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Mutagenic Effectiveness and Efficiency of Gamma Rays in Sesame (*Sesamum indicum* L.)

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Abstract: Mutagenic effectiveness and efficiency of gamma rays were studied in the genotypes of sesame TTVS 51 and TTVS 19. The dosage of gamma rays for mutagenic treatments was fixed based on the LD_{50} values available in the literature in relation to sesame and allied oilseed crops. The mutagenic effectiveness and efficiency is high at 250Gy in both genotypes. The frequency of viable mutants was high in 450Gy on M_2 seedling basis. Mutants with wider spectrum of variation were found at 250Gy and 350Gy of gamma rays. Mutagenic effectiveness and efficiency increased with the decreased in dose or concentration in sesame. Compared to TTVS 19, TTVS 51 produced more economically viable mutations.

Key words: Gamma Rays · Mutagenic Effectiveness · Mutagenic Efficiency · Mutagens

INTRODUCTION

Sesame (*Sesamum indicum* L. syn. *S. orientale* Linn.) (2n = 26), is one of the renowned oil seed crop that belongs Pedaliaceae family. This genus contains over thirty species of that S. indicum is that the most ordinarily cultivated [1]. It was cultivated and domesticated within the Indian landmass throughout Harappan and Anatolian eras [2].

The seed yields of sesame are very low. This is due to lack of improved cultivars, susceptibility to diseases, seed shattering, asynchronous capsule ripening, abiotic stresses and low harvest index. Enhancement in sesame has been low due to limited exchange of germplasm materials.

It is a minor crop with slight or no attention. Research in this crop is limited and in developed countries. Furthermore, it's not a mandate crop by any of the international agricultural research centres. Consequently, it is essential to develop novel approaches in crop improvement programme to improve the yield predominantly for poor/marginal soil under Indian conditions.

Mutation is a sudden heritable change in organism generally the structural change in gene. It's produced by change in the base sequence of genes and it can be induced either spontaneously or artificially both in seed and vegetative propagated crops.

Induced mutations have recently become the subject of biotechnology and molecular investigation leading to description of the structure and function of related genes. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops. These mutations provide beneficial variation for practical plant breeding purpose. During the fast seven decades, more than 2252 mutant varieties have been officially released in world a great majority of mutant varieties (64%) were developed by the use of gamma rays. Hence, mutation-breeding programme has proved to be a successful tool in bringing amelioration in self-pollinated crops.

Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissue. The usefulness of a mutagens in

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mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagens), but also on its mutagenic efficiency (mutation in relation to undesirable changes like sterility, lethality, injury etc.). The selection of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations. In this context, the present investigation was undertaken to study the frequency and spectrum of macro mutations along with the mutagenic effectiveness and efficiency of different doses of gamma rays in sesame.

MATERIALS AND METHODS

Well filled seeds of the genotype TTVS 51 and TTVS 19 having a moisture content of 9 per cent were used for irradiation. One gram of seeds per treatment was packed in butter bags and placed in a Co^{60} gamma cell. The seeds were subjected to irradiation from the cobalt ^{60} gamma source for appropriate time for each dose based on the half-life of the source. Gamma irradiation at 250, 359, 450, 550 and 650 Gy were done through the gamma chamber (GC 1200) installed by the Board of Radiation and Isotope Technology (BRIT), Govt. of India, Mumbai at the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore where cobalt-60 was loaded as the source of gamma irradiator.

After the completion of the treatment, treated seeds were sown immediately in the field along with their respective controls to rise the M_1 generation in a randomized block design with three replications. All the treatments including the controls were raised adopting a spacing of 30 cm in between rows and 30cm in between plants. All the recommended cultural measures namely, irrigation, weeding and plant production methods were carried out during the growth period of the crop.

The seedling height reduction (I) in different M_1 generation was studied. The plant survival (L) was computed as the percentage of plants surviving till maturity. The biological damage (lethality/ injury) was computed as the reduction in plant survival and plant height. At maturity all the surviving M_1 fertile plants were harvested separately and seeds were sown in the next season in plant progeny rows to rise M_2 generation. The respective control and treatment progenies were screened several times for morphological mutations throughout the crop duration. Different kinds of chlorophyll mutants (Xantha, viridis, chlorina and albina) were scored from emergence till the age of four week in M_2 generation by using modified classification of Lampretcht and Kharkwal. Mutation frequency was calculated as percentage of mutated M_2 progenies for both chlorophyll and morphological mutations in each treatment. The mutagenic effectiveness and efficiency were calculated based on the formula given below [3].

Mutagenic effectiveness (%):

$$\text{Mutagenic effectiveness} = \frac{M}{Gy} \times 100$$

Where,

M = Mutation frequency for 100 M_2 plants
Gy = Dose of mutagenic radiation in gray for physical mutagen

Mutagenic Efficiency:

Mutagenic efficiency = M x 100 / L
M x 100 / I
M x 100 / S

Where,

M = Mutation frequency for 100 M_2 plants
L = Percentage of lethality or survival reduction
I = Percentage of injury or reduction in seedling size
S = Percentage of sterility i.e. reduction in seed fertility

RESULTS AND DISCUSSION

The dosage of gamma rays for mutagenic treatments was fixed based on the LD_{50} values available in the literature in relation to sesame and allied oilseed crops. In most of the studies, the LD_{50} valueswere around 450Gy for sesame [4-7]. Therefore, five doses were adopted for the field study keeping two doses above and doses below the LD_{50} with an interval of 100Gy between treatments.

The effectiveness of the mutagens is better judged by studying the progenies in M_2 generation. The frequency of chlorophyll mutations in M_2 has been suggested to provide the most reliable index of mutation rate because of greater accuracy on scoring [8, 9]. The complex interactions between the nature of the biological material and mode of the mutagen acting on it are quantitatively expressed by mutation rate and qualitatively expressed by the spectrum of mutation.

The relative mutation frequencies were scored based on two criteria namely

(I) M_1 plant basis and (ii) M_2 seedling basis. Among the two, the second method proposed by Gaul [9] is preferred since it is proportional to the initial mutation rate

Table 1: Chlorophyll mutation frequencies in M_2 generation

	No. of M_1 plant		No. of M_2 seedlings		Mutation frequency	
Mutagen dose	Studied	Segregating	Studied	Showing Chlorophyll mutants	Per 100 M_1 Plants	Per 100 M_2 seedlings
TTVS 51						
0 Gy	05	--	1311	--	--	--
250 Gy	05	02	1301	14	40.00	1.076
350 Gy	05	03	1272	06	60.00	0.471
450 Gy	05	02	1244	09	40.00	0.723
550 Gy	05	01	1174	08	20.00	0.681
650 Gy	05	02	994	12	40.00	1.200
TTVS 19						
0 Gy	05	--	1330	--	--	--
250 Gy	05	01	1310	17	20.00	1.297
350 Gy	05	02	1293	08	40.00	0.618
450 Gy	05	03	1250	12	60.00	0.960
550 Gy	05	01	1160	08	20.00	0.690
650 Gy	05	02	980	05	40.00	0.510

and rather independent of variations in the progeny size of mutated sector. The estimation of mutation frequency on M_2 seedling basis was found to be the best index as it is rather independent of the variations in progeny size and is also proportional to the size of the mutated sector [9]. In pisum, Blixt [10] has confirmed this phenomenon. Further according to D' Amato [11] the expression of mutation frequency on M_1 plant basis, leads to an over estimation of the mutation rate and it suffers very much from diplontic selection as shown by a drop in mutation frequencies at higher doses.

Chlorophyll Mutation: Chlorophyll mutation frequency was the highest in M_1 plant basis and the lowest on M_2 seedling basis. The frequency of chlorophyll mutations was illustrated in Table 1. The frequency based on M_1 plant basis showed a proportionate increase and reached a maximum almost at middle dose of treatment and thereafter it declined. When comparing the two genotypes, TTVS 19 registered more frequency rate than TTVS 51. The frequency of chlorophyll mutants in general were low in this crop and it may be due to the fact that oil seed crops are resistant to induced chlorophyll mutations as reported by Rajan [12] and Rangaswamy [13]. It may be further attributed to the probable reasons of the elimination of gametes carrying mutations or zygote inviability. The mutagenic spectrum comprised chlorina, xantha, striata and xanthaviridis types. In treatments chlorina occurred more frequently in TTVS 51, xanthaviridis occurred most frequently in TTVS 19. The reason for the appearance of greater number of *Xanthaviridis* type may be attributed to involvement of polygenes in chlorophyll formation [9].

Viable Mutants: Viable mutations were classified as macro and micro mutations, while Swaminathan [14] grouped them as macro mutations and systematic mutations. Viable macro mutations, though induced quantitatively very few, would be more valuable in genetic studies since, plants with altered characteristics or phenotype can serve as markers of the mutability of a variety or species. Frequency of such mutations also serves as an index of mutagenic sensitivity of various mutagenic agents and their dose effects [15]. A number of new commercial varieties have originated from induced macro mutants and they have proved their usefulness in attaining distinct breeding objectives.

In the present investigation, a large number of viable mutants were isolated from all the mutagenic treatments. This includes mutants with alteration in branching habit, plant height, phyllotaxy, nodal distance of the first capsule, flower, nodal length and seed characters *etc.* The frequency of viable mutations was depicted in Table 2.

Among the two genotypes TTVS 51 (99 mutants) produced more number of viable mutants compare to TTVS 19 (78 mutants). The frequency of viable mutants was high in 450Gy on and M_2 seedling basis. Mutants with wider spectrum of variation were found at 250Gy and 350Gy of gamma rays. Among the treatments 450Gy of gamma rays in TTVS 51 and TTVS 19 registered maximum number of viable mutants.

Mutants with multiocules, more number of capsules per plant, increase in capsule length and change in seed colour and altered phyllotaxy occurred which would be useful from the breeding point of view. The occurrence of viable mutants in small proportion in M_2 generation suggested that each of them might represent changes in a single recessive gene from their parents [9].

Table 2: Viable mutation frequencies in M_2 generation

	No. of M_1 plant		No. of M_2 seedlings		Mutation frequency	
Mutagen dose	Studied	Segregating	Studied	Segregating	Per 100 M_1 Plant	Per 100 M_2 seedling
TTVS 51						
0 Gy	5	--	1300	--	--	--
250 Gy	5	3	1282	22	60	1.71
350 Gy	5	2	1258	17	40	1.35
450 Gy	5	3	1193	33	60	2.76
550 Gy	5	2	1102	13	40	1.18
650 Gy	5	3	962	14	60	1.45
TTVS 19						
0 Gy	5	--	1315	--	--	--
250 Gy	5	3	1290	12	60	0.93
350 Gy	5	2	1245	11	40	0.88
450 Gy	5	3	1226	27	60	2.20
550 Gy	5	3	1098	18	60	1.64
650 Gy	5	4	958	10	80	1.04

Mutants for plant height, such as tall and dwarf mutants occurred in the mutagenic treatments in both the varieties. Similar reports of plant height mutants in sesame were observed by many workers [16-21].

In TTVS 51and TTVS 19, mutants for branched plant type with altered phyllotaxy and branched determinate type with normal phyllotaxy and mutants for curved branch were observed. Sasikala and Kamala [22] and Carirgan [4] recorded monostem mutants.

Mutants with bigger leaves and flowers were noted in 350, 450 and 550Gy in both genotypes.

Larger nodes with alternate capsule in 250Gy and larger nodes with shorter capsules 350Gy were observed in TTVS 51.

Mutants exhibiting varied colour*viz.* black, dark brown, light and tan from dull white seeded TTVS 19 and pure white, light black and brown from black seeded TTVS 19 were noticed. The maximum seed colour mutants were observed in the genotypes TTVS 51. Mutants for seed colour were also observed by Prabhakar [17], Pugalendi [18] and Ganesan [19] in sesame. The important economic mutant (pure white seeded type) obtained from TTVS 51 at 350Gy.

Moh [23] working on beans had shown that mutations can occur in any one of the loci governing the seed coat colour*ie*. basic gene, the complementary or the supplementary or the modifiers resulting in array of colour combinations. Moh [24] stated that induced seed coat colour mutations other than the white are probably due to the change in complementary colour factor or modifying factors.

A high yielding mutant with seed yield of more than 50 gram per plant was identified from 550Gy gamma irradiated population in TTVS 51 and from 650Gy in TTVS 19. These mutants have more number of branches (five primary and four secondary branches) and more number of capsules which in turn increase the yield.

Mutagenic Effectiveness and Efficiency: The mutagenic effectiveness and efficiency of chlorophyll and viable mutations were presented in Table 3 and 4 respectively. Konzak*et al.* [3] have presented a detailed account of the mutagenic effectiveness and efficiency. They proposed the term "effectiveness" as a measure of gene mutations in relation to dose and "efficiency" as an estimate of biological effects induced, such as lethality, injury and sterility. To obtain high efficiency, the mutagenic effect must greatly surpass the damage in the cells; such chromosomal aberrations and toxic effects were proposed by Gaul *et al.* [25]. According to them the effectiveness of a mutagen was theoretical importance, but did not have any immediate practical implication. Further, they reported that low biological damage of a mutagen permitted the use of high dose of the mutagen but for practical purpose the aim was to obtain high efficiency.

In TTVS 51, the effectiveness for chlorophyll mutations ranged from 1.24 to 4.30 and viable mutations ranged from 2.14 to 6.84. In TTVS 19 the effectiveness for chlorophyll mutations ranged from 1.25 to 5.20 and viable mutations ranged from1.60 to 4.88.

The efficiency in terms of chlorophyll mutations was in the highest dose at 250Gy on the lethality, injury and sterility basis on both the genotypes. In terms of viable mutations, the efficiency was the highest at 250Gy on TTVS 51. In case of TTVS 19 efficiency in terms of chlorophyll mutations was in the highest dose 250Gy on the lethality, injury and sterility basis. But efficiency in terms of viable mutations was in the highest dose 250Gy on the injury and sterility basis, while the most efficient dose based on lethality was 450Gy.

In conclusion, TTVS 51 responded well for gamma rays to produce more and more viable mutation compare to TTVS 19. Gamma rays proved to be more effective to induce the economically important mutation in TTVS 51 for higher productivity and seed coat color.

Table 3: Mutagenic effectiveness and efficiency for chlorophyll mutants in M_2 generation

Mutagen dose	Percentage survival reduction at 30 days (lethality) (L)	Percentage height reduction at 30 days (injury) (I)	Percentage fertility reduction (sterility) (S)	Mutation per 100 M_2 plants (M)	Effectiveness (M x 100 / krad or C) x 100	Efficiency		
						(M x 100) /L	(M x 100) / I	(m x 100) / S
TTVS 51								
250 Gy	17.91	36.94	2.98	1.076	4.30	6.00	2.91	36.10
350 Gy	31.21	37.40	3.92	0.471	1.35	1.50	1.26	12.01
450 Gy	37.94	44.46	5.64	0.723	1.60	1.90	1.63	12.82
550 Gy	45.39	49.83	15.56	0.681	1.24	1.50	1.36	4.37
650 Gy	49.50	53.00	27.57	1.200	1.85	2.42	2.26	4.35
TTVS 19								
250 Gy	24.23	4.95	6.36	1.300	5.20	5.36	26.26	20.44
350 Gy	33.16	12.82	7.39	0.618	1.76	1.86	4.82	8.36
450 Gy	43.36	12.05	14.99	0.960	2.13	2.21	7.96	6.40
550 Gy	48.76	14.68	18.76	0.690	1.25	1.41	4.70	3.68
650 Gy	56.92	36.92	22.66	0.510	0.78	0.90	1.38	2.25

Table 4: Mutagenic effectiveness and efficiency for viable mutants in M_2 generation

Mutagen dose	Percentage survival reduction at 30 days (lethality) (L)	Percentage height reduction at 30 days (injury) (I)	Percentage fertility reduction (sterility) (S)	Mutation per 100 M_2 plants (M)	Effectiveness (M x 100 / krad or C) x 100	Efficiency		
						(M x 100) /L	(M x 100) / I	(m x 100) / S
TTVS 51								
250 Gy	17.91	36.94	2.98	1.71	6.84	9.54	4.62	57.38
350 Gy	31.21	37.40	3.92	1.35	3.85	4.32	3.60	34.44
450 Gy	37.94	44.46	5.64	2.76	6.13	7.27	6.20	48.93
550 Gy	45.39	49.83	15.56	1.18	2.14	2.60	2.36	7.58
650 Gy	49.50	53.00	27.57	1.45	2.23	2.93	2.73	5.26
TTVS 19								
250 Gy	24.23	4.95	6.36	0.93	3.72	3.83	18.78	14.62
350 Gy	33.16	12.82	7.39	0.88	2.51	2.65	6.86	11.90
450 Gy	43.36	12.05	14.99	2.20	4.88	5.07	18.25	14.67
550 Gy	48.76	14.68	18.76	1.64	2.98	3.36	11.17	8.74
650 Gy	56.92	36.92	22.66	1.04	1.60	1.83	2.81	4.59

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