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INDUCED MUTATIONS IN M_2 GENERATION IN COTTON (*Gossypium arboreum* L.)

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ABSTRACT

Present investigation was undertaken to screen the mutant populations and to identify the desirable mutants for both qualitative and quantitative traits. 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 highest frequency of chlorophyll mutation was observed in 0.4 EMS followed by 20 kR gamma rays in PA 402 cotton variety. While highest frequency of chlorophyll mutant was observed in 0.4 per cent EMS followed by 40 kR gamma rays in PA 255 cotton variety. In the cross combination maximum frequency of chlorophyll mutant was observed in40 kR gamma rays followed by 0.4 per cent EMS treatment. The highest number of chlorophyll mutants was recorded with 20kR gamma rays treatmentin PA 402. While, 30 kR gamma rays treatment recorded highest number of chlorophyll mutants in variety PA 255. Whereas, the cross combination of PA 402 X PA 255 recorded highest chlorophyll mutants in 30 kR and 40 kR treatments.

(Key words : Mutation, segregants, EMS, LD_{50}, chlorina , viridis)

INTRODUCTION

Cotton is the most widely used vegetable fibre and also the most important raw material for the textile industry, grown in tropical and subtropical regions in more than 80 countries all over the world. World consumption of cotton fibre is approximately 115 million bales $year^{-1}$. In India, long and extra long staple cotton is widely grown in South Zone states of Maharashtra, Tamil Nadu, Andhra Pradesh and Karnataka as the climatic requirement for growing this cotton are more conducive and there has been a heavy demand for this cotton in the recent past in textile industry. Besides being the backbone of the textile industry, cotton by-products are also used as livestock feed, seed-oil, fertilizers, paper and other consumer products.

Cotton has been cultivated in India for more than five thousand years. The *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. The use of chemically-induced mutants has been highly successful in most major crops grown across the world but has only occasionally been used in improving cotton. However, the relatively low level of genetic variability currently available in cotton would indicate this would be an ideal tool to increase genetic variability in this species. Mutagenesis has been shown to be an effective tool to create a wide range of phenotypic variation in both diploid and tetraploid *Gossypium* populations.Therefore, there is an urgent need to develop high yielding varieties of *desi* cotton. Mutation based improvement strategies provide an effective way of creating new allelic variations. The improved varieties of crop plants can be developed by pooling available allelic resources from different populations having well adapted, high yielding genotypes. However, mutation breeding have been found 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_2 generation to isolate useful mutants.

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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 seeds are harvested from M_1 generation of two commercial varieties of *Desi* cotton (*Gossypium arboreum* L.) *viz*., PA-402 and PA-255 along with their F_1 (PA-402 x PA-255) were selectedfor growing M_2 generation. The seeds were sown in the field of Cotton Research station, Mehboob Baugh, Parbhani on dated (7^{th} July) *kharif* 2014. One seed was dibbled hill^{-1} at a distance of 60 cm between rows and 30 cm between plants. 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 replications. Recommended package of practices was followed to raise a good crop. Observations were recorded on number of seedlings scored, number of chlorophyll mutants, frequency of chlorophyll muatations, narrow leaf mutants, plant height, mutagenic effectiveness and spectrum of chlorophyll mutations (Albino, Xantha, Chlorina, viridis and xantha viridis).

RESULTS AND DISCUSSION

Number of seedlings scored

In control plot 1489 seedlings of variety PA 402 were scored and in 0.2 per cent EMS lowest number of 984 seedlings were scored. In case of 20 Kr gamma rays treatment 1180 seedlings were scored. With regard to parent PA 255, 1523 seedlings were scored incontrol plot. In case of 40 Kr gamma rays treatment 1020 seedlings were scored. Whereas, in case of hybrid PA 402 X PA 255 sum total of 938 seedlings were scored in 0.2 per cent EMS mutagenic treatment.

Number of chlorophyll mutants

The parent PA 402 recorded 12 chlorophyll mutants in 0.3 per cent of EMS mutagenic treatment. While, 5 chlorophyll mutants were recorded in 30 Kr and 40 Kr gamma rays mutagenic treatment. The parent PA 255, recorded 4 chlorophyll mutants in 0.1 per cent EMS mutagenic treatment. Whereas, the hybrid PA 402 X PA 255 recorded 6 chlorophyll mutants in 10 Kr gamma rays mutagenic treatment. The hybrid has recorded 14 chlorophyll mutants in 30 Kr and 40 Kr gamma rays mutagenic treatments.

Frequency of chlorophyll mutations

Chlorophyll mutations depict the damage caused to the treated material. The data on frequency of chlorophyll mutations in M_2 generation of three cotton populations are presented in Table 1. Chlorophyll mutation frequency expressed on M_2 seedling basis increased linearly with treatment doses of both the mutagens in case of PA-255 and PA-402 x PA-255 populations. However, the trend was not similar with PA-402.Gustafsson (1969) has reviewed the various aspects of directed mutagenesis with particular reference to mutagenic specificity and treatment conditions. The number of seedlings scored in between 938 to 1523 in the experiment. The twelve chlorophyll mutants were recorded by PA 402 in 0.3% EMS mutagenic treatments. Whereas, the highest number of chlorophyll mutants i.e. 15 were recorded by PA 255 in 0.4 per cent EMS mutagenic treatments. Hybrid PA 402 x PA255 exhibited highest number of chlorophyll mutants i.e. 14 in 30 kr and 40 kr gamma rays mutagenic treatment. Deotale *et al*. (2019) tested twenty M_4 Indian mustard mutants for total chlorophyll content. Among these twenty mutants, the highest chlorophyll at 25, 45, and 65 DAS was obtained from ACM18,ACM12, ACM6, ACM8 and ACM4. In case of proximate analysis, the highest chlorophyll was recorded from ACM 18.

A maximum mutation frequency (1.22%) was recorded in PA-402 x PA-255 populations with 40 kR gamma irradiation. While, in case of PA-402 populations highest frequency (1.15%) was observed with 0.3% but in case of PA-255, higher frequency (1.20%) marked with 0.4% EMS concentration of chemical mutagen. In general, the chlorophyll mutation frequency was more in PA-402 x PA-255 populations for both the mutagen followed by PA-255 and PA-402.Kamdi *et al*. (2019) idified entvisible macro-mutants like albino were identified in M_2 generation

Narrow leaf mutants

The parent PA 402 displayed nine narrow leaf mutants in 40 Kr gamma rays mutagenic treatment. While, one narrow leaf mutant was recorded in 0.1 per cent and 0.2 per cent of EMS mutagenic treatments. In case of PA 255 ten narrow leaf mutants were noted in 0.2 per cent of EMS mutagenic treatment. Whereas, 10 Kr gamma rays mutagenic treatment exhibited one narrow leaf mutant.The hybrid PA 255 X PA 402 recorded twelve narrow leaf mutants in 40 Kr gamma rays mutagenic treatment. The hybrid exhibited one narrow leaf mutant in 0.3 per cent of EMS mutagenic treatment.

Plant height

In case of parent PA 402 the plant height ranged between 20 cm to 181cm. The tallest plant was noted in 0.1 per cent of EMS mutagenic treatment. The plant height of 17cm to 167cm was exhibited by PA 255 parent. The maximum plant height of 167 cm was recorded in PA 255 in 0.1 per cent of EMS mutagenic treatment. The range of plant height in hybrid PA 402 X PA 255 was 20 cm to 190 cm. The hybrid recorded maximum plant height of 190 cm in 0.2 per cent of EMS mutagenic treatment. Chavan *et al*. (2019) reported increase in plant height in 150 Gy and 250 Gy. While, decrease was noticed 200 Gy, 300 Gy and 350 Gy over control

Mutagenic effectiveness

It is observed from the table that the mutagenic effectiveness in general was reduced as dosage increased except with 0.3% EMS in PA-402 and 0.4% EMS in PA-255 genotypes.In PA-402, effectiveness was maximum with 10 kR dose of gamma rays followed by 20 kR dose of physical mutagen, while, 0.1% EMS concentration observed highest effectiveness followed by 0.3% EMS concentration of chemical mutagen. Concerning PA-255 populations 20 kR dose of gamma rays and 0.2 per cent EMS of chemical mutagen showed maximum effectiveness. 10 kR and 0.1per cent EMS observed for maximum effectiveness among all treatments in PA-402 x PA-255 populations.Afsari *et al*. (2000) studied effects of gamma radiation on two varieties of cotton. The dosages used were 0, 25, 30 and 35kR. Gamma irradiation decreased plant height, while days to flowering and maturity increased significantly. They observed significant differences mutagenic effectiveness for chlorophyll mutants, narrow leaf mutants and plant height.Ali and Fadlalla (2011) noticed two local cotton varieties, Barac (67) B and Shambat B, belonging to *Gossypium hirsutum*, were treated with gamma irradiation using 0, 100, 150, 200, 250 and 300 GY from CO^{60} source. Most of the plant traits studied decreased with the increasing the dose of gamma irradiation, especially the germination percentage, survival rate. From the results of this study, the optimal dose range of gamma irradiation necessary for mutation induction was in the range of 100 - 250 and 150 - 250 GY, for Barac (67) B and Shambat B, respectively.Asim *et al.* (2015) treated with cobalt source at the rate of 0, 10, 15, 20 and 25 Krades. Three potential cotton varieties i.e Gomal-93, Bt-131 and Bt-CIM-602 previously irradiated @ 15, 20 and 25 KR respectively were selected from among the six varieties. Bt-131 recorded maximum lint perentage (37.7%), lengthy staple (30.9) and highest cotton yield (340.4 kg ha^{-1}) as compared to other varieties. Chavan *et al*.(2019) had conducted an experiment on induction of mutation in lathyrus by gamma rays by using treated seeds of lathyrus cv. NLK-73 with 150, 200, 250, 300 and 350 Gy doses of gamma rays in non-replicated trial along with control. Germinationand mortality percentage were recorded in M_1 and M_2 generation and observed reduction in germination percentage and increase in mortality as compared to control.Khursheed *et al*. (2018) studied effect of gamma radiation (100,200,300 and 400 Gy) and EMS (0.01, 0.03 0.04 %) on faba bean. Both mutagenic effectiveness and efficiency were found to be higher at lower doses of both the mutagens. EMS is more effective than gamma rays.

Spectrum of chlorophyll mutation

Different types of chlorophyll mutants were observed in different mutagenic treatments *viz.,* gamma rays and ethylmethane sulphonate.The spectrum of chlorophyll mutations are given in Table 2. The spectrum of chlorophyll mutants observed were albino, xantha, chlorina,viridis and xanthaviridis. The maximum number of albino chlorophyll mutants were recorded in 20kR gamma rays treatment. While, maximum number of xantha mutants were recorded in 30 kR and 40kR gamma rays treatment. The highest number of viridis chlorophyll mutants was recorded in 0.4% EMS mutagenic treatment. Waghmare *et al.* (2000) conducted a study on *G. arboreum* var. Y1 irradiated using gamma rays (15, 20, 25 and 30 kR) and EMS (0.1, 0.2 and 0.4%). They reported occurrence of 0.15 per cent chlorophyll and 0.17 per cent morphological mutants. In chlorophyll mutations chlorina types were higher in proportion (45.7%) followed by xantha (34.3%). Badigannavar *et al*. (2000), Khatod *et al*. (2002) and Ali *et al*. (2011) observed mutant for high fibre strength of 18.86 g tex^{-1} was isolated from 5kR+0.1% EMS treatment as against 15.72 g tex^{-1} in control. Treatment like 5kR, 0.4% EMS and 5kR + 0.1% EMS induced very desirable mutants for most of qualitative characters.

The albino seedling lacked chlorophyll completely and were almost white, relatively smaller in size. These types survived about 7-8 days and were found lethal.The seedling leaves were distinctly yellowish to white in colour. In these mutants carotenoides were present but chlorophyll was absent. They survived for about 10-12 days and were lethal in nature.The leaves of these mutants were yellow green to pale green in colour and gradually changed to normal green. These mutants were viable and grew as normal plants.The leaves were green with yellow apex. These mutants were viable.The leaves of these types of mutants were golden yellowish to green colour and were found lethal after 15-20 days. The above described mutants *viz*.,albino, xantha, viridis, xanthaviridis and chlorina were found to be prominent in all the populations. On the basis of pooled frequencies of doses over populations, treatments 20 kR gamma rays in PA-402, 20, 30 and 40 kR gamma rays in PA-255 and 30 and 40 kR gamma rays in PA-402 x PA-255 populations while, in chemical mutagenic treatments 0.3% EMS in PA-402, 0.4% in PA-255 and PA-402 x PA-255 showed wider spectrum (all four types) of chlorophyll mutations. Albino, viridis and xanthaviridis types were not noticed in more number except few treatments.Xantha and chlorina appeared in all the treatments, viridis also appeared in all treatment except 40 kR dose of gamma rays in PA-402 and 0.2% EMS in PA-402 x PA-255. Chlorina and viridis appeared in all the treatments of both the mutagen as well as in all the three populations. Chlorina types were expressed more than other types. In all three populations PA-402 x PA-255 showed maximum frequency of chlorina.Kathod *et al*. (2002) noticed seeds of naturally coloured cotton (*G. hirsutum*) were exposed to 15 and 20 kR of gamma radiation and 0.5% ethyl methanesulfonate (EMS). Selection pressure was applied in M_2 generation and selected M_2 mutants were studied for their fibre quality. Mutants with improved fibre quality were obtained with irradiation of cotton seeds with 15 and 20 kR gamma radiation. EMS was effective in improving the maturity coefficient in the crop. Significant differences were noted in the treatment means for uniformity ratio, maturity coefficient and ginning outturn.Remya (2011) reported vast amount of variability was generated for 12 quantitative as well as qualitative characters under study. Mutagenic effectiveness of various doses of mutagens could be revealed by the expression of morphological mutants in M_1 and M_2 generation.Waghmare *et al.* (2000) conducted a study on *G. arboreum* var. Y1 irradiated using gamma rays (15, 20, 25 and 30 kR) and EMS (0.1, 0.2 and 0.4%). They reported occurrence of 0.15 per cent chlorophyll and 0.17 per cent morphological mutants. In chlorophyll mutations chlorina types were higher in proportion (45.7%) followed by xantha (34.3%), virescent (17.1%) and maculate (2.86%).

Table 1.Effect of mutagens on morphological and biochemical parameters in M_2 generation of cotton genotypes

Sr. No.	Treatments	No. of seedlings scored	No. of chlorophyll mutants	Frequency of chlorophyll mutations (%)	Narrow leaf mutants	Plant height (cm)	Mutagenic effectiveness (%)
PA-402							
1	Control	1489	-----	—	0	29-161	—
2	10 kR Gamma rays	1103	7	0.63	-	24-164	6.30
3	20 kR Gamma rays	1180	10	0.85	3	21-165	4.25
4	30 kR Gamma rays	1073	5	0.46	5	34-160	1.53
5	40 kR Gamma rays	1096	5	0.45	9	23-172	1.12
6	0.1 % EMS	1145	6	0.52	1	24-181	5.20
7	0.2 % EMS	984	6	0.61	1	20-167	3.05
8	0.3 % EMS	1042	12	1.15	8	20-162	3.83
9	0.4 % EMS	1027	9	0.88	3	120-160	2.02
PA-255							
10	Control	1523	—	—	0	30-162	—
11	10 kR Gamma rays	1166	5	0.43	1	17-142	4.30
12	20 kR Gamma rays	1109	10	0.90	5	20-158	4.50
13	30 kR Gamma rays	1231	12	0.97	2	19-160	3.20
14	40 kR Gamma rays	1020	11	1.08	3	27-155	2.70
15	0.1 % EMS	1117	4	0.36	-	31-161	3.60
16	0.2 % EMS	1264	11	0.87	2	20-167	4.35
17	0.3 % EMS	1229	11	0.89	10	20-141	2.97
18	0.4 % EMS	1248	15	1.20	-	125-150	3.00
PA-402 x PA-255							
19	Control	1369	—	—	0	110-162	—
20	10 kR Gamma rays	1035	6	0.58	2	31-178	5.80
21	20 kR Gamma rays	1184	13	1.10	-	37-164	5.50
22	30 kR Gamma rays	1234	14	1.13	5	19-158	3.77
23	40 kR Gamma rays	1148	14	1.22	12	37-174	3.05
24	0.1 % EMS	1097	8	0.73	4	28-177	7.30
25	0.2 % EMS	938	8	0.85	2	25-190	4.25
26	0.3 % EMS	1005	11	1.09	1	20-152	3.63
27	0.4 % EMS	1114	13	1.17	3	120-162	2.92

Table 2. Spectrum of chlorophyll mutations in M_2generation of cotton genotypes

Sr. No.	Treatments	Chlorophyll mutants					
		Albino	Xantha	Chlorina	Viridis	Xanthaviridis	Total
PA-402							
1	Control	—	—	—	—	—	—
2	10 kR Gamma rays	—	2	3	1	1	7
3	20 kR Gamma rays	4	1	2	3	—	10
4	30 kR Gamma rays	2	1	1	1	—	5
5	40 kR Gamma rays	3	1	1	—	—	5
6	0.1 % EMS	—	1	2	3	—	6
7	0.2 % EMS	1	2	1	1	1	6
8	0.3 % EMS	—	3	2	4	3	12
9	0.4 % EMS	1	1	3	4	—	9
PA-255							
10	Control	—	—	—	—	—	—
11	10 kR Gamma rays	—	1	1	1	2	5
12	20 kR Gamma rays	—	4	4	2	—	10
13	30 kR Gamma rays	1	5	2	3	1	12
14	40 kR Gamma rays	—	1	7	3	—	11
15	0.1 % EMS	—	1	2	1	—	4
16	0.2 % EMS	3	2	4	2	—	11
17	0.3 % EMS	1	3	3	1	3	11
18	0.4 % EMS	2	2	2	8	1	15
PA-402 x PA-255							
19	Control	—	—	—	—	—	—
20	10 kR Gamma rays	1	2	1	2	—	6
21	20 kR Gamma rays	1	3	6	2	1	13
22	30 kR Gamma rays	2	3	5	4	—	14
23	40 kR Gamma rays	—	5	7	1	1	14
24	0.1 % EMS	—	1	3	2	2	8
25	0.2 % EMS	2	2	4	—	—	8
26	0.3 % EMS	1	3	2	4	1	11
27	0.4 % EMS	—	5	3	5	—	13

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