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MUTAGENIC STUDIES IN M_1 GENERATION IN COTTON (*Gossypium arboreum* L.)

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ABSTRACT

The experiment was conducted to screen the mutant populations to identify the desirable mutants for both qualitative and quantitative traits in cotton. Three populations of cotton *viz.*, PA-402, PA-255 and PA-402 x PA-255 were treated with four doses of gamma rays (10, 20, 30 and 40 kR) and EMS (0.1, 0.2, 0.3 and 0.4%). The observations on mutagenic effects in M_1, $F\text{-}_1M_1$ generation reduced seed germination and survival with the increasing dose of gamma rays.Germination and survival was more pronounced in gamma irradiated populations compared to EMS treatments. Among the genotypes, PA-402 x PA-255 was more sensitive for gamma irradiated mutagenic treatments and genotype PA-255 was more sensitive to EMS. The LD_{50} dose between 30-40 kR gamma rays radiation and 0.3 to 0.4% EMS were found to be ideal for mutagenic treatments irrespective of the population.

(Key words : Mutation, segregants, EMS, LD $_{50}$,chlorina, viridis)

INTRODUCTION

Cotton is one of the most important commercial crop and is popularly known as the "white gold" or "king of fibres". It plays a crucial role in the global economy as well as social and industrial infrastructure. Besides being the backbone of the textile industry, cotton and its by-products are also part of the livestock feed, seed-oil, fertilizers, paper and other consumer products. Cotton has been cultivation in India for more than five thousand years.

Though synthetic fibres have made inroads in many countries in the world, still cotton deserves the prime position in India constituting more than 70 per cent of the total fibre consumption in the textile sector. In India cotton is grown over an area of about 9 million hectares and provides livelihood to over 4 million farming families, along with various allied activities like ginning yarn and fabric production, textile processing, garment manufacture, marketing etc. providing employment to several million people. On the contrary *desi* cottons are well adapted to a wide range of climatic condition, tolerant to both sucking pests and bollworms, of late farmers are inclined to grow desi cotton owing to low input cost required for their cultivation. Therefore, there is an urgent need to develop high yielding varieties of *desi* cotton. However, it appears that cotton being an often cross pollinated crop there is little chance for new recombinants resulting in limited genetic variability in the cultivated diploid species. The improved varieties of crop plants can be developed by pooling available allelic resources from different populations having well adapted, high yielding genotypes. The easiest way is to combine the desirable genes from two parents by crossing and selecting from segregating generations. However, mutation breeding has been found to be promising to induce variability for qualitative and quantitative traits in various crops. It is suggested that the application of mutagenic treatments to hybrids may be one of the wayto induce variability, which is heritable. Complementing the conventional methods, mutation breeding can play a unique role in crop improvement which provides a novel approach to plant breeder for improving the productivity of crop plants. It proves to be the method of choice for obtaining quicker results, when it is desired to rectify small defects in any crop variety.The present investigation was taken up to study the relative effects of physical and chemical mutagens in M_1 generation

MATERIALS AND METHODS

The present investigation was undertaken in *Desi* cotton (*Gossypium arboreum* L.) at Cotton Research station, Mehboob Baugh, Parbhani. The material for the present study was generated in the Cotton Research station, Mehboob Baugh, Parbhani. The pure seed of two commercial varieties of *Desi* cotton (*Gossypium arboreum* L.) *viz.*, PA-402 and PA-255 alongwith their F_1 (PA-402 x PA-255) were

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selected for mutagenic treatment. The seeds of the following one single cross (F_1) were produced during *kharif* 2012 i.e. PA-402 x PA-255 at the Cotton Research station, Mehboob Baugh, Parbhani. Uniform 200 pure dry seeds with about 10 ± 1 per cent moisture each of two varieties *viz*., PA-402 and PA-255, alongwith their F_1 (PA-402 x PA-255) were exposed to 10, 20, 30 and 40 kR dose of gamma rays (CO^{60})with a dose rate of 2.39 kR minute at Nuclear and Agriculture Division, Bhabha Atomic Research Centre, Trombay, Mumbai.The 200 untreated seedsof each variety served as control. The uniform 200 seeds of both varieties along with F_1 hybrid seeds were presoaked in distilled water for 8 hours and then dipped in enough mutagenic solution of chemical mutagens of different concentrations (0.1%, 0.2%, 0.3%, and 0.4% EMS). Seeds treated with chemical mutagens were thoroughly washed in running tap water in order to remove the traces of mutagens, and were immediately sown in the field of Cotton Research station, Mehboob Baugh, Parbhani on dated 5th July *kharif* 2013 by dibbling one seed hill^{-1} at a distance of 60 cm between rows and 30 cm between seeds. The experiment was laid in a randomized block design (RBD) (Panse and Sukhatme, 1954) with three replications. Two hundred seeds of each treatment including control (untreated seeds) were sown in a plot size of 12 x 3 m^2 (5 rows of 12 m length) in each replication. Recommended package of practices was followed to raise a good crop. Observations were recorded on nine characters viz; date of emergence, germination %, survival %, 2.5% span length(%), fibre fineness (ug inch^{-1}), fibre strength (g tex^{-1}), uniformity ratio(%), fibre elongation (%) and short fibre index (%).

RESULTS AND DUSCUSSION

Studies in M_1 generation

The data regarding emergence, germination per cent and survival per cent are presented in Table 1. In general, only pure breeding genotypes have been used in mutation breeding. The usefulness of subjecting hybrid genotypes to mutagenesis for increasing variability was reported by Gregory (1956) in peanut, Rangaswamy (1986) and Mahishi (1983) and Usha and Singh (2005) in cowpea. They hypothesized that mutagenesis of homozygous and heterozygous genotype increase the recombination rate with possibility of increasing variability. However, not many studies have been conducted in this regards to generate valuable conclusions. A few studies conducted so far have expressed conflicting views regarding the role of induced mutations as a supplement to hybridization in releasing variability for quantitatively inherited traits in crop plants.

Studies in M_1, F_1M_1 generation

Mutagenic sensitivity

Mutagenic sensitivity in general is known to be influenced by a populations of factors which include moisture content, temperature, storage of the seed, gaseous composition, pH, concentration of catalytic ions, various pre and post treatment conditions, genetic constitution of the materials treated, strength of the mutagen used, duration of the treatment used etc. (Konzak *et al*., 1965, Blixt, 1967). In the present study, mutagenic sensitivity was worked out through different parameters such as per cent germination, plant survival in M_1, F_1M_1 generation and chlorophyll and viable mutation frequency in M_2, F_2M_2 generation.

Date of emergence and germination

Minimum days (6.66 days) to emergence recorded in 10 kR dose and maximum (10.66 days) observed in 40 kR dose of gamma irradiated treatment in PA-402 population. In case of chemical mutagen i.e. EMS the minimum days to emergence recorded in 0.1% concentration (7.67 days) and maximum days to emergence noticed in 0.4% EMS concentration (11.00 days), as compared to control. In case of PA-255 minimum days (8.33 days) to emergence observed in 10 kR gamma rays dose and highest days to emergence showed by 30 kR dose of gamma rays (11.00 days), while in chemical mutagenic treatment minimum days to emergence noticed in 0.1% EMS concentration (6.67 days) and late emergence observed in 0.4% concentration of EMS (12.00 days). In PA-402 x PA-255 populations minimum days (8.00) to emergence found in 10 kR dose and highest days to emergence (11.33) observed in 40 kR dose of gamma rays, whereas in chemical mutagenic treatment minimum days to emergence marked in 0.1% EMS concentration (6.00 days) and late emergence noted in 0.4% concentration of EMS (8.33 days). Similar types of results have been reported by Badigannavar (1999) high as 64 per cent emergence in Abadhita at 5kR irradiation and as low as 39 per cent at 30 kR. Afsari *et al.* (2000) using were 0, 25, 30 and 35 kR doses of gamma radiation and reported decrease in germination percentages and survival percentages.Remya (2011) observed decrease in germination % and survival % in M1 generation of cotton with 0, 25, 30 and 35 kR doses of gamma radiation.Anasane *et al*. (2019) also recorded reduction in germination percentage in Indian mustard by gamma radiation (1100 Gy).

Survival

Both gamma irradiation and EMS treatments were effective in reducing the survival in all the three populations.The data revealed that survival of plants decreased with an increase in dose or concentrations of gamma rays and EMS in M_1, F_1M_1 generation. The maximum survival was observed in PA-402 population, i.e. 76.25 per cent in 0.1% EMS concentration and minimum survival in 36.42 per cent in 0.4% EMS concentration in PA-402 population respectively. The minimum survival was observed in PA-255 for 0.4% EMS concentration (36.54 per cent) and maximum survival was observed (80.35 per cent) in 10 kR dose of gamma rays. While, in F_1M_1 (PA-402 x PA-255) population the highest survival was observed (82.04 per cent) in 0.1% EMS concentration and lowest survival (32.42 per cent) was observed in 0.4% EMS.The present findings in respect of dose and concentration dependent reduction in survival, mutagen differences and differential response of populations to mutagens are in conformity with the earlier reports of Stoilova *et al*. (1990). They irradiated F_1 seeds of interspecific hybrid (*G. hirsutum* X *G.barbadense*) at 25 kR of gamma rays. They observed, slight sensitivity for emergence and survival of M_1 F_2 plants when *G. hirsutum* was the maternal parent. Aslam *et al*. (1994) studied the response of irradiated cotton pollen at 0.5, 1, 2 and 5 kR of gamma rays prior to cross pollination in various cross combinations. M_1 generation studies showed that higher doses of 2.0-5.0 kR of pollen radiation decreased the emergence and survival. Afsari *et al.* (2000) studied effects of gamma radiation with dosage of 0, 25, 30 and 35 kR. The data on mean germination percentage, survival percentage indicates gamma irradiation decreased survival percentages.

.5. LD_{50} for germination and survival

The genotypic differences for both the mutagenic treatments were observed. PA-402 x PA-255 was more sensitive to gamma irradiation and PA-255 more sensitive to EMS compared to other population. LD_{50} for seed germination in case of gamma rays was found to be 37.58 kR (PA-402), 32.80 kR (PA-255) and 32.16 kR (PA-402 x PA-255), whereas in case of EMS treatments, it was 0.38 per cent (PA-402), 0.34 per cent (PA-255) and 0.36 per cent (PA-402 x PA-255). LD_{50} for survival in case of gamma rays was found to be 33.23 kR (PA-402), 27.85 kR (PA-255) and 27.55 kR (PA-402 x PA-255), whereas in case of EMS treatments, it was 0.33 per cent (PA-402), 0.29 per cent (PA-255) and 0.31 per cent (PA-402 x PA-255). This indicated that the LD_{50} dose between 30-40 kR in case of gamma rays and 0.3-0.4 per cent in case of EMS were ideal for mutagenic treatment in case of cotton. This is in agreement with the finding of Afsari *et al*. (2000), Muthusamy and Jaybalan (2011), and Waghmare *et al*. (2000). They indicated that cotton populations tolerate relatively higher doses (above 30-35 kR of gamma rays and around 0.5% EMS) as against the indications in the present investigation. Findings of these authors and those observed in the present study clearly showed that LD_{50} values vary from genotype to genotype.

2.5 Per cent span length

The data regarding fibre quality are presented in Table 2. In PA-402 populations among all mutagenic treatments 30 kR dose of gamma rays in physical mutagenic treatments recorded highest for 2.5 per cent span length (27.0 per cent) and which is long under category, while, in chemical mutagenic treatments 0.4% EMS concentration recorded highest span length (26.6 per cent) which is also long noted under classification of span length as compared to control (25.8). In case of PA-255 population with 0.4% EMS concentration highest span length (28.1 per cent) wasobserved as compared to control (27.0). Considering PA-402 x PA-255 populations highest span length (27.2 per cent) observed in 40 kR dose of gamma rays of physical mutagen and 0.4% EMS recorded highest span length (26.8 per cent) as compared to their respective control.Asim *et al.*(2015) reported positive increase in flowers plant^{-1}, cotton weight plant^{-1}, 2.5% span length, short fibre index and fibre elongation incotton varieties, when irradiated at the rate of 15 Krad mutagenic treatment.

Fibre fineness/Micronaire value

The mutagenic treatments of 10kR and 40 kR dose of gamma rays and 0.1% and 0.2% EMS concentration recorded highest micronaire value (5.6µg inch^{-1}) as compared to control. Whereas in PA-255 populations all mutagenic treatment recorded lowest micronaire value lower than control. While, in PA-402 x PA-255 populations, 10 kR dose of gamma rays of physical mutagen and 0.4% concentration of EMS marked for highest micronaire value (5.9 and 5.7µg inch^{-1}) respectively over control. Similar results were reported by Khan *et al*. (2014) in four cotton genotypes i.e. Gomal-93, Bt-131, Bt-121 and Bt-CIM-602 when irradiated with 10, 15, 20 and 25 kR doses of gamma irradiation.

8. Fibre strength (g tex^{-1})

The mutagenic dose of 10 kR of gamma rays in physical mutagen and 0.4% EMS concentration of chemical mutagen noted highest for fibre strength (21.0 g tex^{-1}, 21.5 g tex^{-1}respectively) as compared to control. While, in PA-255 populations 10 kR dose of gamma rays and 0.1% EMS concentration observed highest fibre strength (21.7 and 21.9 g tex^{-1}) as compared to control.Whereas 30 kR dose of gamma irradiation and 0.4% EMS concentration of chemical mutagen recorded highest fibre strength (22.8 and 22.2 g tex^{-1}) in PA-402 x PA-255 populations in M_1, M_1 F_1 generation.Muthusamy and Jaybalan (2011) and Si *et al.* (2020) found cotton mutant lines with improved yield and fibre characters viz., span length, fibre fineness, fibre strength, uniformity ratio and fibre elongation.

9. **Uniformity ratio (%)**

In case of , 30 kR dose of gamma rays of physical mutagen recorded highest uniformity ratio (53 per cent), while, 0.1,0.2, 0.3% EMS noticed for highest and similar uniformity ratio (53 per cent) in PA-402 populations. As regard PA-255 populations 40 kR dose of gamma rays and 0.1% EMS concentration showed highest uniformity ratio as compared to control. In PA-402 x PA-255 populations, 10 kR dose of gamma rays and 0.4% EMS concentration observed highest for uniformity ratio (54 per cent) as compared to control. Muthusamy and Jaybalan (2011) found cotton mutant lines with improved yield and fibre characters viz., span length, fibre fineness, fibre strength, uniformity ratio and fibre elongation.

9. Fibre elongation (%)

The maximum fibre elongation was observed in 30 kR dose of gamma rays treatment and 0.2% concentration of EMS (5.4 and 5.3 per cent) in PA-402 populations. While,in PA-255 populations application of 40 kR gamma rays exhibited lower fibre elongation (5.4 per cent) among all treatments of physical mutagen, whereas, 0.4% EMS found higher fibre elongation (5.3 per cent). Asim *et al.*(2015) and Haidar *et al.* (2016) reported positive increase in number of flowers plant^{-1}, cotton weight plant^{-1}, 2.5% span length, short fibre index and fibre elongation in cotton varieties, when irradiated @ 15 Krad mutagenic treatment.

Table 1. Effect of different mutagens on date of emergence, germination and survival in homozygous and heterozygous populations in M_1 and F_1M_1 generation in cotton

Sr. No.	Doses of mutant	PA-402 Date of emergence (DAS)	PA-402 Germination (%) 15	PA-402 Germination (%) 30	PA-402 Survival (%)	PA-255 Date of emergence (DAS)	PA-255 Germination (%) 15	PA-255 Germination (%) 30	PA-255 Survival (%)	PA-402-PA-255 Date of emergence (DAS)	PA-402-PA-255 Germination (%) 15	PA-402-PA-255 Germination (%) 30	PA-402-PA-255 Survival (%)
Gamma rays													
1	10 kR	6.66	66.33	55.33	75.38	8.33	61.33	51.67	80.35	8.00	60.00	50.00	75.00
2	20 kR	7.33	54.33	44.00	68.83	9.00	54.67	44.00	60.92	8.67	45.67	35.00	51.00
3	30 kR	9.33	41.00	32.00	47.34	11.00	41.00	32.67	42.25	10.65	38.33	27.67	45.71
4	40 kR	10.66	35.67	25.33	36.90	10.67	34.67	25.67	40.34	11.33	31.67	19.00	36.16
5	Dry control	6.66	72.33	70.33	89.57	6.67	71.33	70.00	95.89	7.00	71.00	70.00	92.85
EMS													
1	0.1 %	7.67	68.67	59.00	76.25	6.67	64.00	49.00	77.88	6.00	65.67	47.66	82.04
2	0.2 %	8.33	55.33	44.33	64.75	8.67	52.67	41.33	58.94	6.67	55.67	43.67	69.59
3	0.3 %	12.33	43.67	30.33	45.61	10.66	44.67	31.67	45.61	7.66	50.33	38.33	51.27
4	0.4%	11.00	35.66	27.20	36.42	12.00	31.00	20.67	36.54	8.33	31.00	24.00	32.42
5	Wet control	6.33	71.67	68.67	85.53	6.00	75.00	74.33	92.67	5.33	70.00	67.00	94.62

Table 2. Mean performance for quality parameters in homozygous and heterozygous genotypes in M_1, F_1M_1 generation of cotton

Sr. No.	Treatments	2.5% SL length (%)	Fibre fineness (Micronaire) (µg inch^{-1})	Fibre strength (g tex^{-1})	Uniformity ratio (%)	Fibre elongation(%)	Short fibre index (%)
PA-402							
1	10 kR Gamma rays	25.8	5.6	21.0	52	5.3	13.0
2	20 kR Gamma rays	26.0	5.4	20.2	52	5.3	13.9
3	30 kR Gamma rays	27.0	5.5	20.0	53	5.4	13.0
4	40 kR Gamma rays	26.0	5.6	20.2	51	5.1	9.7
5	0.1 % EMS	25.1	5.6	21.4	53	5.2	12.0
6	0.2 % EMS	25.6	5.5	20.3	53	5.3	12.9
7	0.3 % EMS	25.8	5.6	21.4	53	5.1	13.0
8	0.4 % EMS	26.6	5.5	21.5	52	5.2	9.7
9	control	25.8	5.5	20.6	52	5.1	12.8
PA-255							
10	10 kR Gamma rays	27.2	5.1	21.7	50	5.2	10.5
11	20 kR Gamma rays	27.3	5.2	20.3	50	5.2	8.3
12	30 kR Gamma rays	28.0	5.3	21.0	50	5.2	9.0
13	40 kR Gamma rays	27.4	5.2	20.6	51	5.1	9.6
14	0.1 % EMS	26.2	5.5	21.9	53	5.4	10.2
15	0.2 % EMS	26.4	5.7	21.5	51	5.2	9.8
16	0.3 % EMS	26.2	5.1	21.0	51	5.1	11.7
17	0.4 % EMS	28.1	5.3	21.0	50	5.3	8.6
18	Control	27.0	6.0	21.0	50	5.2	9.0
PA-402 x PA-255							
19	10 kR Gamma rays	25.4	5.9	20.2	54	5.1	12.9
20	20 kR Gamma rays	27.0	5.6	21.3	53	5.1	10.2
21	30 kR Gamma rays	27.0	5.3	22.8	51	5.3	12.0
22	40 kR Gamma rays	27.2	5.6	22.4	52	5.2	12.4
23	0.1 % EMS	25.9	5.4	21.3	52	5.2	12.1
24	0.2 % EMS	25.4	5.6	21.6	53	5.1	12.0
25	0.3 % EMS	26.0	5.5	22.0	53	5.3	11.1
26	0.4 % EMS	26.8	5.7	22.2	54	5.2	10.4
27	Control	25.9	5.1	20.6	53	5.9	12.7

10. Short fibre index (%)

The data on short fibre index revealed that the 20 kR gamma rays dose of physical mutagen and 0.3 % EMS concentration of chemical mutagen exhibited highest short fibre index (13.9 and 13.0 percent) as compared to control. In PA-255 populations 10 kR dose of gamma rays 0.3% EMS concentration observed highest for short fibre index (10.5 and 11.7 per cent) against control. Whereas, in PA-402 x PA-255 populations 10 kR dose of gamma rays of physical mutagen exhibited highest short fibre index (12.9 per cent) in all treatments as compared to control.Asim *et al.*(2015) reported positive increase in flowers plant^{-1}, cotton weight plant^{-1}, 2.5% span length, short fibre index and fibre elongation in cotton varieties, when irradiated at the rate of 15 Krad mutagenic treatment. Similar results were reported by Patel *et al.* (2016),who reported that application of EMS positively enhanced lint fiber yield and quality of cotton.

In the present investigation, it was observed that irradiated hybrid generated more variability than that of hybridization and irradiated genotype populations. So in view of this, it may be possible to further increase the variability by irradiation of crosses involving more than two parents. There is a need to test the performance of promising plants selected for high seed cotton yield, other quantitative characters and qualitative characters should to be further tested in advanced generations to confirm their superiority. Identified superior mutants needs to be advanced to test their superior performance in a progeny row trial.

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