filter in petri dishes at 23°± 1° C. Root tips were harvested and fixed in 1:3 acetic alcohol and squashed in 2% acetocarmine for cytological studies. Photomicrographs were taken from temporary preparations. The germination count was taken after 7 days and seedling height after 14 days for all the treatments.

RESULTS

Germination & seedling height: The differential sensitivity of the three varieties of *Allium cepa* L. to the different mutagens can be clearly seen in Figs. 1-6. For the white variety, LD50 was around 30 kR while for Nasik Red it was around 10 kR. Bellari Red seemed most sensitive, where LD50 was about 4 kR. At lower doses of γ-rays, a stimulatory effect on germination and seedling height was seen for the white variety. Nasik Red and Bellari Red showed a decline in percentage germination and seedling height with increasing doses of γ-rays.



Figs. 1–3. Percentage germination of three varieties of *Allium cepa* L. with γ-rays, EMS and mitomycin C, respectively.

Figs. 4–6. Seedling height of three varieties of *Allium cepa* L. with γ-rays, EMS and mitomycin C, respectively.

With lower concentrations of EMS, a stimulatory effect on germination was seen for the white variety while Nasik Red and Bellary Red showed a decline with increasing concentrations. However, the white variety exhibited a reduction in seedling height with increasing concentrations of EMS, while there was a stimulatory effect on the seedling height at lower concentrations of EMS for Nasik Red & Bellari Red.

All the varieties showed a decline in percentage germination and seedling height with increasing concentrations of Mitomycin C. Bellari Red appeared to be most sensitive, exhibiting the least percentage germination and lesser seedling height amongst the three varieties in all the treatments, while the white variety seemed to be the most resistant.

Growth, survival and sterility: At higher doses of irradiation (10, 25, 50 kR) the seedlings of all the varieties failed to grow into mature plants. The plants from seeds irradiated at lower doses showed only vegetative growth. With EMS, for all the varieties, reduction in survival was seen. The surviving plants, in all the varieties showed early flowering.

Plants obtained from seeds treated with 0.03%, and 0.25% EMS for the white variety, flowered 32 days before control, which took 170 days to flower. However, in the case of Nasik Red, plants obtained from seeds treated with 0.25% EMS flowered 42 days before the control, while plants from seeds treated with 0.35% EMS, flowered 32 days before the control which took 164 days to flower. In the case of Bellary Red variety, the plants from seeds treated with 0.25% and 0.35% EMS flowered 5 days before the control, which took 162 days to flower. For the higher concentrations of EMS i.e. 0.35% and 0.50%, in the White variety only vegetative growth was observed whereas Nasik Red and Bellari Red varieties exhibited only vegetative growth at the lowest and highest concentrations i.e. 0.03% and 0.50% of EMS. For all the three varieties, plants obtained from seeds treated with all the concentrations of Mitomycin C were sterile and showed only vegetative growth.

Mitotic abnormalities: These included misorientation of the spindle, precocious movement of chromosomes at metaphase, single, double, criss-cross and multiple bridges and fragments and laggards at anaphase. Stickiness and clumping of chromosomes and bloating of cells were some of the abnormalities observed at mitosis with all the treatments with higher doses/concentrations (Table 1, Figs. 7-14).

Arrestation of cell division accompanied in general by bloating of cells, seemed to be indicative of the highest degree of cytological damage caused by the mutagens, and the more sensitive variety i.e. Bellari Red, showed such a condition in almost all the treatments.

DISCUSSION

Although much work has been done during recent years on radiosensitivity of seeds to ionizing radiations and chemical mutagens, there is hardly any record of relative radiosensitivity of closely similar genetic systems. Effects of mutagenic treatments are usually measured by parameters like percentage germination, seedling height, survival at maturity, chromosomal aberrations at mitosis and meiosis etc. (Gregory 1955, 1964; Blixt *et al.* 1963, Santos 1964). Inhibition of seedling growth after treatment of seeds is a convenient technique for studying effects of ionizing radiations and radiomimetic chemicals in plants (G. Ahnstrom 1974). Inhibition of seedling growth

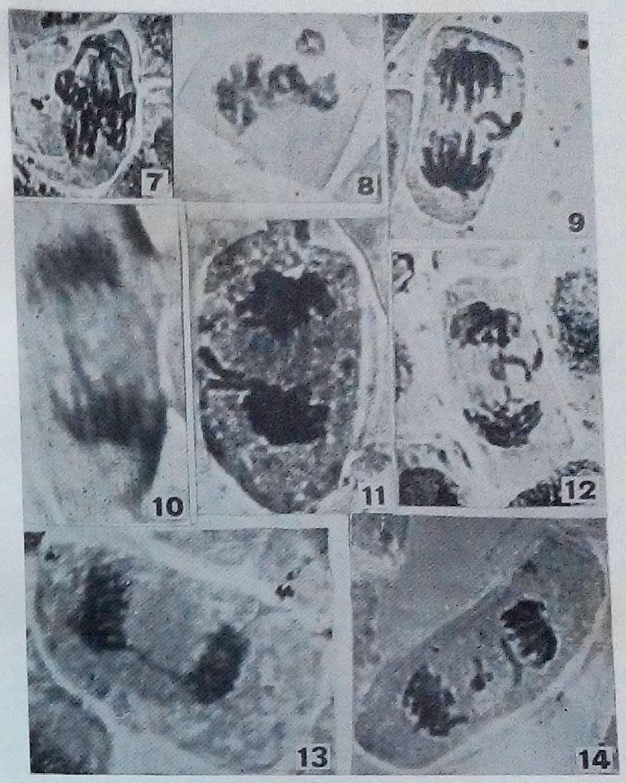


Fig. 7. Precocious movement.
Fig. 8. Misorientation.
Fig. 9. Laggards. Fig. 10. Criss-cross bridge. Fig. 11. Laggards.
Fig. 12. Bridge and laggards. Fig. 13. Single bridge.
Fig. 14. Laggards and fragments.

seems also to be correlated with the amount of chromosome damage (Conger & Stevenson 1969).

TABLE 1

Effect of mutagens on mitosis in three varieties of Allium cepa L.

Variety	Treatment	Dose in kR/% conc.	Aberrant metaphases	Aberrant anaphases	Aberrant telophases	Other aberrations	Total % of aberrant cells
White	γ-rays	1	4.8	3.2	2.0	—	10
		2	5.1	6.7	1.8	—	13.6
		4	5.0	4.3	4.2	—	13.5
		5	—	7.22	5.78	5.0	18.0
		10	6.2	21.4	10.7	10.1	48.5
		25	4.0	12.0	10.0	—	26.0
		50	Only interphases	Mitotic arrest			
Nasik Red	γ-rays	1	8.0	10.2	8.9	3.1	3.02
		2	10.0	13.6	10.8	4.2	38.6
		4	16.3	18.9	12.4	6.3	53.9
		5	20.0	16.6	26.0	18.0	80.0
		10	10.0	25.0	10.0	15.0	60.0
		25	Only interphases	Mitotic arrest			
		50		–do–			
White	EMS	1					
		2					
		4					
		5	Interphases	Mitotic arrest			
		10	–do–				
			–do–				
		50	–do–				
		0.03	20.0	25.0	—	—	45.0
		0.25	26.0	20.0	—	—	46.0
		0.35	Bloating of cells, clumped nuclei				
		0.50	do				
Nasik Red	EMS	0.03					
		0.25					
		0.35	do				
		0.50					
Bellari Red	EMS	0.03					
		0.25	do				
		0.35					
		0.50					
White	Mitomycin C	0.001	—	4.8	4.8	7.1	16.7
		0.005	16.6	25.0	8.4	—	50.0
		0.01	8.3	22.2	23.0	10.0	63.0
Nasik Red	do	0.001					
		0.005	Bloating of cells & clumped nuclei				
		0.01					
Bellari Red	do	0.001					
		0.005	do				
		0.01					

A stimulatory effect of lower doses on seedling height has been reported by various workers (Johnson 1937, Shull & Mitchell 1953, Kersten *et al.* 1943). The stimulatory effect may be due to increased activity of auxins (Ehrenberg 1955). Reduction of seedling height after irradiation is reported by several authors (Caldecott *et al.* 1963, Gunckel 1957, Jagathesan 1960). Complete cessation of cell division was observed by

Mackie (1952) in barley. Haskins *et al.* (1958) did not find any appreciable effect of dose levels on cell size. But they found drastic mitotic inhibition at increasing doses. Vant Hoff & Sparrow (1963) and Gunckel (1957) have attributed growth inhibition to mitotic arrest and inhibition. Growth inhibition may also be due to auxin destruction (Smith & Kerstein 1942) or inhibition of auxin synthesis (Gordon 1954). Other workers have offered various other explanations.

Shah & Patel (1975) observed failure of embryos of irradiated seeds of *Solanum melongena* and *Capsicum* to grow into mature plants. Sterility in M_1 generation, caused by irradiation is actually the first sign of genetical effectiveness of the treatment (Kivi 1962). The failure of flowering and sterility indicates correlation in a harmful way. Chromosome aberrations have long been known to be intimately associated with mutagenicity for radiations and certain chemicals like EMS. The cytological effects of ionizing radiations have been described in detail by various workers (Lea 1955, Sparrow 1951, Bacq & Alexander 1955).

Clumping of chromosomes has been reported following treatments using radiations, chemical mutagens, pesticides and a variety of other agents (Pritchard & Count 1968, Amer & Ali 1969, Abraham & Cherian 1978). Clumped chromosomes and stickiness might be due to some disturbances at the cytochemical level (Evans 1962). Precocious movement and unoriented chromosomes at metaphase could be interpreted as arising from a disturbance in the formation of the spindle. Misorientation of the spindle can be interpreted thus that the mutagen might alter the gene controlling the biochemical pathways of the substances which determine the number and position of poles of the spindle and thus it may disturb the position of the spindle (Prasad 1974).

The fragments might be due to interstitial deletions due to breaks caused by ionizing radiations (Lea 1955). They may be very small acentric rings which cannot be observed well due to microscopical difficulty (Moutschen 1968). The presence of single and double anaphase bridges might be due to inversions of different types (Lewis & John 1966).

From the data it is obvious that the three varieties thus differ in their radiosensitivity for different biological characters, the extent of which was variable for different doses and treatments of the mutagens. Large varietal differences were earlier found by Gelin *et al.* (1958). These are in fact very large with varying LD50s from 2 to 40 kR. This variation in sensitivity has been shown to originate from effects of several systems. Similar intervarietal differences have been reported for barley by Joshi & Sharma (1975) and the present observations seem to fall in line with the above workers. One peculiar feature observed in the response to flowering time was that the most resistant variety flowered earlier than the control, indicating that there need not necessarily be direct correlation between mutagen sensitivity and induction of early flowering.

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J. Cytol. Genet. **15**: 186–190, 1980.

CYTOGENETIC STUDIES IN THE GENUS *NARCISSUS* L. II. CYTOLOGY OF *N. JONQUILLA* L. AND ITS HYBRIDS WITH *N. PSEUDONARCISSUS* L.

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SUMMARY

Narcissus jonquilla has been treated as a hybrid species by some because of heteromorphicity in its karyotype. In order to assess whether heteromorphicity is basic in the species or has arisen secondarily, cytological observations were carried out on 3 varieties belonging to *N. jonquilla* and *N. jonquilla* x *N. pseudonarcissus* hybrids. The studies have revealed that *N. jonquilla* is a true species with perfect chromosome homology. The observed heteromorphicity in the karyotype may be a result of interspecific hybridity, to which the species is quite amenable, or structural alterations undergone by its chromosomes.

INTRODUCTION

Cytological studies in *Narcissus* have been carried out by several workers. These have resulted in detailed elucidation of systematic position and interrelationships of the genus (Fernandes 1951, 1975). One of the problems that, however, still remains to be settled is the nature of species *N. jonquilla*. It has been treated as a true species by some, a contention supported by its homomorphic karyotype (Sato 1938, Fernandes 1939 and Hirahara & Tatuno 1967) and regular meiosis (Nagao 1933). Kurita (1955) on the other hand reported heteromorphicity in the karyotype of his material of *N. jonquilla*, thus suggesting that the species may be hybrid in origin. Fernandes (1939) reported irregular meiosis in one of the clones investigated by him, though he did not ascribe it to lack of homology between the two genomes. Like most other species of the genus which interbreed freely, *N. jonquilla* hybridizes with *N. pseudonarcissus*. The hybrid *N. jonquilla* x *N. pseudonarcissus* resembles true *N. jonquilla* in plant size and shape of the leaf and flower, thus leaving scope for confusion in its identification. With this possibility in view studies on karyotype and meiosis were carried out on 3 varieties belonging to *N. jonquilla* and its hybrids with *N. pseudonarcissus*.

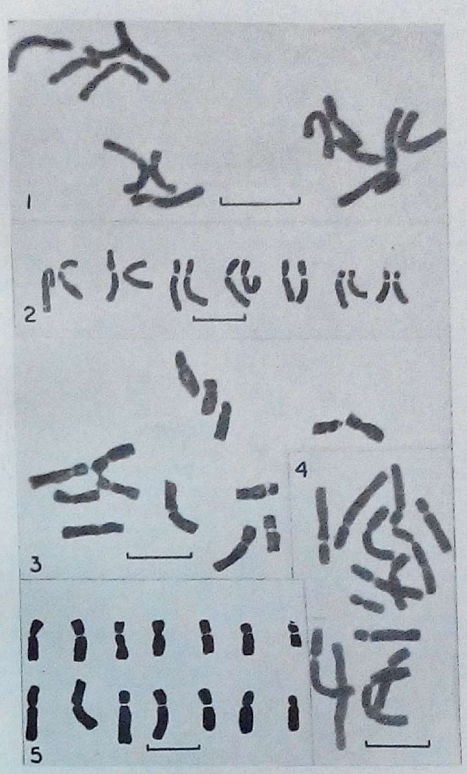
MATERIALS AND METHODS

Three varieties: *jonquilla* (belonging to *N. jonquilla*), 'Orange Queen' and 'Odorus Plenus' (*N. jonquilla* x *N. pseudonarcissus* hybrids) were collected from gardens and parks in Kashmir. Root tips obtained by placing bulbs in moist sand were prefixed in 0.002 M aqueous hydroxyquinoline solution at 10° C for 3 hr. Anthers at proper stages of meiosis were obtained by dissecting open the bulbs in September-October. All materials were fixed in 1:3 acetic alcohol and squashed in 1% acetoorcein. The terminology of Battaglia (1955) was used for chromosome classification.

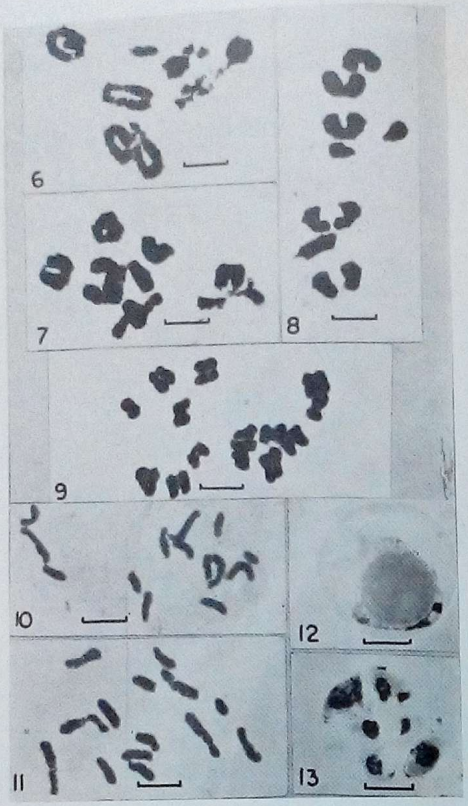
OBSERVATIONS

Karyotype

N. jonquilla var. *jonquilla*: Fourteen chromosomes were observed in the root tip cells of this variety (Fig. 1). These can be grouped into 7 perfect pairs, 5 long and 2 medium



Figs. 1–5. Karyotype and karyoidiogram of *Narcissus jonquilla* var. *jonquilla* (Figs. 1 & 2) and *N. jonquilla* x *N. pseudonarcissus* hybrids 'Odorus Plenus' (Figs. 3 & 5) and 'Orange Queen (Fig. 4). Note only one satellited chromosome in Figs. 3 & 4. (Scale 10 μ).



Figs. 6–9. Meiosis in *Narcissus jonquilla* var. *jonquilla* showing 7 II at diakinesis (Figs. 6 & 7) and metaphase I (Fig. 8) and 7:7 disjunction at anaphase I (Fig. 9). Note the presence of a pseudoquadrivalent in Fig. 6. (Scale 10 μ)

Figs. 10–13. Meiosis in *Narcissus jonquilla* x *N. pseudonarcissus* hybrid 'Orange Queen' showing 14 I at diakinesis (Fig. 10) and metaphase I (Fig. 11), a monad (Fig. 12) and a polysporad (Fig. 13). (Scale 10 μ).

(Fig. 2). All long chromosomes are submetacentric, the second and third pairs however tend toward metacentric and acrocentric types respectively. Both the medium sized pairs are acrocentric. The two chromosomes constituting the smallest pair bear satellites on the short arms. The satellite stalk of one of the chromosomes is distinctly longer than that of the other. The chromosomes range between 6.4—12.1μ. Total complement length of the karyotype is 136.8μ. Karyotype formula: 10 L Sm+2 M St+2 M^{t} St.

N. jonquilla x *N. pseudonarcissus* hybrids: The two varieties 'Orange Queen' and 'Odorus Plenus' have similar karyotypes and have been described together. Root tip cells of these varieties bear 14 chromosomes (Figs. 3, 4) ranging between 4.0—11.0μ. Chromosomes of *N. jonquilla* and *N. pseudonarcissus* genomes stand out distinctly from one another and do not match into homomorphic pairs (Fig. 5). 7 chromosomes comprising the *N. pseudonarcissus* set group into two size classes; 6 long and 1 short. Of the long chromosomes 5 are submetacentric and one is acrocentric. The short chromosome is submetacentric and bears satellite on the short arm. The *jonquilla* set matches with that of var. *jonquilla* detailed above. Interestingly however, in both the varieties none of the chromosomes of this set bears a satellite.

Karyotype formula: *pseudonarcissus* set: 5 L Sm+1 L St+1 S^{t} Sm

jonquilla set: 5 L Sm+2 M St.

Meiosis

N. jonquilla var. *jonquilla*: The 14 chromosomes of this variety were observed to form 7 perfect bivalents (Figs. 6-8). In about 12% pollen mother cells the 2 large bivalents were involved in pseudoquadrivalent association (Fig. 6). Since none of these persist till metaphase I, they seem to be transitory, getting resolved into bivalents as the chromosomes condense. At metaphase I many cells show precocious separation of chromosomes of the smallest bivalent (Fig. 8). This, however, does not disrupt normal disjunction of chromosomes at anaphase I (Fig. 9). The percentage of stainable pollen in this variety is as high as 92%. 'Orange Queen': The 14 chromosomes of this variety represent a set each of 7 *jonquilla* and 7 *pseudonarcissus* chromosomes. At no stage during meiosis do these chromosomes pair into bivalents (Figs. 10, 11) though at earlier stages 1 to 2 pseudobivalents may be observed (Fig. 10). No typical metaphase plates are formed resulting in random distribution of univalents at anaphase. The ensuing monads and polysporads (Figs. 12, 13) are highly sterile.

DISCUSSION

N. jonquilla has been treated as a true species by some and as a hybrid by others. This difference of opinion is largely due to diversity reported in chromosome complement of the clones studied from time to time (Sato 1938, Fernandes 1939, 1966; Kurita 1955, Hirahara & Tatuno 1967). Karyotype of the material employed by these

authors is at some variance; this variation is reflected in the karyotype formulae produced below:

Sato (1938)	: $2n=14=10\ L+2M+2\ M^{t}$
Fernandes (1939)	: $2n=14=2\ Li+2\ Lm+2\ Lm+2\ Lp+2\ li+2\ lp+2lp^{t}$
Kurita (1955)	: $2n=14=2L_1^{sm}+2L_2^{sm}+3\ L_3^{sm}+2L_4^{sm}+2\ L_6^{st}+L+e^{st}$ T $L_7^{st}+t\ L_8^{st}$.

Hirahara & Tatuno (1967) have not provided karyotype formula for the materials which they investigated but have stated categorically that the chromosomes match into 7 perfect pairs. Thus, except for Kurita (1955) who observed 4 heteromorphic chromosomes in the somatic complement, all others have reported that the two genomes are seemingly quite homologous.

The present investigation on *N. jonquilla* from Kashmir reveals that the two haploid sets are karyomorphically alike. Pairing of chromosomes at meiosis indicates perfect genetic homology between the two genomes. These observations support the view that *N. jonquilla* is a true species.

It is difficult to make conjectures about the origin of material worked out by Kurita on the basis of karyotype alone. In the hybrids 'Orange Queen' and 'Odorus Plenus' none of the 7 chromosomes of the *pseudonarcissus* and *jonquilla* genomes match perfectly; prominent differences in size and arm ratio are observed in second, third, fourth and seventh pairs. Absence of pairing at meiosis shows complete lack of homology between the two. The 4 heteromorphic chromosomes observed by Kurita include the 2 smallest which are heteromorphic in the present material also. However, the absence of data on meiosis in this variety makes it difficult to conclude whether the heteromorphicity in its chromosomes is due to hybridity or structural alterations.

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HISTOCHEMICAL AND ULTRASTRUCTURAL INVESTIGATIONS ON THE FOOT OF THE MOSS *PHYSCOMITRIUM CYATHICARPUM* MITT. —EVIDENCE FOR THE PRESENCE OF A Ca^{2+}-ACTIVATED ATPase

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SUMMARY

The presence of Ca^{2+}-activated adenosine triphosphatase has been reported for the first time in the foot cells of the moss *Physcomitrium cyathicarpum* Mitt. Dense reaction product of this enzyme is observed along the inner face of the outer walls of the peripheral cells of the foot, and the cells of the vaginula abutting the foot cells. The distribution of the reaction product for Mg^{2+}-activated ATPase follows almost the same pattern as for Ca^{2+}-activated adenosine triphosphatase. Electron microscopic observations reveal the presence of extensive cell wall labyrinth in foot and vaginula cells. The labyrinths increase the surface area of the wall as well as the cell membranes.

INTRODUCTION

The occurrence of several ATPases has been reported in many prokaryotes (Downie *et al* 1979) and some eukaryotes (Hodges 1976). Convincing evidence exists to suggest that some of these ATPases function as energy transducers in the transport of ions across the membranes, including tonoplast. In prokaryotes the ATPases are located in the plasma membranes of both aerobic and anaerobic species. In addition to being involved in ATP synthesis, the ATPases may also play a role in coupling ATP, produced by substrate-level phosphorylation, to the energization of the membrane (Downie *et al.* 1979). In eukaryotes, the ATPases associated with plasma membrane (Hodges *et al.* 1972, Williamson & Wyn Jones 1972, Hodges 1973) have been shown to mediate cation transport across the cell. An ATPase, especially sensitive to anions, has also been found to be associated with the tonoplast (Rungie & Wiskich 1973, Balke *et al.* 1974). Although no direct evidence exists to link this enzyme to ion transport, the unique sensitivity of this ATPase to anions suggests that it may also have a transport function (Hodges 1976).

The foot region of the sporophyte in mosses has been shown to contain Mg^{2+}-activated adenosine triphosphatase, especially in the peripheral layer (Maier & Maier 1972). The present report is the first record of the demonstration of another ATPase, namely, Ca^{2+}-activated ATPase, in addition to the above mentioned enzyme in the foot and the gametophytic cells (vaginula) immediately surrounding the foot.. Corroborative electron microscopic observations reveal that high intensity of the reaction products of these ATPases, which are reliable "markers" of the plasma membrane, results from highly increased surface area of the plasma membrane and walls of the enzyme rich cells.

MATERIALS AND METHODS

Leafy plants of *Physcomitrium cyathicarpum* Mitt., bearing sporophytes were collected from the Delhi University Botanical Garden The plants were washed free of mud and embedded in gelatin-sucrose supporting medium (Chayen *et al.* 1973). Blocks of

the embedded material were fixed on pre-cooled (−30°C) metal block holders and quenched in liquid nitrogen (Gahan *et al.* 1967). Sections ranging from 12-16 μm were cut in an IEC Cryostat precooled to −30° C.

For the localization of Mg^{2+} -activated adenosine triphosphatase, the sections were incubated at 37°C for 1 h in the reaction mixture standardized by Shaykh & Roberts (1974) at pH 7.2. Ca^{2+} -activated ATPase was localized at pH 9.4 following the procedure given by Chayen *et al.* (1973). Sections incubated in substrate-free buffer served as controls. Incubation of the experimental as well as control sections was carried out under the same conditions. At the completion of reactions, the sections were mounted in glycerine jelly on glass microslides, and photographed immediately.

For electron microscopy, the foot was excised in a solution of 5% glutaraldehyde in 0.05 M phosphate buffer (pH 6.9). The tissue remained in the fixative for 6 h at room temperature. The material was washed in buffer and postfixed in a solution of 1% aqueous OsO_4 for 2 h. The material was dehydrated in acetone, transferred to propylene oxide and embedded in Durcupan ACM mixture (Fluka, Switzerland). Ultrathin sections were cut on Porter Blum MT-2 ultramicrotome and collected on copper grids coated with formvar film. Sections were stained for 10 min with aqueous saturated uranyl acetate solution and for 20 min in lead citrate (Reynolds 1963). The sections were observed and photographed in Philips transmission electron microscope (EM 300) at 80 kV.

OBSERVATIONS AND CONCLUSIONS

The sporogonium in *P. cyathicarpum* has a short seta, the end of which burrows into the gametophyte and serves as the haustorial foot. It is conspicuously glandular (Lal 1962, Lal & Bhandari 1968) having a peripheral layer of richly cytoplasmic elongated cells. It also has a basal quadrant of similar cells arranged in two tiers. The tissue interior to the peripheral layer, 4-6 cells across, is composed of elongated, narrow, thin walled and lightly staining cells.

Enzyme localization studies reveal that there is a dense deposition of enzyme reaction product for Ca^{2+} -activated ATPase along the inner face of the outer walls of the peripheral cells of the foot (Fig. 4). Activity of this enyzme is also observed in the cytoplasm of these cells and is comparatively more than in the other cells of the foot. This is due to the abundance of plastids and mitochondria in the peripheral cells (Fig. 7). Lead precipitation is very weak in the core cells of the foot (Fig. 4).

Cells of the vaginula (gametophytic tissue) at the sporophyte-gametophyte interface also show a heavy deposition of lead precipitate along the walls facing the foot cells (Fig. 4). The cytoplasmic contents, however, show a feeble reaction for Ca^{2+} -activated ATPase. Since the presence of Mg^{2+} -activated ATPase is more widely known in plants, the material was also tested for this enzyme. Heavy deposition of its reaction product was also noted (Fig. 2) and its distribution followed almost the same pattern as described for Ca^{2+} -activated ATPase. Similar findings have been reported regarding the distribution of Mg^{2+} -activated ATPase in *Polytrichum piliferum* (Maier & Maier 1972). Ultrastructural studies of the foot revealed extensive cell wall invaginations. These finger-like ingrowths of the secondary wall fuse at their extremities to form a complex labyrinth containing pockets of cytoplasm (Figs. 5, 6). Extensive

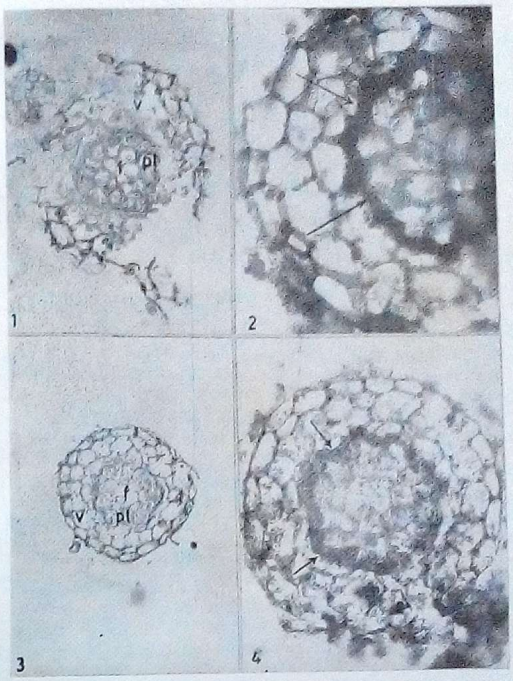

Fig. 1. T.S. foot (f) surrounded by vaginula (v) [control]. x 217.
Fig. 2. Section showing deposition of Mg^{2+} -activated ATPase reaction product in the thickened peripheral walls of the foot and vaginula cells surrounding it (arrows). x885.
Fig. 3. T.S. foot and vaginula representing control for Ca^{2+}-activated ATPase. x 217.
Fig. 4. Section showing Ca^{2+}-activated ATPase activity. The deposition of the reaction product is slightly less but follows the same pattern. x 563.

cell wall labyrinths result in a marked increase in the surface area of the wall as well as plasma membrane. There would thus be a corresponding increase in the amount of ATPases present. The amount and pattern of distribution of the reaction products confirm this belief. Similar wall labyrinths were observed in the cells of the vaginula (Figs. 5, 6) abutting the foot cells, which likewise show relatively high activity of ATPases.

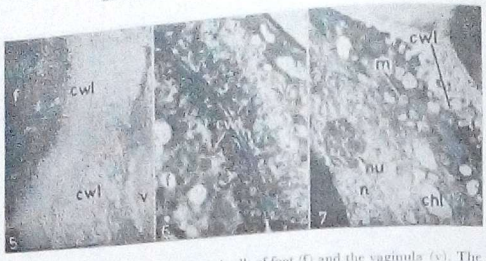

Fig. 5. Portion of interface between the peripheral cells of foot (f) and the vaginula (v). The cell walls in both these regions show extensive labyrinth (cwl). x 1470.

Fig. 6. An enlarged view of cell wall labyrinth. x 2200.

Fig. 7. Portion of a cell from the peripheral layer showing abundance of plastids (chl) and mitochondria (m) along with a nucleus (n) and a large nucleolus (nu). x 2200.

Adenosine triphosphatases are thought to be involved in a range of physiological activities associated with different cellular membranes (Sexton & Hall 1978). In prokaryotes extensive literature exists on the occurrence of membrane-bound ATPases and their biochemical characterization (Abrams & Smith 1974, Simoni & Postma 1975, Haddock & Jones 1977, Downie *et al.* 1979). However, very few reports are available in other plants (Poux 1967, Robards & Kidwai 1969, Hall 1971, Edwards & Hall 1973, Gilder & Cronshaw 1973a, b, 1974; Yapa & Spanner 1974, Hodges 1976). A detailed study of Ca^{2+} -activated ATPase has been made in animal systems and it is believed that the energy required for the synthesis of ATP may be derived from the transmembrane Ca^{2+} gradient in sarcoplasmic reticulum (Barlogie *et al.* 1971, Makinose & Hasselbach 1971, Makinose 1972, de Meis & Vianna 1979).

The demonstration of the activity of Ca^{2+} -activated ATPase assumes special significance because of the uniqueness of the tissue involved. Sporophyte is obligatorily dependent upon the gametophyte for its nutrition. Foot is the only point of contact between the gametophyte and the sporophyte, and solute uptake by the sporophyte from the gametophyte must take place in this region. In this context the presence of Ca^{2+} -activated ATPase may be indicative of an additional transport ATPase which can be mobilized for the synthesis of ATP. The exact role played by this enzyme in the present system, however, must await further work.

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J. Cytol. Genet. **15**: 196–201, 1980.

CYTOCHEMICAL STUDIES ON THE ORIGIN AND DEVELOPMENT OF PROTEIN BODIES IN THE COTYLEDONS OF PIGEON PEA

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SUMMARY

The origin and development of protein bodies has been investigated in the cotyledons of pigeon pea, *Cajanus cajan* (Linn.) Millsp., an important pulse-yielding plant. The protein deposition starts in very small vacuoles which are subsequently converted into protein bodies. The synthesis and deposition of proteins in vacuoles is rapid and most of the vacuoles are replaced by protein bodies in a short time. The protein bodies are without any inclusions. Their number and size in the cotyledonary cells increases as the seed matures. A higher concentration of these bodies is observed in the peripheral layers as compared to the central zone. Later on due to the dehydration of cotyledons, protein bodies come close and form a proteinaceous matrix in the cytoplasm.

INTRODUCTION

The utmost importance of pulses in our daily diet because of their high protein content cannot be over-emphasized. In India pulses are in great demand because most of us are vegetarians and do not eat meat. Besides proteins the pulses are also rich in carbohydrates and a few of these (groundnut and soybean) contain a fairly high amount of fatty oils. All these metabolites are stored in the cotyledons during the development of seed.

Because the cotyledons in legumes are the chief organs to store food, recently they have attracted a lot of attention. However, the studies on the synthesis, mobilisation and storage of various metabolites in the cotyledons of pears (Bain & Mercer 1966, Smith 1971), beans (Öpik 1968, Walbot 1973, Manteuffel *et al.* 1976) and soybean (Tombs 1967) are mostly restricted to the storage parenchyma. Very little data is available to show the subcellular events occurring in other zones of the cotyledons. The present communication deals with the cytochemical studies on the origin and development of protein bodies in different zones of cotyledons of pigeon pea, *Cajanus cajan* (Linn.) Millsp. during various stages of seed maturation.

MATERIALS AND METHODS

Seeds of *Cajanus cajan* (Linn.) Millsp. a local variety belonging to the family Fabaceae (Papilionaceae) were procured from Pulse Division, Indian Agricultural Research Institute, New Delhi. The seeds were sown in the month of July 1977 in the University Botanical Gardens, Delhi. To obtain a high-yielding and healthy crop, *Rhizobium* inoculum was mixed at the time of seed sowing (for details *see* Gandhi 1979).

The plant bears fruits during October to April. However, in order to have uniform material, collections were made only during the months of February and March. Seeds at various stages of development were fixed in FAA (5 ml formalin, 5 ml glacial acetic acid, 90 ml 70% ethanol) for 24 hours. The material was dehydrated in a tertiary

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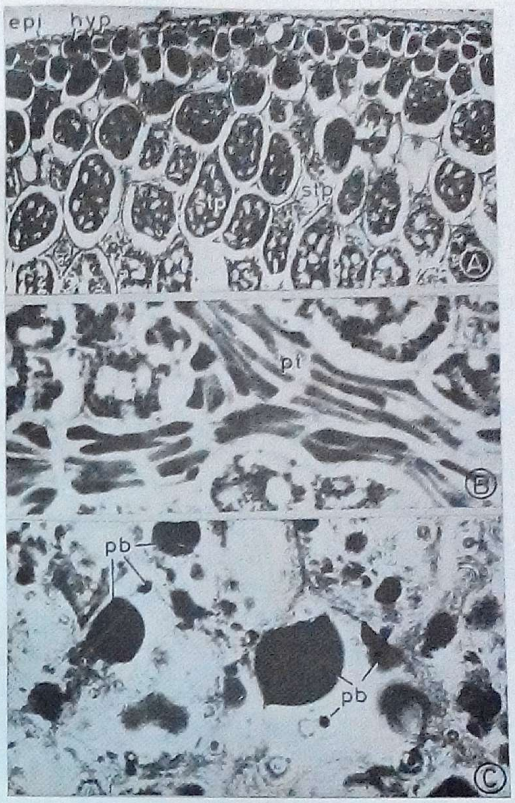

Fig. 1A-C. Longisections of portions of cotyledons stained with haematoxylin-erythrosin (*A*, *B*) and mercuric bromophenol blue (*C*). A. Portion of cotyledon (30 DAP) from the peripheral region showing epidermis (epi), hypodermis (hy) and storage parenchyma (stp). x **240**. B. Same, passing through procambial tissue (pt.); note narrow and elongated cells of procambial strand. x **465**. C. A highly enlarged portion of the cotyledon (19 DAP), showing protein bodies (pb) of different sizes and shapes. x**1200**.

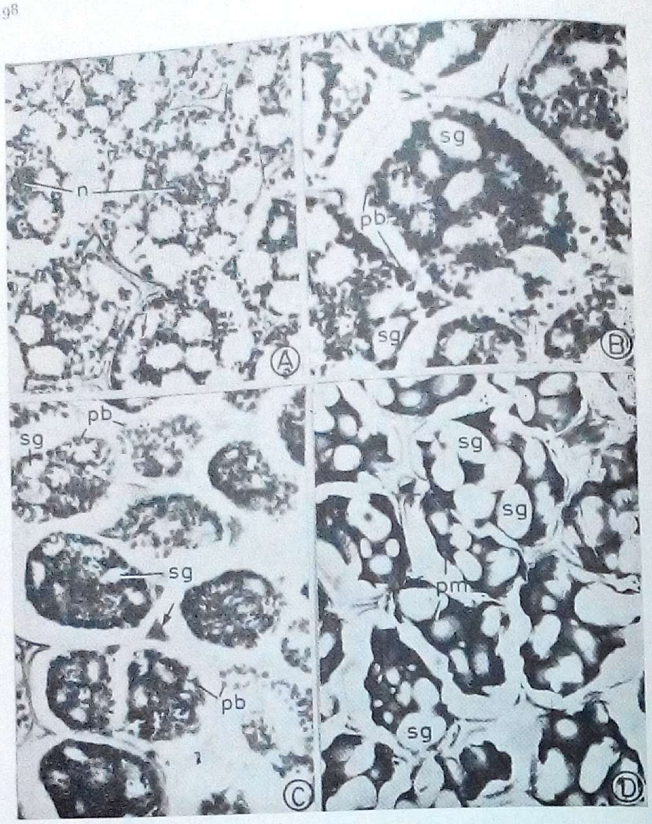

Fig. 2A-D. Longisections of portions of cotyledons stained with mercuric bromophenol blue. A. Portion of cotyledon (30 DAP) showing a few central cells in the storage parenchyma zone. Note the distribution pattern of protein bodies (arrows) in cells. x **610**. B, C. A few cells of cotyledon (34 DAP) full of protein bodies (Pb). Note protein bodies (dark stain) surround starch grains (sg) which are unstained. Arrows indicate partially (*B*) and totally (*C*) filled intercellular spaces. B. x **740**; C. x **520**. D. Portion of cotyledon (38 DAP). The entire cytoplasm, with the exception of starch grains (sg) shows dense staining due to the formation of proteinaceous matrix (pm). x **492**.

butyl alcohol series and embedded in paraffin wax. To soften the hard material the blocks were soaked in water at 35°C for 10-12 days. The mature seeds were kept in water for about a month and re-embedded. Sections were cut at 12 µm and for histological studies stained with haematoxylin-erythrosin combination (Johansen 1940). For the cytochemical localization of proteins the sections were stained either with mercuric bromophenol blue (Mazia *et al.* 1953) or aniline blue black (Sehgal & Parikh 1977).

OBSERVATIONS

Development of Cotyledons: Twelve days after pollination (DAP) a young creamish-white dicotyledonary embryo is formed. The cotyledons attain their maximum size 30 DAP and occupy the entire seed cavity. After this, the phase of dehydration sets in and there is a gradual dehydration of cytoplasm. During the subsequent 8-10 days the entire cotyledon dehydrates. Due to dehydration the cytoplasm appears shrunken and is separated by a large space from the cell wall. Each cotyledon is made up of 4 distinct regions. These are outer epidermis, hypodermis, procambium and storage parenchyma. The cells of the epidermis are small, narrow and elongated. The hypodermis is made up of more or less isodiametric cells (Fig. 1A). The cells of the procambial strands are narrow and elongated (Fig. 1B). Storage parenchyma comprises large, isodiametric cells having large intercellular spaces (Fig. 1A).

Development of protein bodies: During the cell expansion phase (13-17 DAP) of the embryo, development of protein bodies is observed. The protein deposition starts in very small vacuoles which are subsequently converted into protein bodies. The synthesis and deposition of proteins in the vacuoles occur rapidly with the result that most of the vacuoles are replaced by protein bodies which stain densely with aniline blue black or mercuric bromophenol blue (Fig. 1C). The protein bodies are devoid of any inclusions like globoids or crystalloids. The size and number of protein bodies increases as the seed matures (Figs. 1C; 2A, B). The increase in size of the protein body is due to the following reasons:

(i) The increase in the size of vacuoles which later on get filled with proteinaceous material.

(ii) Small protein bodies coalesce to form bigger ones.

A higher concentration of protein bodies is observed in the peripheral layers as compared to central zone (Figs. 1A, 2A). The corners of intercellular spaces in storage parenchyma are partially or totally filled with proteinaceous material (Fig. 2B, C). Occasional unstained areas in the protein bodies have been observed. Due to rapid accumulation of reserve metabolites the cells of the cotyledons are seen laden with protein bodies (Fig. 2B C). Later on due to the dehydration of tissue, protein bodies come close and form a proteinaceous matrix in the cytoplasm (Fig. 2D.)

DISCUSSION

The formation of protein bodies during different stages of seed development has been described in legumes e.g. *Arachis hypogaea* (Dieckert & Snowden 1960), *Glycine max*

(Bils & Howell 1963), *Pisum sativum* (Bain & Mercer 1966), *Phaseolus vulgaris* (Öpik 1968), *Vicia faba* (Briarty *et al.* 1969), Neumann & Weber 1978), and other flowering plants e.g. *Triticum aestivum* (Graham *et al.* 1962), *Hordeum vulgare* (Jones 1969), *Zea mays* (Khoo & Wolf 1970), *Ricinus communis* (Sobolev *et al.* 1972), *Linum usitatissimum* (Dhar & Vijayaraghavan 1979).

Mottier (1921) believed that protein bodies owe their origin to plastids. Graham *et al.* (1962) and Morton *et al.* (1964) from their studies on kernals of *Triticum* species stated that the protein bodies are localized within the characteristic structures the 'proplasts' or 'protein-forming plastids', which consist of distinct outer membranes and ribosomes. Khoo & Wolf (1970) working on maize endosperm have noticed that a few vacuoles are produced from endoplasmic reticulum and dictyosomes. Protein synthesized outside the membrane gets deposited in these vesicles and they turn into protein granules. In *Vicia faba* (Briarty *et al.* 1969) the storage protein appears as a single unit but ontogenetically it is derived from several individual bodies.

In *Cajanus cajan* (present study) the protein deposition occurs gradually in the vacuole and with the passage of time the contents become dense. These observations are in general agreement with those made on other legume seeds (Quilichini 1952, Öpik 1968), cereal grain (Buttrose 1963) and linseeds (Dhar & Vijayaraghavan 1979).

Rost (1972) has classified protein bodies into 3 types, those with no inclusions, those with only globoid inclusions and those with globoid and crystalloid inclusions. Generally, in all legumes including *Cajanus cajan* (present study) protein bodies (without inclusions) fall in the first type (*see* Bils & Howell 1963, Bain & Mercer 1968, Öpik 1968, Briarty *et al.* 1969). However, in *Vicia faba* (Rost 1972) and many other legumes (Lott & Buttrose 1978) a few protein bodies with globoid inclusions are also observed. Recently, Buttrose & Lott (1978) have theorised that protein body structure is related to taxonomic grouping.

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MUTAGEN INDUCED STRUCTURAL ABNORMALITIES IN LEAVES OF *TURNERA ULMIFOLIA* LINN.

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SUMMARY

Considerable variation in the size and shape of leaves was observed in plants raised from gamma rays and EMS-treated seeds. Mutagen-treated plants, in general, showed reduction in the size of leaves. The leaves were also comparatively thicker. The shape varied from lanceolate, elliptical to ovate as against elliptic-lanceolate in control plants. In some plants leaves were spathulate. In a few plants leaves with involuted margins giving somewhat boat-shaped appearance were noticed. Fusion of leaves was observed in higher doses of gamma rays. The chlorophyll deficiency and chlorotic sectors (albinism) were found in plants obtained from the mutagen-treated seeds. The abnormalities were less in the second generation while in the third generation it was almost equal to that in the control.

INTRODUCTION

Turnera ulmifolia var. *angustifolia*, belonging to the family Turneraceae, is an ornamental plant with attractive flowers. It has good potential in respect of caffeine (Tarar & Dnyansagar 1977) and antifungal activity (Nicolis 1970). The cytogenetic work on this plant is meagre. Therefore, present authors have undertaken a detailed cytogenetic study on the various aspects in this plant (Dnyansagar & Tarar 1971, Tarar & Dnyansagar 1972, 1974, 1976, 1977, 1979, and 1980). The present paper deals with some changes induced in leaf by gamma rays and ethyl methane sulfonate.

MATERIALS AND METHODS

Dry (9.8% moisture) and mature seeds of *Turnera ulmifolia* Linn. were irradiated with gamma radiation doses ranging from 10 to 100 kR from the ^{60}Co source of the Bhabha Atomic Research Centre, Trombay, Bombay. The irradiated seeds were sown in garden along with the control. The seedlings raised from the treated seeds were carefully transplanted in pots. Control plants were also planted in pots in separate rows.

The chemical mutagen ethyl methane sulfonate (EMS) of the Eastman Kodak Company* was used. The seeds were treated with different concentrations of EMS solution ranging from 0.5 to 2% for different durations ranging from 8 hours to 24 hours. The pH of the solution was adjusted to 7 with phosphate buffer. The treated seeds were washed and post-soaked in deionised water for 4 hours. They were then sown in posts in separate rows alongwith the control. Length and breadth of leaf were noted for each plant per treatment. The average of such values for 10 plants was calculated.

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OBSERVATIONS

The average length and breadth of the leaf in the control plant was 9.5 cm and 3.5 cm, respectively. These values decreased with an increase in exposure (Table 1). It was observed that 10 kR and 20 kR exposures of gamma rays had no effect on the mean leaf size and it was equal to that of control. However, beyond 30 kR reduction in the leaf size was observed (Fig. 6). At 30 kR the leaf had a mean length 9 cm and breadth 3 cm while at 100 kR it had 4 cm and 1 cm, respectively. Leaves were thicker and the decrease in the number and size of the stomata was observed in the case of exposures from 40 kR to 100 kR. The mean length and breadth of leaf at 100 kR was 5.4 cm and 1.5 cm, as well 7 cm and 2.2 cm in R_2 and R_3 generations, respectively, indicating the tendency towards normalisation (Table 1).

TABLE 1

Data on leaf size of plants raised from gamma-irradiated seeds in R_1, R_2 and R_3 generations

Exposure	R_1 Leaf		R_2 Leaf		R_3 Leaf	
	Mean length cm	Mean breadth cm	Mean length cm	Mean breadth cm	Mean length cm	Mean breadth cm
Control	9.5	3.5	9.5	3.5	9.5	3.5
10 kR	9.5	3.5	9.5	3.5	9.5	3.5
20 kR	9.5	3.5	9.5	3.5	9.5	3.5
30 kR	9.0	3.0	9.5	3.5	9.5	3.5
40 kR	8.5	2.8	9.0	3.0	9.5	3.5
50 kR	7.8	2.6	9.0	3.0	9.5	3.5
60 kR	7.0	2.4	8.5	2.8	9.0	3.0
70 kR	6.5	2.2	8.0	2.5	9.0	3.0
80 kR	6.0	2.0	7.0	2.3	8.5	2.8
90 kR	5.5	1.4	6.6	2.2	7.5	2.5
100 kR	4.0	1.0	5.4	1.5	7.0	2.2

The leaves of the control plants were elliptic-lanceolate whereas in the case of plants obtained from irradiated seeds the shape varied in general from lanceolate-elliptical to ovate (Fig. 1). A few plants showed chlorophyll deficiency. The colour difference ranged from dark green to light green. The chlorophyll deficient sectors were also observed in the plants in the case of exposure from 70 kR to 100 kR (Fig. 2). In some plants raised from seeds exposed at 60 kR, leaves were with involuted margins giving a somewhat boat-shaped appearance (Fig. 3). In a few plants raised from seeds irradiated at 80 kR, leaves were spathulate (Fig. 4).

In all EMS treatments, the size of the leaf was reduced. However, in the case of 0.5% EMS concentration and 8 hour treatment, it was equal to that of control. Leaves were thicker and the number and size of stomata decreased at higher concentrations and duration of EMS treatment (i.e., 0.5% for 24 hour duration and 1%, 1.5% and 2% concentration for 16 and 24 hour duration). The maximum reduction in the size of leaves was observed in plants obtained from seeds treated with 2% EMS concentration for 24 hours (mean length 6.6 cm and breadth 2.2 cm). However, in E_2 and E_3 generations, they were almost similar to those of control plants. A few of the seedlings

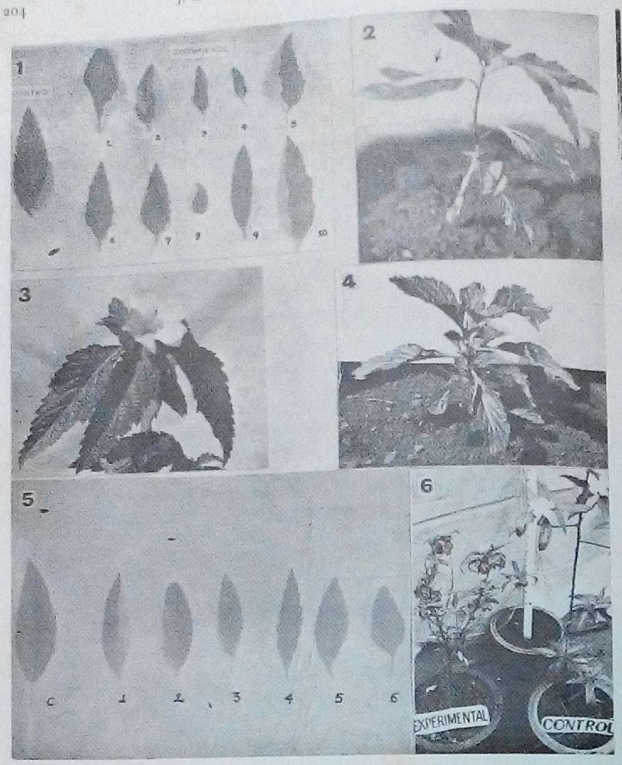

Fig. 1. Variation in size and shape of the leaves. C—Leaf of control plant. 1-10. Leaves from the 'experimental plants' raised from gamma irradiated seeds.
Fig. 2. Seedlings raised from seeds treated with gamma rays (70 kR) showing chlorophyll deficient sectors (albinism).
Fig. 3. Plant raised from the seed irradiated at 60 kR. Leaves are with involuted margin giving a somewhat boat-shaped appearance.
Fig. 4. 'Experimental plant' showing spathulate leaves raised from the seed irradiated at 80 kR.
Fig. 5. Variation in size, shape and colour of the leaves. C—Leaf of control plant. 1–6. Leaves from the 'experimental plants' raised from EMS-treated seeds.
Fig. 6. 'Experimental plant' raised from the seed irradiated at 60 kR showing inhibition of growth and reduction in the size of leaves and flower.

showed chlorophyll deficient sectors, which, however, did not persist and gradually disappeared as the plants reached maturity (Fig. 2). These sectors were of varying sizes and sometimes covered the complete half of the leaf (Fig. 5-1). Similarly many plants showed variation in respect of size and shape. Variation in shape was observed from lanceolate, elliptical to ovate as against elliptic-lanceolate in control plants (Fig. 5).

TABLE 2

Data on leaf size of plants raised from EMS treated seeds in E_1, E_2 and E_3 generations

Duration of treatment	Concentration of EMS %	E_1 Leaf Mean length cm	E_1 Leaf Mean breadth cm	E_2 Leaf Mean length cm	E_2 Leaf Mean breadth cm	E_3 Leaf Mean length cm	E_3 Leaf Mean breadth cm
	Control	9.5	3.5	9.5	3.5	9.5	3.5
	0.5	9.5	3.5	9.5	3.5	9.5	3.5
	1.0	9.0	3.0	9.0	3.0	9.5	3.5
8 hours	1.5	8.5	2.8	9.0	3.0	9.5	3.5
	2.0	8.5	2.8	9.0	3.0	9.5	3.5
	0.5	9.5	3.5	9.5	3.5	9.5	3.5
	1.0	8.8	3.0	9.0	3.0	9.5	3.5
16 hours	1.5	8.0	2.7	8.8	2.9	9.0	3.0
	2.0	7.8	2.6	8.5	2.8	9.0	3.0
	0.5	9.0	3.0	9.5	3.5	9.5	3.5
	1.0	8.0	2.8	9.0	3.0	9.0	3.0
24 hours	1.5	7.8	2.7	8.5	2.8	8.8	2.9
	2.0	6.6	2.2	8.2	2.6	8.8	2.9

DISCUSSION

In the present investigation it was observed that plants of *Turnera ulmifolia* var. *angustifolia* raised after mutagenic treatments showed reduction in the size of leaves. The reduction was pronounced at the higher exposures of gamma rays and higher EMS concentrations. The leaves were also comparatively thicker.

Singh *et al.* (1939) reported variations in the shape and size of leaves of cotton after irradiating the seeds with X-rays. Hagberg & Nybom (1954) found that the X-ray-treated potato plants showed reduction in the number of leaflets and the leaves were thicker. Schwartz (1954) noticed that after irradiating dry maize seeds, the leaves showed reduction in size corresponding to an increasing dose of radiation up to 100 kR. Pratt *et al.* (1959) found that the size of the leaf was reduced at higher exposures of chronic gamma irradiation in the case of apple plants. Similarly Moh (1962) found a decrease in the size of the leaf in coffee plants raised from seeds irradiated with gamma rays, thermal neutrons and X-rays and attributed it to the chromosomal deficiency. Haber & Foard (1964) concluded that the reduction in size of the leaf in gamma irradiated wheat seedlings might be attributed largely or almost entirely to the radiation induced mitotic inhibition rather than to other actions of radiation. Besides these explanations, Irvine (1940) held the view that the abnormalities observed in leaves after irradiation

could be due to the disturbances of phytohormones as a result of irradiation. Pelc & Howard (1956), however, expressed the view that the inhibition of DNA synthesis could be the reason, while Mikaelsen *et al.* (1968) suggested the chromosomal aberration as the cause. Dhanaraj (1971) reported reduction in size of the leaves obtained from irradiated or EMS-treated seeds. Roy *et al.* (1971) observed that the leaves of *Cucumis sativus* were crippled and smaller in size in the lower doses of X-irradiation, while in higher doses plants had much fragmented leaves. Kamaluddin & Abraham (1973) noticed narrow leaves in X-irradiated chilli plants.

In the present investigation, the reduction in the size of leaves was proportional to the increase in the exposure of gamma rays and concentration and duration of EMS treatment. It was also observed that (Tarar & Dnyansagar 1972, 1974) the mitotic inhibition and chromosomal aberrations were more in higher exposurers and concentrations of EMS treatment. Therefore, it is likely that the reduction in the size of leaves of *Turnera ulmifolia* var. *angustifolia* may be chiefly due to mitotic inhibition and chromosomal aberrations. It may also be due to disturbances in phytohormones as a result of mutagenic treatment.

Haskins & Moore (1935) observed various leaf abnormalities in *Citrus* seedlings grown from X-ray-irradiated seeds. Chlorophyll deficiency noted by them was many-fold higher in treated plants. They also found partially bifoliate and trifoliate leaves in treated plants. Johnson (1936) noticed altered phyllotaxy and deformed leaves in *Zinnia* after X-ray-irradiation.

Singh *et al.* (1939) reported various leaf abnormalities in cotton following seed treatment with X-rays. Asymetrical variegated lamina showing puckering and tumor-like structures were also observed by them. Bishop & Aalders (1955) noticed leaf bifurcations in apple scions exposed to thermal neutrons and X-rays. They attributed these to the delayed expression of some radiation effect. Gunckel (1957) in his review on the effect of ionizing radiations in plants (morphological characters) has stated that leaves of irradiated plants usually show induced changes in their colour, form and texture. The irregular development of leaf blade was reported by Gunckel & Sparrow (1954). They suggested that these morphological changes were due to chromosomal aberrations induced by the mutagen.

Jana (1962) observed a rolled leaf mutant in X_2 generation of *Phaseolus mungo*. He found that in this mutant, the margin of the leaves was bent downwards giving the rolled effect. He also obtained some other leaf mutants in the above mentioned plant with X-ray treatment. Kumar & Das (1971) reported a cup-leaf mutant in *Brassica campestris* var. Brown sarson from gamma ray exposures. They found that this mutant had margins involuted in the form of a cup. He attributed this phenotypic change to the induced chromosomal aberrations.

Roy *et al.* (1971) observed chlorophyll deficient leaves in plants of *Cucumis sativus* subjected to X-ray-irradiation. Similar observations were made by Gohal *et al.* (1972) in Guar (*Cyamopsis tetragonoloba* L.) They obtained two variants with chlorophyll deficiency in the plants obtained from EMS-treated seeds.

In the light of above findings, the change in shape of leaves, chlorophyll deficiency and chlorotic sectors (albinism) observed in the present investigation may be chiefly attributed to the chromosomal aberrations induced by the mutagenic treatment.

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EFFECT OF SOME CHEMICAL MUTAGENS ON MUTATION FREQUENCY AND SPECTRUM IN PEARL MILLET (*PENNISETUM TYPHOIDES* (BURM.) S. & H.)

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SUMMARY

Dry seeds of inbred PIB-251 of pearl millet were treated with 0.04M EMS, 0.12M HA and 0.12M HZ. In M_2 generation, EMS produced highest frequency and wider spectrum of chlorophyll and viable mutations followed by HA and HZ. Some degree of mutagenic specificity was noticed as EMS induced dark xantha and HA treatment induced exclusively chlorina mutants. A number of viable mutations affecting one or more than one associated morphological characters were observed in treated populations. Knotched grain mutant and mutants having more than one midrib occurred only in EMS treatment. A positive correlation was observed between the frequency of chlorophyll and viable mutations.

INTRODUCTION

Chemical mutagenesis is regarded as an effective and important tool in improving the yield and quality characters of crop plants. Mutagenic actions of a number of chemicals either singly or in different combinations have been reviewed (Konzak *et al.* 1965) , but very little information is available on their efficiency and effectiveness in pearl millet. This investigation reports the effects of EMS, HA and HZ in pearl millet, inbred PIB-251, on chlorophyll and viable mutation frequency and their spectrum in M_2 generation. It is hoped that this information would help in planning chemical mutagenesis experiments to increase the frequency and widen the spectrum of desirable mutants for use in the improvement of this important grain and fodder crop.

MATERIALS AND METHODS

Dry seeds of inbred PIB-251 of pearl millet were presoaked for 6 hr in distilled water and were treated for 6 hr with freshly prepared phosphate buffered mutagenic solutions of 0.04M EMS (ethyl methane sulphonate), 0.12M HA (hydroxylamine) and 0.12 M HZ (hydrazine) at pH 7.0, 5.5 and 8.5, respectively, at room temperature (22°C ± 1°C). The treated seeds were thoroughly washed in running tap water for 4 hr. For control, seeds were soaked for 4 hr in distilled water. The seeds of all the treatments were sown in the field to raise M_1 generation. At maturity all the M_2 plants were harvested individually and in the next season the M_2 generation was raised as plant to row progenies. The M_2 populations were carefully screened for both chlorophyll and viable mutations and expressed as per 1000 M_2 plants.

RESULTS

Chlorophyll mutation: The frequency of chlorophyll mutants per 1000 M_2 plants was highest in case of EMS (18.74), followed by HA (13.48) and HZ (6.9). Eight distinct types of chlorophyll mutants were observed among different treatments (Table 1). Dark xantha was quite rare and occurred only in EMS treatment, whereas albino, virescence,

maculata and striata appeared in all the treatments. Chlorina appeared only in HA treatment

TABLE 1

Frequency of chlorophyll mutants (per 1000 M2 plants) in the inbred PIB 251 of pearl millet

Treatment	Total No. of M2 plants	No. of chlorophyll mutants	Chlorophyll mutants per 1000 M2 plants	Chlorophyll mutants A	X	DX	C	T	V	S	M
Control	1240	—	—	—	—	—	—	—	—	—	—
EMS 0.04M	960	18	18.74	4.16	2.07	1.04	—	2.07	2.07	3.12	4.16
HA 0.12M	890	12	13.48	1.12	3.37	—	2.24	2.24	2.24	2.14	1.12
HZ 0.12M	720	5	6.9	2.77	—	—	—	—	1.38	1.38	1.38

A—Albina, X—Xantha, DX—Dark Xantha, C—Chlorina, T—Tigrina, V—Virescence, S—Striata, M—Maculata.

Viable mutations: The treatment showed significant differences in the induction of viable mutation. HZ was least effective in inducing mutations, whereas EMS was most effective and HZ had an intermediate effect. Some 19 different viable mutant types were identified (Table 2). The mutants such as tall, vigorous, dwarf and late, bifurcated ear, notched grain were observed only in the EMS treatment, whereas weak,

TABLE 2

Frequency of viable mutants (per 1000 M2 plants) in the inbred PIB 251 of pearl millet

Treatment	No. of M2 plants	No. of viable mutants	Viable mutants/ 1000 M2 plants	Types of viable mutations
Control	1240	—	—	—
EMS 0.04M	942	38	40.3	19
HA 0.12M	878	27	30.7	15
HZ 0.12M	715	9	12.5	5

leaves with two midribs, twisted and protruding ear mutants were rare and exclusive to HA treatment. Semidwarf, thin, short ear and narrow leaf appeared in all the treatments. Brachytic, long leaf, broad leaf, late, thick, short ear, drooping and knobbed grain mutants occurred only in EMS and HA treatments and not in HZ. Late dwarf and knobbed grain mutants occurred both in EMS and HA treatments but their frequency was greater in former than in latter. HA treatment was more effective than EMS in inducing drooping ear mutants.

DISCUSSION

The frequency of both chlorophyll and viable mutations was higher in EMS than HA and HZ treatments, which is in agreement with the previous report (Augustine *et al.* 1975) Chlorophyll mutations may originate because of mutations in chlorophyll gene(s) (Goud 1967), which are situated on several chromosomes loctaed near the

centromere or proximal segments of the chromosome (Swaminathan 1965). The relative efficiency of mutagens in inducing chlorophyll mutations depend upon their specifications on DNA. EMS treatment causes gaps or minor deficiencies through depurination, incorporating mistakes in replication and repair processes in DNA. HA treatment, instead, induces one-way transition (Freese *et al.* 1961) by reacting with cytosine (Tessman *et al.* 1964) in such a way that altered cytosine prefers pairing with adenine in place of guanine. Hence the effect of HA on chromosomal level is weak as compared to EMS. HZ reacts with the pyrimidine bases breaking the pyrimidine ring leading to the removal of the base from the nucleotide chain of DNA. Besides, it produces H_2O_2 after reacting with O_2 and causes damage to the biological system if the radicals produced are not neutralized *in situ*. EMS induced drastic dark xantha mutations can be attributed to the gaps induced by EMS through depurination of DNA bases. Chlorina mutants induced by HA exclusively indicate that chlorina mutation sites might be cytosine rich. HZ treatment induced a number of mutants such as albina, virescence, striata and muculata. HA has been reported to be a weak mutagen (Travis *et al.* 1972).

The effects of mutagens on a constellation of morphological characters such as dwarfness with early flowering, short thin ears, bushy semidwarf mutants either without flowering or very late with long and broad leaves and ears can be attributed to mutations in genes having pleiotropic effects (Sjodin 1971). The mutagens also showed specificity in inducing viable mutations, the frequency of which was positively associated with the frequency of chlorophyll mutations and this observation is in agreement with earlier report (Doll & Sandfaer 1969). This information may be efficiently and effectively used in deciding a particular treatment (on the basis of chlorophyll mutation frequency) for use in mutagenesis programme for obtaining higher frequency of viable mutations.

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INDUCED POLYGENIC VARIABILITY IN BREAD WHEAT (*TRITICUM AESTIVUM* L.) III. COMBINING ABILITY FOR THREE YIELD CONTRIBUTING CHARACTERS

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SUMMARY

A six parent diallel cross was made separately in the normal and the treated (12 Kr gamma rays) parents. The derived F_1 and F_2 populations were studied to study combining ability for spike length, spikelets per spike and kernels per spike. Both G.C.A. and S.C.A. mean squares were significant for all the three characters in both F_1 and F_2 in the normal as well as in the treated sets except S.C.A. for spike length and kernels per spike in normal F_2 and the G.C.A. for kernels per spike in normal F_1. In general the estimates of S.C.A. component increased for the three characters due to treatment. Generally the S.C.A. variance was also relatively smaller in F_2's as compared to F_1's. The *per se* performance of the parents was a good indication of high general combining ability of at least two characters i.e. spike length and spikelets per spike. Both high x low and low x low combinations exhibited higher S.C.A. effects.

INTRODUCTION

Combining ability analysis, in general is utilized for selecting the parents for any hybridization programme, irrespective of whether it is meant for developing inbreds or for exploiting heterosis. There is, however, some evidence that combining ability variances and hence the inheritance pattern can be altered to some extent through mutagenic treatments as shown by Romirez *et al.* (1969) in wheat. If it is so, it would be worthwhile to find out to what extent mutagenic treatment can alter the estimates of combining ability variances and effects and nature of gene effects as well. Keeping this in view, a 6 x 6 diallel cross was attempted in wheat utilizing untreated (normal) material in one set and the material raised after seed treatment with 12 Kr gamma rays in another set. In the first two papers (Balyan & Gupta 1978a, b) of this series, results pertaining to protein content, pelshenke value, days taken to heading, days from heading to maturity, yield per plant, tillers per plant and 100-grain weight were presented. The results pertaining to spike length, spikelets per spike and kernels per spike are presented in this paper.

MATERIAL AND METHODS

The experimental material for the present study consisted of six diverse wheat varieties of bread wheat (*Triticum aestivum* L.) belonging to three height groups viz. tall (C 273 and K-68), double dwarf (Sharbati Sonora and Shera) and triple dwarf (Hira and UP 301). In each variety, a part of the seed was treated with 12 Kr dose of gamma rays. The two populations (normal and treated) were grown during the rabi season of 1973-74. All possible 15 cross combinations in each treatment excluding reciprocals were prepared. F_1 seed along with parents was sown during the rabi season of 1974-75 to advance the material to F_2 generation.

The thirty six progenies (including 6 parents + 15 F_1's + 15 F_2's) each for the normal and treated sets were raised in a randomized block design with three replications during

rabi season of 1975-76. The parents and F_1's were sown in two plots each whereas F_2's in four plots each. Each plot was a two meter row of 13 plants each spaced at 15 cms within the row and 30 cms between the rows. The seeds were hand dibbled at the depth of 4 to 5 cms. Non-experimental rows were planted all around the border to eliminate the border effects. Normal recommended cultural practices were adopted for raising the crop. The data were recorded for parents and F_1's on five plants each and for F_2's on fifteen plants in each replication.

For carrying out statistical analysis, the means of the above observations were used. The analysis of variance was carried out according to the method given by Panse & Sukhatme (1967). The procedure of estimation of general and specific combining ability as developed by Griffing (1956) was used for the present investigation using Method 2, Model I.

RESULTS AND DISCUSSION

The mean squares and appropriate degrees of freedom are given in Table 1 for analysis of variance. The F-test indicated that there were highly significant differences among progenies for three characters under study in both normal and treated sets.

TABLE 1

Mean squares and coefficient of variability in a 6 x 6 F_1 and F_2 diallel cross (normal and treated sets) for three characters in wheat (values in parentheses belong to F_2)

Source	D.F.	Mean squares: Spike length Normal	Spike length Treated	Spikelets per spike Normal	Spikelets per spike Treated	Kernels per spike Normal	Kernels per spike Treated
Replications	2	5·44** (4·17*)	2.77 (0.20)	0.31 (0.03)	6.25 (10.85**)	17.81 (6.37)	130.07* (137.00*)
Progenies	20	4·15* (4·55**)	5·25** (4·35**)	2.43** (1.65**)	5.91** (2.67**)	76.41** (59.64**)	223.66** (120.93**)
Error	40	0.47 (0.89)	1.18 (0.49)	0.61 (0.55)	1.17 (0.90)	25.20 (24·13)	27·13 (22.83)
C.V.		18.98 (20.12)	21.17 (21.47)	8.43 (7.21)	13·78 (9·45)	15.85 (15.45)	29.36 (22.69)

*Significant at 5%; **Singificant at 1%.

It would be interesting to note that for all the three characters, the magnitude of progeny mean squares and coefficient of variability were higher in treated set than in the normal set in both F_1 and F_2 generations. This indicated that irradiation treatment has released variability for these characters. However, t-test indicated that mean squares in the treated populations were significantly higher than in the normal set only for spikelets per spike (F_1) and kernels per spike (F_1).

During the present study it could be noticed that both G.C.A. and S.C.A. mean squares were significant in normal as well as mutagen treated sets (Table 2), indicating complex nature of characters studied. Therefore both additive and non-additive gene effects are important in the present material. Similar findings were reported by Borojevic (1963), Paroda & Joshi (1970) and by Smocek (1971) for yield

TABLE 2

Mean squares for combining ability in 6 x 6 F_1 and F_2 diallel cross (normal and treated sets) for three characters in wheat (values in parentheses belong to F_2)

Source	D.F.	Mean squares: Spike length Normal	Spike length Treated	Spikelets per spike Normal	Spikelets per spike Treated	Kernels per spike Normal	Kernels per spike Treated
G.C.A.	5	4·13** (4·52**)	4·29** (4·30**)	1·03** (0·83**)	2·47** (0·97**)	17.67 (38.10**)	67·15** (15·30*)
S.C.A.	15	0·46** (0·51)	0·90* (0·47**)	0·74** (0·46*)	1·80** (0·86**)	28.05** (13.80)	77·02** (48.64**)
Error	40	0.15 (0.29)	0.30 (0.16)	0.20 (0.18)	0.39 (0.30)	8.40 (9.04)	9.04 (7.61)
Components							
$\frac{1}{5}\sum_{gi}^{\wedge}$		0.49 (0.52)	0.48 (0.52)	0.82 (0.63)	0.26 (0.08)	0.16 (3.75)	7.26 (0.96)
$\frac{1}{15}\sum_{i<j}^{\wedge}\sum_{sij^2}$		0.31 (0.22)	0.51 (0.30)	0.53 (0.27)	1.41 (0.56)	20.03 (5.76)	67.98 (41.03)
$\frac{1}{5}\sum_{gi}^{2/}-\frac{1}{5}\sum_{i<j}^{/\wedge}\sum_{s\,i\,j^2}$		1.60 (2.40)	0.95 (1.73)	1.54 (2.31)	0.18 (0.14)	0.05 (0.65)	0.10 (0.02)

*Significant at 5%; **Significant at 1%.

and other attributes in F_1 as well as F_2 generation. It can also be seen from the ratio of G.C.A. and S.C.A. components (Table 2) that mutagen treatment increased the non-additive genetic variance relative to additive for spike length and spikelets per spike. However, for kernels per spike there was a relative increase in additive component due to treatments. Burton & Powell (1969) for forage yield in pearl millet (*Pennisetum typhoides*) and Micke (1969) for green matter in sweet clover (*Melilotus albus* L.) also reported an increase in non-additive variance in treated populations. Conversely, Lawrence (1968) for flowering time in *Arabidopsis thaliana*, Gardner (1969) for grain yield in maize (*Zea maize*) and Sharma & Singh (1975) for brown rust resistance in spring wheat reported an increase in additive genetic variance in treated populations. However, Gupta & Virk (1977) also reported an incerase in additive type of gene effects, but only at higher doses of gamma irradiation (20 Kr and 30 Kr) and not at lower doses of 10 Kr. The properties of induced variability depend on genetic architecture of a particular character of the genotypes utilized (Mather 1960, Lawrence 1965). Therefore, the induced variability and changed gene effects could be due to interaction between the mutations and other genes. In the normal set, G.C.A. mean squares in respect of kernels per spike however, became significant only in estimates obtained for F_2. The reverse was the situation for spike length and kernels per spike with respect to S.C.A. mean squares which became non-signifi-

cant in F_2 generation. Both these trends can be attributed to breakage of linkages favouring non-additive genetic variance.

It is obvious from Table 3 that no new positive and significant G.C.A. effects were induced in treated set. However, in the parent K-68, for spike length and in the parent Sharbati Sonora for spikelets per spike, the magnitude of G.C.A. effects increased in treated set over the normal in both F_1 and F_2 generations, indicating thereby that mutagen treatment has increased the additive gene effects in these cases. This suggests that majority of genes with additive effects can be accumulated in the crosses where the above parents are involved and selection would be more effective in these crosses of treated population compared to the normal one.

TABLE 3

Estimates of g.c.a. effects of parents in F_1 and F_2 generations of 6x6 diallel cross (normal and treated sets) for three characters in wheat (values in parentheses belong to F_2)

Parents	Spike length		Spikelets per spike		Kernels per spike	
	Normal	Treated	Normal	Treated	Normal	Treated
1. C273	0.82**	0.65**	−0.59**	−0.34	0.47	−1.58
	(0.47)	(0.66**)	(−0.27)	(−0.18)	(−1.25)	(−2.09**)
2. K-68	0.96**	1.10**	0.04	−0.26	−0.77	−2.57**
	(1.25)	(1.17**)	(0.02)	(0.17)	(1.69)	(−0.55)
3. Sharbati Sonara	−0.83**	−0.67**	0.37*	1.00*	1.17	−5.60**
	(−0.74)	(−0.55**)	(0.49**)	(0.56**)	(2.62**)	(2.17**)
4. Hira	−0.37**	−0.35	0.10	−0.06	−2.65**	−0.18
	(−0.66)	(−0.45**)	(−0.41**)	(−0.17)	(−3.06**)	(0.43)
5. UP310	−0.23	−0.04	−0.23	−0.59**	1.22	1.56
	(−0.11)	(−0.21)	(0.18)	(−0.44**)	(−1.40	(0.01)
6. Shera	−0.36**	−0.69**	0.30*	0.22	0.56	0.10
	(−0.23)	(−0.64**)	(−0.02)	(0.07)	(−1.10)	(0.04)
S.E.± (gi)	0.13	0.20	0.15	0.20	0.94	0.97
	(0.18)	(0.13)	(0.14)	(0.18)	(0.92)	(0.89)
S.E.± (gi–gj)	0.20	0.31	0.23	0.31	1.45	1.50
	(0.27)	(0.20)	(0.22)	(0.27)	(1.27)	(1.38)

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

There are some cases where G.C.A. effects were significant in F_1 generation only (Table 3). This situation could be attributed to the presence of dominance and/or dominance x dominance type of interactions in F_2 generation. Similarly, in some cases the G.C.A. effects are significant only in F_2 generation. This could be attributed to the breakage of linkages contributing to non-additive effects.

The analysis of S.C.A. effects indicated (Table 4) that for all the three characters some new positive S.C.A. effects (desirable) were induced in treated set which were absent in the normal set. However, in some of the cases, S.C.A. effects were significant in the normal set, but became non-significant in treated set. In general *per se* performance of the parents was a good indication of high general combining ability for at least two characters i.e. spike length and spikelets per spike. However, crosses which performed better were either high x low or low x low combinations.

Thus it appeared that both additive and non-additive components of genetic variance were important for these characters in both normal and treated set. However, as pointed

TABLE 4

Estimates of s.c.a. effects in F_1 and F_2 generations of 6x6 diallel cross (normal and treated sets) of three characters in wheat (values in parenthesis belong to F_2)

Crosses	Spike length		Spikelets per spike		Kernels per spike	
	Normal	Treated	Normal	Treated	Normal	Treated
1. C273 X K-68	−0.44	0.11	−1.46**	−1.66	−8.50**	−0.89
	(−0.83)	(−1.19**)	(−0.59)	(0.42)	(−4.31)	(1.23)
2. C273 X Sharbati Sonora	1.26**	0.68	0.13	0.61	3.04	4.83
	(−0.21)	(1.22**)	(−0.26)	(−0.06)	(−2.48)	(0.56)
3. C273 X Hira	0.16	0.99	1.22**	1.07	1.03	7.53**
	(−0.13)	(0.37)	(0.72)	(0.61)	(4.88)	(4.26)
4. C273 X UP301	0.49	1.03	1.00	0.84	4.33	3.88
	(0.36)	(0.43)	(−0.71)	(1.07*)	(−2.56)	(5.12*)
5. C273 X Shera	−0.82*	−0.95	−1.03**	1.32*	−1.10	4.47
	(−0.84)	(0.33)	(−0.22)	(0.32)	(−4.37)	(0.98)
6. K-68 X Sharbati Sonora	−1.26**	−0.37	−1.08**	−0.20	−4.46	2.10
	(−0.39)	(−0.18)	(−0.71)	(−0.45)	(−0.64)	(−1.20)
7. K-68 X Hira	−0.20	0.28	0.89*	1.15**	3.73	5.46*
	−(0.19)	(0.23)	(−0.29)	(−0.32)	(−0.11)	(6.29*)
8. K-68 X UP 301	−0.35	1.00	0.04	0.78	4.51	1.56
	(0.01)	(0.53)	(0.18)	(1.23*)	(2.29)	(7.04**)
9. K-68 X Shera	0.27	0.96	1.19**	0.89	−0.65	0.29
	(−0.33)	(1.25**)	(−0.40)	(0.84)	(−3.52)	(3.48)
10. Sharbati Sonara X Hira	0.03	1.36	0.39	1.23*	4.97	5.88
	(−0.27)	(0.17)	(−0.48)	(0.59)	(3.17)	(2.77)
11. Sharbati Sonora XUP301	−0.28	0.16	−0.32	1.61**	2.54**	12.09**
	(−0.36)	(1.15)	(0.27)	(0.50)	(0.84)	(0.77)
12. Sharbati Sonora X Shera	−0.29	0.57	0.69	−0.21	2.71	2.91
	(0.14)	(0.20)	(0.14)	(−0.18)	(3.02)	(0.02)
13. Hira X UP301	−0.17	−0.38	0.13	−0.41	3.54	2.60
	(−1.13)	(−0.03)	(0.57)	(0.17)	(−1.13)	(1.84)
14. Hira X Shera	0.36	0.44	−0.08	0.27	0.34	−0.60
	(−0.22)	(−0.02)	(−1.32**)	(0.26)	(−5.49*)	(3.18)
15. UP301 X Shera	0.91*	0.55	0.18	0.99	5.16	6.88**
	(1.29)	(−0.13)	(0.17)	(0.52)	(0.52)	(7.09**)
S.E.± (Sij)	0.35	0.56	0.40	0.53	2.57	2.67
	(0.54)	(0.36)	(0.38)	(0.48)	(3.51)	(2.45)
S.E.± (Sij–Sik)	0.53	0.83	0.60	0.83	3.83	3.98
	(0.80)	(0.53)	(0.57)	(0.72)	(5.24)	(3.65)
S.E.± (Sij–Sikl)	0.48	0.77	0.55	0.77	3.55	3.68
	(0.74)	(0.50)	(0.53)	(0.67)	(4.85)	(3.37)

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

out earlier, the magnitude of total variance for progenies (Table 1) was higher in case of treated set than that in the normal set. Therefore, the scope for improvement shall be more in treated population than in the normal. Simultaneously, it is also envisaged that due to the use of different mutagenic treatments (physical and chemical) the inheritance of different characters are altered in different ways in different crop species. To be more precise, in wheat itself, different studies (Sharma & Singh 1975, Gupta & Virk 1977, Balyan & Gupta 1978a, 1978b) revealed different types of changes in gene effects with the use of different mutagens and their doses. Therefore, it would be inferred that the nature of induced genetic variability is genotype and dose specific. This observation is also supported by Gupta & Virk (1977), who pointed out that the magnitude of induced variation in gene effects varied with the genetic constitution of the cross and the dose of mutagen applied. Since different materials and

mutagens (including their doses) were utilized in different studies, further studies are needed to appreciate fully the nature of induced genetic variation.

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