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ARTICLE

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POLLEN GRAIN CHARACTERS - A USEFUL PARAMETER FOR TESTING RADIOSENSITIVITY AND CHARACTERIZATION OF MUTANTS

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Determination of radio-sensitivity is pre-requisite for large scale irradiation in mutation induction for breeding experiments and a wide range of parameters (growth inhibition, chromosomal aberrations, mutation etc.) have already been standardized. Appreciable amounts of literature have been accumulated on pollen grains after mutagen treatment. Pollen grains and their morphological features are genetically stable characters and the effects of mutagens on these features have been studied critically on different ornamental plants and other crops. Changes in pollen grain sterility, size and morphology (ornamentations) were significant after mutagen treatment. Increase in pollen grain sterility after mutagen treatment was observed in all the experimental materials. The responses to irradiation with regard to pollen sterility/fertility appeared to be plant specific. Apertural character and exine ornamentation pattern are the two most stable morphological features of pollen to be considered for diagnostic value of a taxa. Differential conspicuous changes in exine and apertural characters were recorded both after mutagens treatments and in mutants. Significant changes in pollen apertural and exine surface sculptures due to mutation at several independent loci controlling these characters and their differential sensitivity to mutagens have been clearly established. Such study will provide additional information for understanding the genetic control over pollen aperture and exine surface ornamentation which are of potential as markers in plant biosystematics.

Introduction

Study of induced mutations commenced in 1927 by Muller[1] who discovered the mutagenic property of x-ray in *Drosophila* Stadlar[2,3] demonstrated that similar mutation could be induced in plants (Maize & Barley) by x-ray. In the same year mutation has also been induced in *Nicotiana* by Goodspeede[4] and Olsom[5]. Mutation breeding is now an established method for crop improvement. There are wide range of important publications in the mutation breeding field regarding all the basic and applied aspects of induced mutagenesis.The effect of mutagens on chromosome and genes was the main aspect of study after the discovery of mutagenic effects of radiations/mutagens. In course of time, the subject 'radiosensitivity' has been developed. It deals with the sensitivity of plant or animal tissues to radiations and helps in understanding the mechanism of action of ionizing radiations. Radiosensitivity provides valuable information on radiation protection and radiation therapy which have been generated through radiosensitivity studies. Such studies yield valuable information to induce geneticvariability in crop improvement programme through induced mutation. Effects of physical and chemical mutagens have been studied by many workers for

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radiological studies on morphological, cytological and physiological characters in higher plants[6-12].

Determination of radiosensitivity of every material is pre-requisite for large scale irradiation in mutation breeding experiment for development of new variety. Appreciable knowledge and literature have been generated on radiosensitivity during early stages of crop improvement through induced mutagenesis. Recently, determination of radiosensitivity before initiation of mutation breeding work has declined. Radiosensitivity i.e. mode of action of radiations can be assayed by a number of parameters like growth inhibition (reduced germination, reduced height of seedling), lethality, chromosome breakage/aberrations, vegetative and floral abnormalities, mutation frequency and spectrum etc.) It has been determined that various biological, physical and chemical factors can modify the effects of radiation in plants. The number of variables related to radiosensitivity are many and the problem is complex. Different factors which determine the relative radiosensitivity of different plant species have been studied for a long time. Studies generated sufficient information on mutagen doses and their sensitivity to plants. Radiation induced growth inhibition in plants is an outcome of genetic loss following chromosome aberrations or physiological changes. In this regards, structural organization of nucleus and chromosomes (nuclear geometry, number and size of nucleoli, amount and distribution of heterochromatin, chromosome number and number and position of centromeres) were compared in relation to radiosensitivity.

To determine the sensitivity (LD_{50} or optimum working dose) to mutagens, a number of parameters may be selected. The quickest method is seed germination or sprouting 15 day seedling/sprout height may be considered. Morphological abnormalities at the age of 30 days is a good parameter. Another very quick method is chromosomal aberrations during root / shoot tip mitosis.

Pollen grain sterility/fertility is also a good parameter for detecting sensitivity of mutagens. Appreciable amounts of literature have been accumulated on pollen grains after mutagen treatment[13-17]. Pollen grains and their morphological features are genetically stable characters and the effects of mutagens on these features have been studied critically[14,15] on different ornamental plants and vegetable, medicinal and oil yielding plants. Pollen morphological features are well accepted as unique stable characters which may be used as important diagnostic features for identification even at cultivar level. The pollen morphological characters are categorized as apertural, exine ornamentation, exine strata, shape and size in order to their importance and stability. As these characters are accepted as genetically stable, any variation in these characters indicates some changes in those parameters which are responsible for the expression of these characters, in other words it is expected that any change in any of the regulating factors would bring out variation in corresponding characteristic features. Though much work has been done to understand the inheritance pattern of pollen morphology there are controversial views specially on the nature of genetic control of exine patterning[18-21].Effect of polyploidy and hybridization on pollen grain characters have been extensively studied by many scientists[22-27], but literature on effects of mutagens on pollen grains is limited. Effects of mutagens on pollen grains have been reported by many workers. This is, perhaps, first conceptual plan to use pollen grains for testing sensitivity of a mutagen and characterization of mutants.

Materials and Methods:

Plant Materials and Mutagens **-** Different type of propagules (seed, cuttings, suckers, bulbs, corms, eyes etc.) and Mutagens (X-ray, Gamma ray, colchicine, EMS, MMS etc.) were used for experiments.For testing sensitivity of pollen grains to mutagens a wide range of materials and explants were selected for experiments : Ornamentals – Canna *indica* L., Cannaceae, rhizome, Chrysanthemum *morifolium* Ramat., Asteraceae, cuttings/ suckers – gamma rays – 1.0 to 3.5Krad; colchicine - 0.0625 and 0.125%), Gladiolus L. (Iridaceae, bulb –250 rad – 5 Krad), *Mesembryanthemum criniflorum* L.f., Aizoacae, seed –5, 10, 20, 30, 40 and 50 Krad gamma rays), *Narcissus tazetta* L. (Amaryllidaceae, bulb – gamma rays – 250, 500, 750 rad), *Lantana depressa* Naud. (Verbenaceae, stem cuttings – gamma rays – 1 to 4 Krad), *Rudbeckia laciniata* L. cv. Golden glow (Asteraceae, rhizome - gamma rays - 0.5, 1.0, 2.0 and 4.0 krad); Vegetable crops - *Trichosanthesanguina* L. (Cucurbitaceae, dry seed – x-ray – 6 to 30 kR; colchicine – 0.25 to 1.00% for 18 hrs.); Medicinal - Fenugreek - *Trigonella foenum-graecum* L. (Fabaceae,seed - gamma rays – 30 to 50 Krad; MMS - 0.02–0.06% 6 hrs.; EMS - 0.3–0.6% 6 hrs.); Oil bearing crop - *Jatropha curcas* L. (Euphorbiaceae, seed – gamma rays - 6–24 Krad; colchicine - 0.25–1.00%).

For testing pollen grain sterility/fertility - Pollen grains were mounted in malachite green-glycerine jelly. The pollen grains which were regular in shape, full and had uniform stain were considered as fertile, while those which were irregular in shape, empty and hyaline were regarded as sterile[28]. Pollen preparation for pollen morphological studies

was made by the acetolysis method following Erdtman[29] for both SEM and light microscope.The terminology used in the present pollen morphological studies is based on Erdtmann[30], Faegri and Iversen[31] and Punt *et al*. [32].

Results

All types of mutagens were found to affect nearly all pollen characters, including morphology, sterility and dimension of pollen grains in all the experimental materials:

Ornamentals: *Chrysanthemum* – Significant (P<0.05 to P<0.001) increase in pollen grain sterility and reduction in pollen grain size were recorded after gamma irradiation and with an increase in exposures in cultivars 'Nimrod', 'D-5', 'Lalkila' and 'Lilith'. Pollen grains of 'Nimrod', 'D-5' and 'Lilith' were uniform (Fig. 1.A) whereas the pollen grains of 'Lalkila' were of two distinct sizes, which were termed 'big' and 'small' (Fig. 1.B). The size of 'small' and 'big' pollen grains were 45.47+0.40 µm and 64.26+0.73 µm respectively and the frequency of big pollen grains was 3.98%. In vM_1 generation the polar diameter and exine thickness were reduced after irradiation and with increase in exposures. The differences in mean polar diameter and exine thickness between the control and irradiated populations were significant (P<0.05 to P<0.01) in some cases. The extent of reduction (relative to control) in polar diameter and exine thickness (in parenthesis) in 'D-5', 'Lalkila' and 'Lilith' were about 9(12), 3(11) and 6(12) percent respectively after treatment with 2.0Krad.The size of the bigger pollen grains and their frequency were also reduced after irradiation. It was interesting to note that only in one plant after treatment with 1.5Krad some of the pollen grains became bigger even than the largest pollen

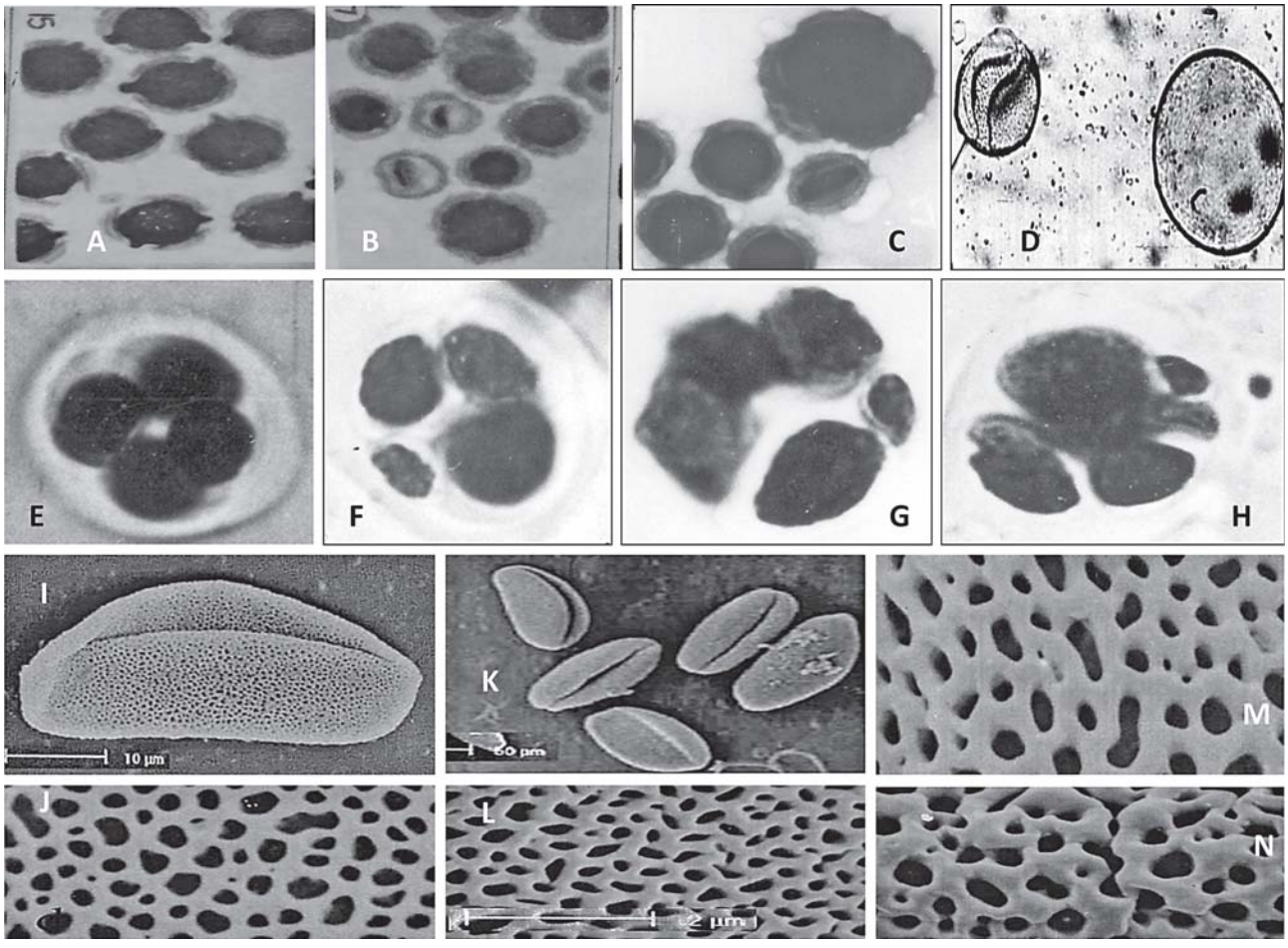

Fig. 1. A. Chrysanthemum cv. 'D-5' normal pollen grains (unacetolysed) . B. cv. 'Lalkila' showing dimorphic pollen grains. C. cv. 'Lalkila' showing increased pollen size (c.f. Datta and Gupta 1980). D. Large and small fertile pollen grains Trichosanthes anguina (c.f. Basu and Datta 1977). E-H. Pollen mother cells with different number of pollen grains. I-N. SEM - Pollen grain and exine ornamentation of *Narcissus tazetta.* I. lateral equatorial view showing full grain and aperture (colpus). J. Part of reticulate exine surface with circular lumina and flat-topped muri. K. Pollen grains of plants irradiated with 250 rad. L. Part of pollen exine surface of plants irradiated with 250 rad showing elongated lumina and raised muri. M. Pollen exine surface of plants irradiated with 500 rad showing compact brochi with small angular lumina and narrow muri. N. Pollen exine surface of plants irradiated with 1000 rad showing loose brochi with large irregular lumina with raised muri of varying width (I-N c.f.Datta et al. 2003).

grain of the 'big' category in the cultivar 'Lalkila' (Fig.1.C). The size of these largest pollen grains were 85.58+1.22µm. Colchicine treatment significantly increased pollen grain sterility and decrease in pollen grain size in chrysanthemum cv. 'D-5' (Table 1)[33].

Pollen fertility decreased with increase in exposure to gamma rays in ***gladiolus*** cv. 'White Friendship' (Table 1).

Rudbeckialaciniata cv. Golden glow – Pollen grain fertility and polar diameter of control plants were 97.34 (%) and 32.3+0.60 µm. Pollen fertility was reduced to 84.06 per cent after irradiation with 1.0 Krad. Pollen grain fertility (%) and polar diameter (µm) in parenthesis of few induced abnormal flowers were determined - 0.5Krad small flower with lesser floret 98.21 (28.9+0.44**), 0.5Krad small flower with abnormal shape 98.75 (36.0+0.33**), 0.5Krad flower with narrower and lesser florets 97.26 (30.0+0.32**), 1.0Krad small flower with lesser florets 84.06 (30.3+0.33**). Both increase and decrease in polar diameter were recorded. Pollen grains were observed in two distinct size (36.0 and 28.9 µm) in plants irradiated with 0.5 Krad. Oval shape pollen grains were also found after irradiation with 1.0 Krad with maximum polar axis 36.0 µ and 28.9 µm. However, equatorial axis was almost constant (25.0 µm)[34].

Table 1. Effects of mutagens on pollen grain sterility (%), polar diameter (µm) and wall thickness (µm) in different ornamentals.

Material : Chrysanthemum	Mutagen : Gamma rays (Krad)		
cv. 'Nimrod'	control	1.5	2.0
Sterility	0.38	1.05	1.25
Polar diameter	37.34+0.22	36.27+0.22	36.21+3.30
cv. 'D-5'			
Sterility	1.85	2.48	4.52***;
Polar diameter	52.88+0.29	50.35+0.32***	48.26+0.34***
Wall thickness	10.66+0.18	10.55+0.19	9.39+0.12***
cv. 'Lilith'			
Sterility	0.85	1.94***	3.86***
Polar diameter	47.69+0.30	47.11+0.29	44.98+0.40***
Wall thickness	11.14+0.15	11.08+0.17	9.83+0.12***
cv. 'Lalkila'			
Small pollen			
Polar diameter	45.47+0.40	45.42+0.49	44.09+0.47***
Wall thickness	9.99+0.20	9.88+0.19	8.90+0.16***
Big pollen			
Polar diameter	64.26+0.73	65.45+1.31	64.24+0.85
Wall thickness	13.07+0.37	12.04+0.31	11.23+0.27***

Chrysanthemum	Colchicine treatment		
cv. 'D-5'	control	0.0625	0.125
Sterility	0.98	1.85	2.25**
Pollen grain size	36.00+0.38	34.09+0.39**	35.42+0.29

Gladiolus	Gamma rays (Gy)					
cv. 'White Friendship'	0.00	250	500750		1000	1250
pollen fertility	93.85	94.20	87.18	78.68	70.07	0.000

Mesembryanthemum	Gamma rays (Krad)					
Control	5	10	20	30	40	50
Sterility	76.00	49.00	47.6	91.7	4.76	73.00	9.00

**=P<0.01, ***=P<0.001

Narcissus**:** Pollen grains of control and gamma irradiated plants were ellipsoidal, monocolpate (1-aperturate) and reticulate. The colpus i.e. single aperture in control and irragiated plants was elongated and situated parallel to the 1st equatorial axis and extended to the proximities of the longer axis of the pollen (Fig.1.I). Striking differences in pollen exine ornamentation pattern were observed among the control and irradiated plants under SEM. The pollen exine ornamentation pattern in control was reticulate where the brocholi (Brochus –it consists of one lumen of a reticulum and half of the width of the adjacent muri, Erdtman 1952) consisted of large circular lumina interspersed with smaller lumina and the top of the muri was flat (Fig. 1.J). The number of lumina per 2µm^2 was 8 to 9. The lumina became larger and elongated mixed with some in between smaller and round lumina (Fig. 1.K) in pollen of 250 rad gamma ray treated plants. The muri were raised and the number of lumina per 2µm^2 was 6 to 7 due to increase in size. Exine ornamentation significantly changed in pollen of 500 rad irradiated plants where the brochi became compact consisting of small angular lumina and narrow muri (Fig. 1.L). The number of lumina per 2µm^2 has been increased to 13-14 in this pollen due to reduction in size. 1000 rad gamma ray treated plants showed very conspicuous change in exine pattern. A loose brochi comprising of large lumina of various shapes was found. The muri was raised and varied in width from

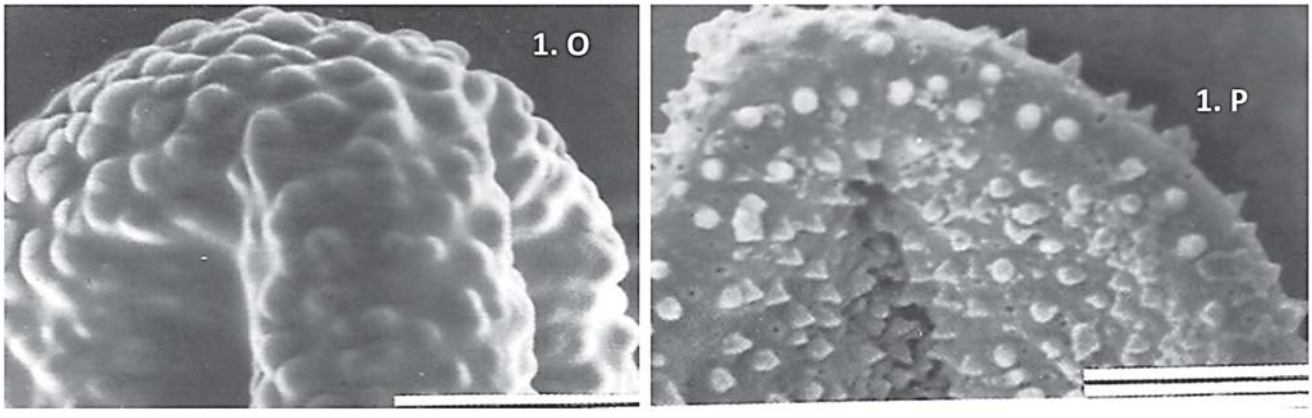

Fig. 1. O. Mesembryanthemum pollen grain showing 'microverrucate' exine pattern after gamma ray treatment and Fig. 1. P. showing 'spinulose' exine (c.f. Chaturvedi et al. 1997).

narrow to considerable wide at the joints (Fig. 1. M,N) and the number of lumina per $2mm^2$ was 4–5 due to larger size35[25].

***Mesembryanthemum criniflorum* L.f.:** Patternless variation in pollen grain sterility was observed after gamma irradiation. Both increase and decrease in sterility were observed after treatment. It was interesting to note that pollen fertility increased after treatment with 30 krad and 50 krad dosages. Pollen grain size was found to be comparatively stable. Not much change was noticed after irradiation (Table 1). The pollen morphology of control and 5, 20, 30, 40 and 50 Krad dosages were same. The exine ornamentation of 10 Krad treated plants was completely changed showing 'Microverrucate' (a wart-like sexine element, more than 1 µm wide, Fig.1. O) pattern as against the 'spinulose'(tip is angular as seen from the periphery, Fig.1. P) pattern of control. There are no puncta in the exine. A few dyads with similar pattern of exine also occured among the monads. The basic apertural character in these grains remains the same as in the controls, being 3-colpate (colporoidate)[36].

Vegetable Crop: *Trichosanthesanguina* L.: Few treated plants showed fertile pollen grains of two distinct sizes 'large' and 'small' (Fig. 1. D). The polar axis in M1 generation after x-irradiation became smaller and pollen sterility increased (Fig.2). In colchicine treated population, the polar axis significantly enlarged and pollen sterility was significantly higher with increase in concentration (Fig. 3). A good relationship was observed between exine thickness and polar axis. The longer was the polar axis the thicker was the exine (Fig.4). Wide range of pollen size was observed in induced tetraploid plants. Size of polar axis in control was 64.3+0.19µm and in tetraploid ranged from 78.0+0.22 to 96.2+0.48 µm. Polar axis in triploid was 87.0+0.91µm. There was no evidence of variation in chromosome number in the treated plants[37].

Oil Bearing Plants: *Jatropha curcas*: Pollen sterility decreased after gamma irradiation more prominantly in lower doses (6, 12 Krad). Both small and large pollen grains were observed in *Jatropha* in addition to normal grains. Small pollen grains were observed only in 18 Krad and the frequency was more over control. Large pollen grains

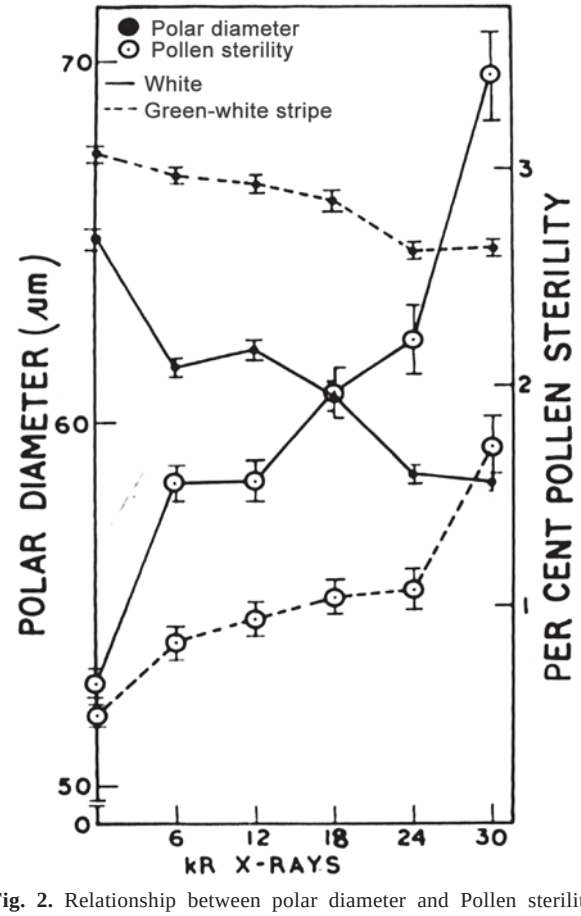

Fig. 2. Relationship between polar diameter and Pollen sterility to x-ray dose

were recorded in 12 and 18Krad and the frequency were slightly higher than control. Random variations in size of three types of pollen grains were observed on plant to plant basis.Pollen sterility decreased after colchicine treatment. Both small and large pollen grains in addition to normal grains were recorded in colchicine treated population and control and patternless variations in frequency were observed after colchicine treatment. No significant variation in size of all types of pollen grains were observed in control and treated populations. Both increase and decrease in pollen sterility and size of normal and small pollen grains were recorded after combined treatment and the variation in some cases were significant (Table 2)[38,39].

Medicinal Plants: *Fenugreek*: Both physical and chemical mutagens significantly increased the pollen sterility (Table 2)[40].

Mutations: In course of study with the present materials, a number of beneficial mutants have been developed due to permanent genetic changes after mutagen/s treatments. All the mutants were detected within

Table 2. Effects of mutagens on pollen grain sterility (%) and polar diameter(µm) in *Jatropha* and Fenugreek.

Jatropha curcas Gamma rays (Krad)							
	Control	6	12	18			
Pollen sterility	1.25–4.83	0.64–1.38	0.68	0.65–4.35			
Small pollen	1.40–3.44	-	-	3.52–6.07			
Size	48.15+1.62	-	-	48.60+1.71			
Large pollen	0.40–1.11	-	2.00	1.44-2.45			
Size	81.54–88.92	-	81.00+0.72	82.53–87.84+2.16			
Normal pollen size	69.12–70.11	65.25-69.75	72.81+0.81	53.72–69.03+0.90			
Jatropha curcas Colchicine treatment							
	Control	0.25	0.50	1.00			
Sterility	1.25–4.83	0.17–3.03	0.16–1.59	0.29–1.60			
Small pollen	1.40–3.44	7.72-10.69	0.28–2.21	2.50–4.87			
Size	45.90–48.15+1.62	49.23+2.16	45.54–50.04+1.44	45.90–50.40+2.34			
Large pollen	0.40–1.11	1.20–4.24	1.00–5.45	0.52–0.91			
Size	80.00-88.92+3.60	81.00–93.19+6.03	80.00–92.25+2.13	80.10–83.97+5.85			
Normal pollen							
Size	55.80–72.00+0.72	59.13–69.03+0.61	45.54–64.80+0.72	59.40–68.64+0.55			
Combined treatment – gamma rays + colchicine (%)							
	Sterility	Small pollen	size small pollen	size normal pollen			
Control	1.26	3.44	45.27+1.17	59.76+0.45			
12Krad+0.50	0.18–1.78	2.31–6.15	42.07–47.97+1.71	57.60–63.90+0.90			
0.50+12Krad	1.06	5.44	44.76–48.42+0.90	59.40–62.37+0.73			
12Krad+1.00	0.41–0.69	2.19–5.44	44.76–48.87+0.72	57.69–60.57+1.08			
1.00+12Krad	0.18–0.84	3.16–4.75	45.90–48.78+1.35	58.85–62.55+0.81			
18Krad+0.50	0.91	2.12	46.12+1.02	59.85+0.95			
0.50+18Krad	0.21–1.42	2.57–2.69	46.98+1.08	60.48–67.32+0.36			
18Krad+1.00	0.35–9.73	1.58–4.40	44.37–48.60+1.98	63.45–75.42+5.40			
1.00+18Krad	0.35–1.48	2.40–2.45	45.72+2.70	58.86–60.30+0.70			
Fenugreek (*Trigonella foenum-graecum* L.)							
Control	MMS	0.02	0.03	0.04	0.05	0.06	Critical difference
7.21		11.43	11.48	19.03	28.55	29.99	1.30
	EMS	0.3	0.4	0.5	0.6		
		33.51	43.82	44.03	50.35	5.11	
Gamma rays [Krad]		30	35	40	45	50	
		24.16	30.27	33.34	42.85	35.50	13.14

*=P<0.05, ***=P<0.001

LD_{50} dose of respective materials.Pollen sterility and morphology were studied on some selected materials as per availability of pollen grains. The details of mutants were as follows:

Chrysanthemum: cv. 'D-5' (magnolia purple) produced mutants 'Alankar' (Spanish orange, 1.5 Krad) and 'Agnisikha' (erythrite red, 2 Krad), cv. 'Kingsford Smith' (purplish mauve) produced 'Rohit' (Rhodonite red, 2.0 Krad), cv. 'Himani' (white) produced 'Sheela' (Canary yellow, 2.0 Krad), cv. 'Flirt' (Chrysanthemum crimson) produced 'Man Bhawan' (Reddish-yellow, 1.5 Krad), cv. 'E-13' (mauve) produced Five mutants ('Anamika' (light reddish), 'Basanti' (yellow), 'Himani' (white), 'Kapish' (brownish) and 'Lohit' (dark reddish), cv. 'M-24' (light purple, ray florets tubular with spatula like open tips) produced 'Tulika' (light purple, brush like florets), cv. 'SharadBahar' (purple) produced 'ColchiBahar' (terracotta red, 0.0625%); Rose: cv. *R. damascena* (pink) produced 'Mutant' (white, 1 Krad), cv. 'Junior Miss' produced 'Sukumari', cv. 'Queen Elizabeth' (pink) produced 'Sharada' (white, 3 Krad); Fenugreek: Produced Five (M1, M2, M3, M4 and M5, EMS -0.3, 0.5, 0.4%) morphological mutants (leaf character, duarf, early flowering, reduced plant height, increased branching, late flowering, small chocolate coloured seeds), 'small seed' mutant (40 Krad), 'green seed' mutant (0.06% MMS), 'bold seed' mutant (0.04% MMS); *Trichosanthusanguina*: Green White Stripe fruit produced 'Short Thick Fruit' mutant (30 kR x-rays), Two selections 'Early Flowering Mutants' (24 and 30 kR); White fruit produced 'Dwarf Thick Fruit' mutant (18 kR x-rays), 'Yellow Fruit' mutant (18kR –rays), 'Crinkled Leaf' mutant (24 kR x-rays), Two selections 'Early Flowering Mutants' (18 and 30 kR)[6,37,40].

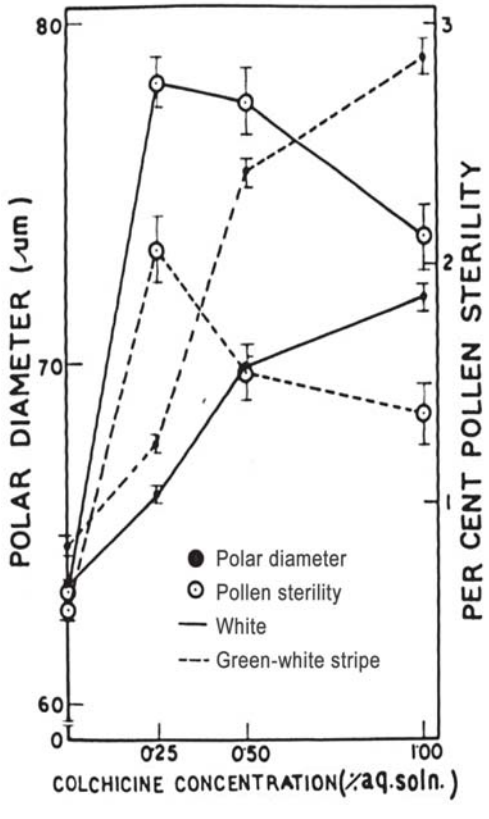

Fig. 3. Relationship between polar diameter and Pollen sterility to colchicine concentration

All the chrysanthemum flower colour / shape mutants were detected in vM1 generation.

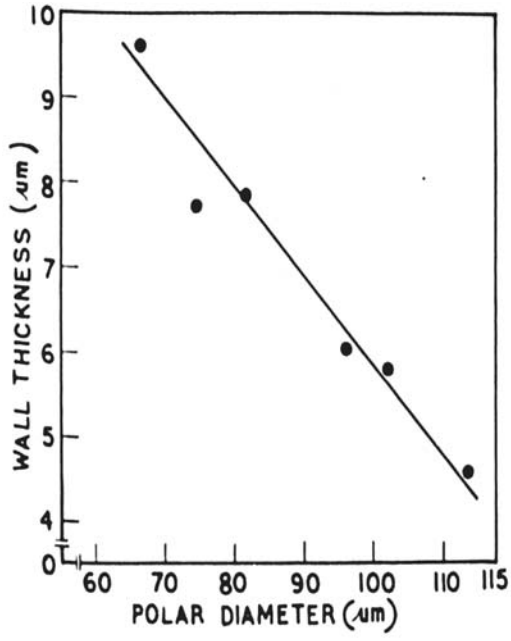

Fig. 4. Relation of wall thickness to Polar axis

Analysis of Mutants

'D-5' and its Mutant 'Alankar': Pollen grain sterility was significantly ($p<0.01$) higher in the mutant than control.All the pollen grains of 'D-5' were of same size which were considered as 'normal' whereas in the mutant there were 'big' and 'small' pollen grains in addition to 'normal' grains. The percentage of normal pollen grains was 88.43 while 7.71 and 4.07 per cent pollen grains were 'small' and 'big' respectively in the mutant. The average size of the 'small' pollen grains was 28.13 µm while that of the 'big' pollen grains was 45.82 µm. There was no significant change in the polar diameter and length of the spine of the pollen grains in 'D-5' and its mutant except the thickness of the wall of the pollen grains of the mutant was significantly ($P<0.01$) more than the control (Table 3)[17].

'Megami' and 'Hemanti': There was no significant difference in pollen grain sterility between 'Megami' and 'Hemanti'. In 'Hemanti' there were 'big' and 'small' pollen grains in addition to normal pollen grains. In 'Megami'

Table 3. Pollen grain characters in original cultivars and their mutants in ornamentals.

Characters		
	'D-5'	'Alankar'
Pollen grain sterility (%)	0.67+0.16	1.80+0.31 **
Pollen type		
Normal (%)	100.00	88.43+0.74
Small	0.00	7.61+0.61
Big	0.00	4.07+0.31
Polar diameter (um) of normal	38.22+0.24	37.52+0.28
Wall thickness	7.50+0.10	8.09+0.13
Length of spine	2.68+0.04	2.64+0.05

	'Megami'	'Hemanti'
Pollen grain sterility (%)	3.67+0.36	3.80+0.40
Pollen type		
Normal (%)	95.68+0.39	91.82+0.67
Small	4.32+0.38	5.21+0.55
Big	0.00	2.97+0.42
Polar diameter (um) of normal	34.43+0.34	35.86+0.44
Pollen grains +S.E.		
Wall thickness normal	6.67+0.30	8.27+0.15***
Length of spine of normal pollen	2.18+0.15	2.59+0.07*
Polar diam. Normal	34.43+0.34	35.86+0.44
Small	21.31+0.42	25.48+0.47
Big	-	45.77+0.64

	'Otome Zakura'	'White'	'Yellow'
Pollen sterility (%)	1.80+0.30	2.72+0.39	4.40+0.56***
Pollen g size pol.diam.	41.43+0.27	42.06+0.36	38.61+0.33***
Pol. G. wall thcknes	9.14+0.19	8.95+0.20	8.82+0.19
Size of p.g. spine	3.41+0.11	3.55+0.11	3.27+0.15

	'E-13'	'Anamika'	'Basanti'	'Himani'	'Kapish'	'Lohit'
Pollen sterility	1.2+0.2	3.5+0.4***	1.7+0.3	3.1+0.4***	4.8+0.6***	2.2+0.3+
Normal(%)	100.0	97.4+0.3	95.7+0.5	100.0	100.0	97.9+0.3
Large(%)	0.0	0.4+0.1	1.3+0.3	0.0	0.0	0.0
Small(%)	0.0	2.3+0.3	2.9+0.4	0.0	0.0	2.1+0.3
Polar diameter						
Normal	50.5+0.4	52.8+0.5***	52.8+0.5***	50.2+0.5	50.8+0.6	50.5+0.5
Large	-	64.5+0.9	70.4+1.2	-	-	-
Small	-	41.6+0.7	41.9+0.6	-	-	36.6+1.5
Normal pollen						
Wall thickness	9.7+0.2	10.0+0.2	9.6+0.1	9.6+0.2	10.1+0.2	9.8+0.2
Length of spine	2.7+0.1	3.1+0.2	2.9+0.1	2.7+0.1	3.3+0.1**	3.0+0.1

	'M-24'	'Tulika'
Sterility	4.45+0.46	7.04+0.53**
Polar diameter	32.88+0.18	31.55+0.18

	'Sharad Bahar'	'Colchi Bahar'
Pollen grain sterility (%)	15.17+0.86	10.30+0.64***
Pollen type		
Normal (%)	91.16	92.65

Small	4.32+0.38	5.21+0.55
Big	1.09	2.04
Small	7.75	5.31
Polar diameter (um) of normal	31.53+0.23	30.63+0.18
Pollen grains +S.E.		
Small	19.93+0.32	16.78+0.47
Big	38.88+0.41	42.30+0.52
Rose		
	R. damascena (pink) and its mutant (white 1krad gamma rays)	
	Control	Mutant
Pollen sterility	45.99+1.54	48.38+1.50
Polar diameter (µm)	32.84+0.40	32.99+0.43
	'Junior Miss'	'Sukumari'
Pollen fertility (%)	12.97+0.82	16.47+1.00*
Polar diameter (µm)	42.00+0.69	39.89+0.66+
	'Queen Elizabeth'	'Sharada'
Pollen sterility (%)	30.81+1.02	35.26+1.15**
Size (µm)	39.73+0.44	39.78+0.30

+=P<0.02; *=P<0.05; **=P<0.01; ***=P<0.001

only 'small' pollen grains were found in addition to normal pollen grains. There was no significant change in polar diameter of 'Megami' and 'Hemanti'. But significant increase in wall thickness and size of the spine of the pollen grains were recorded in 'Hemanti' (Table 3)[41].

'Otome Zakura' and 'White' and 'Yellow' mutants: In the yellow mutant significant decrease in pollen grain size and increase in pollen sterility were observed. No change in thickness of wall and size of the spine of pollen grain were observed between the control and its mutants (Table 3)[14].

'E-13' and its mutants ('Anamika', 'Basanti', 'Himani', 'Kapish' and 'Lohit'): Pollen grain sterility was significantly higher in all the mutants except 'Basanti'. All the pollen grains of 'E-13', 'Himani' and 'Kapish' were of the same size, which were considered normal. In 'Anamika', 'Basanti' and 'Lohit' there were 'large' and 'small' pollen grains in addition to normal grains. The polar diamter of 'Anamika' and 'Basanti' was significantly greater than that of 'E-13'. There was no significant difference in wall thickness and size of the spine of pollen grains except in 'Kapish', where spine length increased significantly. Size of 'large' and 'small' pollen grains have been mentioned in the Table3[42].

'Sharad Bahar' and 'Colchi Bahar': Pollen grain sterility was significantly (P<0.001) less in 'ColchiBahar'. In both the cases 'small' and 'big' pollen grains were observed in addition to 'normal' pollen grains(Table 3)[43].

'M-24' and 'Tulika': Pollen grain sterility was significantly (P<0.001) more in 'Tulika' than control. Pollen grain diameter (µm) in 'M-24' and 'Tulika' was same (Table 3)[44].

Pollen Morphology in Original and Mutant Cultivars of Chrysanthemum: For pollen morphological studies following original cultivars of Chrysanthemum and their gamma ray (Krad) and colchicine induced somatic flower colour mutants (in parenthesis) were selected for studies : 'D-5' -Magnolia purple ('Alankar', Spanish orange, 1.5 krad; 'Agnisikha' - Erythrite red, 2.0 krad); 'Kingsford Smith' - Purplish mauve ('Rohit' - Rhodonite red, 2.0 krad); 'Himani' – White ('Sheela' - Canary yellow, 1.5 krad); 'Sharadbahar' – Purple ('ColchiBahar' - Terracotta red, Colchicine 0.0625%).

Pollen grains of all the original and their respective mutants were 3-(4) zonocolporate, prolate-spheroidal, or oblate-spheroidal in shape and possess tectatespinoseexine with perforations forming reticulate to punctuate patterns. Endocolpium shape was variable – most common was lalongate type but lolongate, circular, square, and indiscernible types were also found.

'D-5' having a fosso-reticulate pattern with narrow muri and irregularly shaped lumina, changed to reticulate exine with broad muri and uniformly circular lumina in the mutants 'Alankar' and 'Agnisikha' .The tips of the spines also changed from straight to bent.Exine pattern (reticulate undulated) and spine shape (broad base) of 'Kingsford

Smith' changed to a scrobiculate wrinkled surface and bulbous base respectively in the mutant cv. 'Rohit'. Punctuate exine surface pattern of 'Himani' changed to a scrobiculate pattern in the mutant cv. 'Sheela'. Exine surface and spine character were significantly changed in colchicine induced mutant 'ColchiBahar'. 'SharadBahar' has reticulate exine with narrow muri, closely packed lumina and spines with broad bases abruptly taper to form acute tips (Fig.5.A,B). Mutant 'ColchiBahar' showed foveolateexine having broad muri and undulated exine surface and the spines were conical in shape (Fig.5.C,D).

***Lantana depressa* and its two mutants**: Pollen grains of original *L. depressa* were 3-4 colporate, more or less circular in polar view, colpa narrow, endocolpium circular (Fig. 5.E). Exine surface undulate with perforations and granules (Fig.5.F). '*L. depressa*variegata' pollen grains 3-4 colporoidatesubspheroidal, more or less circular in polar view, colpa narrow, endocolpium not properly delimited (Fig.5.G.). Exine surface undulated with perforations (Fig.5.H). '*L. depressa*Bicoloured' pollen grains 3-4 colporoidatesubspheroidal, triangular in polar view, amb straight, colpa narrow, margin undelimited (Fig.5.I). Exine surface undulated with perforations (Fig.5.J)[14].

Rose

***R. damascene* (pink) and its mutant** (white 1krad gamma rays) :There was no significant difference in pollen grain sterility and size between the control and mutant (Table)45[37].

'Junior Miss' and mutant'Sukumari': Normally after meiosis four pollen grains are formed from each pollen mother cells. The number of pollen grains per pollen mother cell varied from 4-6 and their size and frequency were also different in both the cases. Pollen grain sterility was significantly more ($P<0.02$) in the mutant, whereas the pollen size was significantly reduced ($P<0.02$) in the mutant (Table 3)[46].

'Queen Elizabeth' (pink) mutant 'Sharada' (3krad, white): Number of pollen grains varied from 4 to 8 in both the original cultivar and mutant. The percentage of pollen mother cells with different numbers of pollen grains were recorded as follows:

Frequency of Pollen Grains Per Pollen Mother Cell	Original Cultivar	Mutant
4	55.75+1.83	60.20+1.85
5	34.64+1.75	30.17+1.74
6	7.98+1.00	7.04+0.97
7	1.22+0.40	2.01+0.53
8	0.41+0.23	0.57+0.29

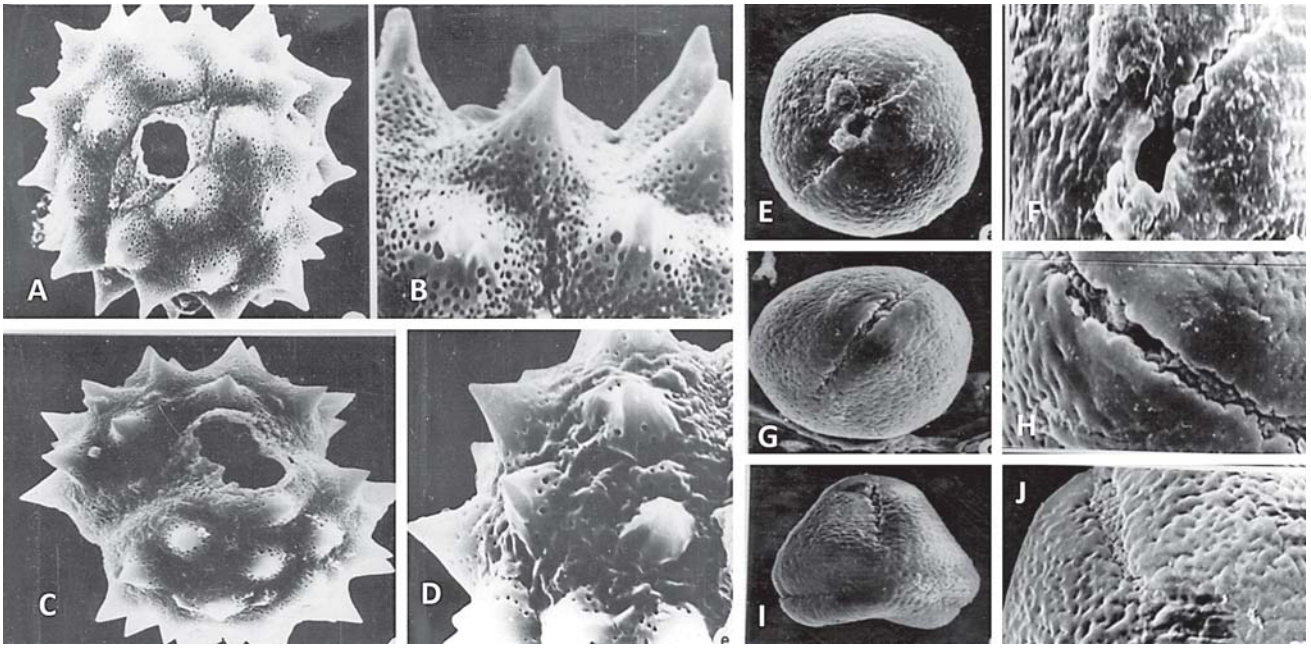

Fig. 5. A-B. Chrysanthemum cv. 'Sharad Bahar' A. equtorial view showing circular endocolpium, B. reticulate surface. C,D. cv. 'Colchi Bahar', C. equtorial view showing lalongate endocolpium, D. perforated, undulated surface. E-F. *Lantana depressa*, E. equtorial view showing circular endocolpium, F. undulated granulated surface with perforations. G-H. L. depressa variegata, G. equtorial view showing undelimited endocolpium, H. striate perforated surface. I-J. *L. depressa* bicoloured, I. polar view, J. undulated perforated surface (c.f. Datta 1977).

Size of pollen grains within each pollen mother cell varied (Figs. 1E-H). No difference in pollen grain size was observed between the two, whereas significant (P<0.01) higher sterility was recorded in the mutant (Table 3)[47].

Fenugreek: EMS (0.3, 0.5, 0.4%) induced five morphological mutants (leaf character, duarf, early flowering, reduced plant height, increased branching, late flowering, small chocolate coloured seeds) :All the mutants had higher pollen sterility and reduced pollen grain size (both equatorial diameter and polar axis) than the control. However, differences were significant only in M1 and M2 (Table 4)[48].

Significant increase in pollen sterility and decrease in pollen grain size were recorded in a gamma ray (40Krad) induced 'small seeded' mutant of Fenugreek (Table)[49].

Increase in pollen sterility and significant decrease in pollen size were observed in a 0.06% MMS induced 'green seed' colour mutant (Table 4)[50].

MMS (0.04%) induced 'bold' seed mutant of fenugreek showed increase in pollen grain sterility and decrease in pollen grain size over the control (Table 4)[51].

***Trichosanthes*:** Following mutants were established - *T. anguina* (GWS) and 'Short Thick Fruit'mutant (30 kR x-rays); *T. anguina* (white) and 'Dwarf Thick Fruit' mutant (18 kR x-rays); *T. anguina* (White) and 'Yellow Fruit' mutant (18kR –rays); *T. anguina* (white) and 'Crinkled Leaf' mutant (24 kR x-rays)

Pollen grain sterility was significantly higher in all the the mutants in M_2, M_3 and M_4generations (Table 4).

Table 4. Pollen Grain Characters in Fenugreek and Trichosanthes Anguina

Fenugreek control and EMS induced five morphological mutants						
	Control	M1	M2	M3	M4	M5
pollen sterility (%)	1.65+0.41	10.72+1.08***	3.45+0.56**	2.39+0.75+	1.46+0.59	1.95+0.65
Equatorial Diameter(µm)	38.15+0.27	34.32+0.29***	33.75+0.28***	36.02+0.28	37.82+0.23	37.90+0.47
Polar axis	27.58+0.22	25.00+0.24***	25.32+0.26***	27.57+0.18	27.50+0.40	27.57+0.22
Gamma ray (40Krad) induced small seeded mutant of Fenugreek						
Control Mutant						
Equatorial diameter(µm)	38.15+0.27		33.82+0.26***			
Pollar axis (µm)	27.58+0.22		24.75+0.18***			
Sterility (%)	1.65+0.41		3.37+0.49*			
0.06% MMS induced 'green' seed colour mutant of Fenugreek						
Pollen sterility (%)	0.41+0.12		0.99+0.23			
Equatorial diam.(µm)	41.00+0.28		37.22+0.20*			
Polar axis (µm)	30.85+0.23		28.08+0.25*			
MMS (0.04%0 induced 'bold' seed mutant of fenugreek						
Sterility (%)	1.65+0.41		3.21+0.66***			
Equatorial diameter (µm)	38.15+0.27)		32.90+0.29***			
Polar axis (µm)	27.58+0.22		25.82+0.47***			
T. anguina (GWS) and Short Thick Fruit Mutant (30 kR x-rays)						
	M2		M3		M4	
	Control	Mutant	Control	Mutant	Control	Mutant
Pollen sterility(%)	0.5+0.1	1.3+0.1***	0.5+0.1	1.2+0.1***	0.4+0.1	1.3+0.1**
T. anguina (white) and Dwarf Thick Fruit Mutant (18 kR x-rays)						
Pollen sterility(%)	0.48+0.08	1.02+0.14***	0.52+0.09	1.24+0.12***	0.56+0.18	.21+0.11***
T. anguina (White) and Yellow fruit Mutant (18kR –rays)						
Pollen sterility(%)	0.48+0.08	1.32+0.11**	0.53+0.09	1.24+0.10**	0.56+0.18	1.02+0.12*
T. anguina (white) and Crinkled Leaf Mutant (24 kR x-rays)						
Pollen sterility(%)	0.48+0.08	1.21+0.14**	0.52+0.09	1.12+0.12**	0.56+0.18	1.09+0.11*

*=P<0.05; **=P<0.01; ***=P<0.001

Polar diameter in 'Yellow Fruit' mutant (68.26+0.20) was more than the control (64.31+0.19). Pollen grains in mother line (*T. anguina*, White) and 'Crinkled Leaf' mutant were triporate and + circular in polar view. The polar diameter (µm) in the control was 64.31+0.19 whereas it was 84.68 + 0.21 in the mutant. Annulus was present in both cases and the pore was elongated. The length (µm) and breadth (µm) of pore in the control and in the mutant were 10.23 and 7.49 and 13.20 and 3.72 µm, respectively. The exine in the control was thin and slightly undulating whereas thick and undulating in the mutant. Thickness (µm) of exine, sexine and nexine was 2.25, 1.45 and 0.80 µm in the control and 2.80, 2.10 and 0.70 µm, respectively in the mutant. There was a granulated area around the pore in the mutant, the exact nature of which could not be established (Table 4)[52,53,54].

Discussion

Parameters like germination/sprouting, plant height, abnormal plant growth, chromosomal aberrations etc. are immediate early effects of mutagens. The percentage of plants with induced morphological abnormalities decreases with aging of the plants. Diplontic selection gradually eliminates the abnormal cells and the plants appear to be slowly returning to near normalcy. In some cases, however, the percentage of abnormal leaves increase at later stage. It is assumed that under exceptional conditions retention of affected cells and their participation in growth and development in later stages of growth is possible. Progeny tests of the abnormal plants showed only normal plants in second and subsequent generations, indicating no immediate genetic background of the aberrations[6,55-58].

All the mutations in chrysanthemum took place in the first generation (vM1) after gamma irradiation which were due to recessive mutations. It was interesting to note that pollen ornamentation patterns were also changed in the first generations. The flower colour/shape mutations and pollen morphological mutations were maintained in subsequent generations through vegetative propagations. It is worth mentioning that all present day ornamentals (*Chrysanthemum* and *Mesembryanthemum*) varieties are highly heterozygous as a result of of centuries of natural cross pollination, selective crossing between elemental species, indiscriminate inter-varietal hybridization with appropriate and careful genotype selection and selection pressure and natural and induced mutations. All the mutants in seed propagated plants (*T. anguina*, *T. foenum-graecum*) were isolated in the second generation after treatment with mutagens, bred true in subsequent generations and inheritance of mutant characteristics suggested their recessive nature. Changes in pollen ornamentation pattern like shift in the exine surface pattern from spinulose to 'Microverrucate' in 10 krad treatment in *Mesembryanthemum* and changes in exine and apertural features in other materials (chrysanthemum, Lantana, Narcissus) may be considered as separate parts like other parts of a plant controlled by different genes, or sets of genes. The occurrence of a number of changed characters (flower colour, flower shape, pollen ornamentations etc.) in one mutant and also individually in different species/ variety suggest that the changes were perhaps induced at several independent loci. However, pleiotropic or epistatic effect of a mutated gene affecting all these characters cannot be ruled out.

Apertural character and exine ornamentation pattern are the two most stable morphological features of pollen to be considered for diagnostic value of a taxa. Pollen grain characters are often very helpful not only for identification of genus or species[59,60] but also for identification at cultivar level[61].Prominent differential changes in exine and apertural characters were observed in the present experimental materials both after mutagen treatment and also in mutants. Maximum deviation in pollen exine surface ornamentation pattern was observed in the present mutagen treated populations and mutants. Changes in exine ornamentations were very conspicuous between the original and mutant varieties. These changes are inconsistent and not dose specific.Significant changes in pollen apertural and exine surface sculptures due to mutation at several independent loci controlling these characters and their differential sensitivity to mutagens have been deistinctlyestablished[16]. Studies also argue that the apertural feature is more stable pollen morphological character than the exine features as the regulatory gene/s of the later character is more susceptible to radiation. The present investigation is in agreement with the view ofHaqet al.[62] who, while working with seed irradiated plants of Lactuca and Cichorium, suggested genetic control of exine pattern and also supposed that different features of exine pattern are controlled by different genes. Chaturvedi[63] also reported a similar type of endocolpial variation in small flower cultivars of chrysanthemum.

The chromosome number remained unchanged in all cases. Changes in pollen grain sterility, size and morphology (ornamentations) were significant after mutagen treatment. Increase in pollen grain sterility after mutagen treatment was observed in all the experimental materials except in few treatments. The responses to irradiation with regard to pollen sterility/fertility appeared to be plant specific. Increase in pollen grain fertility percentage after treatment

with 30 and 50 krad dosages in the present experiment with Mesembryanthemum provide good information which may be used to advantage in experiments of breeding.

Considerable variations in size and shape of pollen grains have been reported in experimentally raised polyploids and colchicine raised plants[64-67]. Colchicine treatment has been shown to affect exine patterning[16]. Dimorphic pollen grains have been found in some of the original cultivars and their mutants. Variation in pollen size within a species has been thought to be due to environmental and nutritional conditions[68,69], population variation within a single plant[70]. The formation of unequal pollen grains has earlier been considered to be due to movement of unequal number of chromosomes at anaphase I and unequal amount of chromatin material in the tetrad[71]. In the present experimental materials there was no evidence of increased chromosomal material in the tetrads. The increase in pollen grain size may be considered to be due to increase in cell volume without increase in chromosomal material but possibly due to differential nutritional conditions.Pollen grain size was reduced after irradiation and with increase in exposure in some cultivars. In addition, some pollen grains became larger after irradiation. Reduction of polar axis and equatorial diameter with low x-ray dose and enlargement of these characters with the highest dose have been reported in *Trigonella foenum-graecum*[72]. Movement of unequal amount of chromatin material in the tetrad has been considered to the reason behind formation of unequal pollen grains[70]. Ghatnekar[73] reported loss of huge amount of chromatin materials are the cause of formation of 'Minipollen'. No change in chromosome number was observed after mutagens treatments in the present experiments and also in the mutants. Spontaneous mutant of Abiespindrow (Royle) Spacleposses four-winged pollen as opposed to two-winged pollen found in the species[74].The radiological aspects of pollen grains have been reviewed by Brewbaker and Emery[75]. Present study will provide additional information for understanding genetic control over pollen aperture and exine surface ornamentation which are of potential marker value in plant biosystematics.

Conclusion

From the above study of radiobotanical aspects and unique morphological features, pollen grain merits as a supplementary parameter in determining radiosensitivity and characterization of mutants. ❐

References

1. L.J. Stadler, LJ, *Science,* **68** : 186-187 (1928a).
2. L.J. Stadler, L.J., *Proceedings of the National Academy of Science*, **14** : 69-75 (1928b).
3. H.J. Muller, H.J., *Science*, **66** : 84-87 (1927).
4. T.H. Goodspeed, *Jour. Hered*., **20** : 243-259 (1928).
5. T.H. Goodspeed and A.R. Olson, *Science*, **67** : 46 (1928).
6. S.K. Datta, Ornamental plants- Role of mutation (Daya Publishing House, Delhi, 1997).
7. S.K. Datta, Parameters for Detecting Effects of Ionizing Radiations on Plants In: Plant Responses to Environmental Stress [eds.: R.D. Tripathi, K. Kulshreshtha, M. Agrawal, K.J. Ahmad, C.K. Varshney, S.V. Krupa, P. Pushpangadan], (International Book Distributing Co., Lucknow, 2006) p.257-265.
8. S.K. Datta, Success Story of Induced Mutagenesis for Development of New Ornamental Varieties. In : Bioremediation, Biodiversity and Bioavailability (Global Science Books, 2012), 6 (Special Issue I), p15-26.
9. S.K. Datta, Induced Mutagenesis: Basic Knowledge for Technological Success. In: Mutagenesis: Exploring Genetic Diversity of Crops [Eds. N.B. Tomlekova, M.L. Kozgar and M.R. Wani], (Wageningen Academic Publishers, 2014) p. 95-137.
10. S.K. Datta, Improvement through Induced Mutagenesis: Ornamental Crops. In : Advanced Technologies for Crop Improvement and Agricultural Productivity [Eds. C.P. Malik, Shabir Hussain Wani, Himakshi Bhati – Kushwaha and Ritesh Kaur], (Agrobios (India), Jodhpur, 2017) Chater 4, p. 49-86.
11. J.E. Gunckel and A.H. Sparrow, *Encyclopedia of Plant Physiology*, **16** : 555-617 (1961).
12. A.H. Sparrow, A.F. Rogers and S.S. Susan, *Rad. Bot.* **8** : 149-186 (1968).
13. S.K. Datta, Advances in Horticulture and Forestry 1, Article No. 31 : 241-254 (1990).
14. S.K. Datta, Indian Floriculture ; Role of CSIR. Regency Publications, A Division of Astral (International (P) Ltd., New Delhi, 2015).
15. S.K. Datta, Induced Mutation Breeding, *Springer* (2023).
16. K. Datta and S.K. Datta, *Israel Journal of Plant Sciences*, **46,** 199-207 (1998).
17. S.K. Datta and M. N. Gupta, *The Chrysanthemum*, **34** (4) : 193-200 (1981).
18. H. Godwin, *New Phytol*, **67** : 667-676 (1968).
19. C.M. Rogers and B.D. Harris, *Amer. J. Bot*., **56** : 1209-1211 (1969).
20. J. Heslop-Harrison, *Science*, **161** : 230-237 (1968).
21. J. Heslop-Harrison, Sporopollenin in the biological context. In: Sporopollenin [Eds. Brooks, J. et al.], (Academic Press, London, 1971) p. 1-30.
22. F.W. Gould, *Brittonia*, **9**: 72-75 (1957).
23. L.G. Kessler and D.A. Larson, *Pollen Spores*, **11** : 203-221 (1969).
24. C.F. Quiros, *J. Hered*, **66** : 45-47 (1975).
25. B.R. Rajendra, A.S. Tomb, K.A. Mujeeb and L.A. Bates, *Pollen Spores*, **20** : 145-156 (1978).
26. C. Ravikumar and P.K.K. Nair, *Can. J. Bot*., **64** : 3134-3140 (1986).
27. M. Chaturvedi, K. Datta, and S.K. Datta, *Taiwania,***42** (4) : 289-295 (1997).

28. S.K. Datta, Rose News, 5(6) : 3 (1986).
29. G. Erdtman, Svensk Botanisk Tidskrift, 54: 561-564 (1960).
30. G. Erdtman, Pollen Morphology and Plant Taxonomy. Angiosperms. An Introduction to Palynology 1 (Almquist and Wiksell, Stockholm, 1952).
31. K. Faegri and J. Iversen, Text Book of Pollen Analysis. 2nd ed. Munksgaard, Copenhagen (1964).
32. W. Punt, P.P. Hoen, S. Blackmore, S. Nilsson and A. Le Thomas, Glossary of pollen and spore terminology, *Rev. Palaeobot Palynol*, 143: 1–81 (2007).
33. S.K. Datta and M.N. Gupta, *New Botanist*, **VII**: 73-85 (1980).
34. R. Shukla, P. Nath, H.M. Jugran and M.N. Gupta, *J. Nuclear Agric. Biol*., **15** (4) : 207 210 (1986).
35. K. Datta, R. Shukla and S.K. Datta, *Cytologia*, **68**(3): 225-230 (2003).
36. M. Chaturvedi, K. Datta and M. Pal, *Feddes Repert*., **108**: 325-333 (1997).
37. R.K. Basu and S.K. Datta, *Grana*, **16**: 105-109 (1977).
38. S.K. Datta and R.K. Pandey, *J. Ind. Bot. Soc*., **71**(I-IV) : 213-215 (1992).
39. R.K. Pandey, Cytogenetical studies on Jatropha curcas. L. Ph.D. Thesis, Kanpur University, Kanpur (1993).
40. V. Laxmi and S.K. Datta, *Biological Memoirs*., **13** (1) : 64-68 (1987).
41. S.K. Datta and M.N. Gupta, *Progressive Hort*., **14** (1) : 33-37 (1982).
42. S.K. Datta and M.N. Gupta, *SABRAO Journal*, **134** (2) : 136-148 (1981).
43. S.K. Datta and B.K. Banerji, *J. Indian Bot. Soc*., **67**(3&4): 275-277 (1988).
44. S.K. Datta, B.K. Banerji and M.N. Gupta, *J. Nuclear Agric. Biol*., **14** (4) : 160 (1985).
45. M.N. Gupta, P. Nath and S.K. Datta, S.K. 1988. *The Indian Rose Annual*, **11**: 55-61 (1988).
46. S.K. Datta and M.N. Gupta, *J. Nuclear Agric. Biol*., **11**(2) : 32-33 (1982).
47. S.K. Datta and M.N. Gupta, *Sci. & Cult*. , **50** : 200-201 (1984).
48. S.K. Datta and V. Laxmi, *J. Ind. Bot Soc*., **71** (I-IV) : 65-68 (1992).
49. V. Laxmi and S.K. Datta, *J. Nuclear Agric. Biol*. **15** (3) : 183-184 (1986).
50. V. Laxmi, M.N. Gupta and S.K. Datta, *SABRAO Journal*, **14** (1) : 83 (1983a).
51. V. Laxmi, M.N. Gupta and S.K. Datta, *Cytologia*, **48** : 473-78 (1983b).
52. S.K. Datta, *Crop Improvement*,**14** (2) : 182-184 (1987).
53. S.K. Datta, *Indian J. Gent. & Pl. Breed*., **54** (1) : 13-17 (1994).
54. S.K. Datta, *Biological Memoirs*, **15**(1) : 18-22 (1989).
55. S.K. Datta, *J. Indian Bot. Soc*., **67**(3&4) : 271-274 (1988).
56. S.K. Datta and R.K. Basu, *Trans. Bose Res. Inst*., **40**(3) : 63-67 (1977).
57. S.K. Datta, D. Chakrabarty, A.K. Verma and B.K. Banerji, *Caryologia*, **64**(4) : 388-397 (2011).
58. Z. Hossain, A.K.A. Mandal, R. Shukla and S.K. Datta, *Plant Science*,**166** : 215-220 (2004).
59. C.W. Compton, *Can. J. Bot*., **60** : 294-300 (1982).
60. L.Z. Diaz, *Grana*, **35** : 24-32 (1996).
61. M. Chatrvedi, K. Datta, P. Nath and M. Pal., *Grana*, **32** : 168-174 (1993).
62. M.Z. Haq and M.B.E. Godwara, *Pollen Spores*, **15** : 421-436 (1983).
63. M. Chaturvedi, *New Botanist*, **11** : 91-101 (1984).
64. S.S. Raghuvanshi and S. Joshi, *Cytologia*, **29** : 61-78 (1964).
65. S. Joshi and S.S. Raghuvanshi, *Grana Palynol*.,**6** : 186-190 (1965).
66. R.P. Roy and J. Ghosh, *Nucleus*, **14** : 111-115 (1971).
67. A.K. Singh and R.P. Roy, *Cytologia*, **40** : 13-20 (1975).
68. E.B. Kurtz and J. Livermann, *Bull. Torrey Bot. Club*, **85** : 136-138 (1958).
69. C.R. Bell, *Am. J. Bot*., **46** : 621-624 (1959).
70. K.E. Clausen, *Pollen et Spores*, **2** : 169-174 (1960).
71. M.A.Q. Shaikh and M.B.E. Godward, *Cytologia*, **37** : 489-495 (1972).
72. S. Chanda and S. Sahay, *Grana*, **10** : 136-140 (1970).
73. M.V. Ghatnekar, *Caryologia*, **17** : 220-224 (1964).
74. P.N. Mehra and P.D. Dogra, *Palynol. Bull*., **1** : 16-23 (1965).
75. J.L. Brewbaker and G.C. Emery, *Radiat. Bot*., **1** : 101-154 (1962).