

Original Research Article

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Gamma Ray Induced Macro Mutants in Sesame (*Sesamum indicum* L.)

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ABSTRACT

An experiment was made to determine the spectrum and frequency of different macro mutants induced by gamma rays on sesame with two genetic backgrounds. Treatment concerned seeds of the two cultivars Rama and Tillotoma, predominantly grown in West Bengal were irradiated by five doses (250, 300, 350, 400 and 450 Gy) of gamma rays at the Bhaba Atomic Research Centre (BARC), Trombay. Irradiated seeds along with control (unirradiated) were sown in University experimental farm, Visva-Bharati during pre-kharif in 2015, 2016 and 2017 to grow M₁, M₂ and M₃ generation, respectively. All putative mutants were selected from M₂ population were grown in the M₃ generation to confirm their breeding behavior. A wide spectrum of the mutants under studies *i.e.* shattering resistance, determinate growth habit, early matured types etc. were observed. Among the all traits, shattering resistant and early matured type mutants were induced from both cultivars at each applied doses. Depends upon all traits under studies, Rama had the highest mutation frequency (8.64×10^{-3}) followed by Tillotoma (6.28×10^{-3}). These results indicated that selection of unique mutants is not a matter of chance; rather it depends upon population size, the response of the cultivars to effective mutagenesis and careful screening.

Keywords

Sesame, Gamma rays, Selection, M₂ generation, Macro-mutation, M₃ generation.

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Introduction

Sesame (*Sesamum indicum* L.; $2n = 26$) is rich in limiting amino acid methionin, and provides a valuable source of nutritious protein as well as high quality oil that resists oxidative rancidity due to the presence of the phenylpropanoid, lignins, sesamin and sesamol. In India, it is the fifth important edible oil seed crop after groundnut, rapeseed-mustard, sunflower and soybean with the annual average production around 6,80,000 metric tons, though, the productivity of sesame stands alarmingly poor comparing to other oil yielding crops. As a quality oil seed crop, it can reduce the shortage of other edible

oil production not only in India but also in major sesame growing countries in the world, if the production and productivity are enhanced using modern breeding techniques like mutation breeding with irradiation.

Yet, sesame is unimproved in India and many collections have been made from local land races, but little genetic information is available which can lead to its utilization in breeding programmes. A number of factors affecting sesame improvement programmes have been identified. Firstly, the germplasm of sesame is not as large as in other crops

(Ashri, 1981). Secondly, the architecture of sesame is poorly adapted to modern farming system because of its indeterminate growth habit, sensitivity to wilting under intensive management and seed shattering at maturity (Cagirgan, 2001; Uzun and Cagirgan, 2006). Although many research has been made on through irradiation and chemical mutagen to improve: yield (Wongyai *et al.*, 2001), capsules number, leaves maturation, male sterility, plant architecture (Cagirgan, 2006), seed size and seed color (Hoballah, 2001), narrow leaf (Sengupta and Dutta, 2005), resistant to fusarium wilt (Silme, 2010) and early matured plant (Mensah *et al.*, 2007), however, studies on growth habit, maturity, shattering habit and plant architecture are still scanty.

Indeterminate growth habit is a challenge for sesame breeders. Due to continual flowering and non-uniform ripening of the capsules, it is difficult to decide when to harvest the seeds. Flowering can continue over a period of 2 months if the environmental conditions are favorable for sesame growth (Cagirgan, 2006). It has become an active area of research after the discovery of “dt-45” by Ashri (1981), which was the first determinate mutant type, selected from M₂ population of the Israeli sesame cultivar “No-45”, irradiated with 500 Gy Gamma rays. It is encouraging that Cagirgan induced six such mutants whose breeding value and affinity to the ‘id’ allele are now under study in Turkey. Seed shattering is also a major issue to lose the production and productivity, early harvesting causes low seed yield, and poor quality due to the consist of immature seeds on the top of the plant. The late harvesting too reduces seed yield from the lower matured capsule because of seed shattering. In addition, this crop is predominantly cultivated in state like West Bengal, India during pre-*kharif* season after harvest of paddy, rapeseed-mustard and potato. For more acceptances among farmers to grow sesame as catch crop in rice based

cropping system, there should be easy availability of short duration early maturing (60-65 days) varieties with high yield potential which are currently lacking in this crop. To address this challenges and finding a solution, the goal of the present study has been undertaken to select determinate mutant, shatter resistant, early maturing mutant types and some desirable traits to make the crop suitable for multiple cropping system, to ensure higher yield and possibly create to choose new ideotypes in further generation.

Materials and methods

The study was carried out at the University Agriculture Farm of Visva-Bharati, Sriniketan (23⁰29' N latitude and 87⁰42' E longitudes) in pre-*kharif* season of 2015, 2016 and 2017. The elevation of the site is 58.9 m above the mean sea level with sub-humid, sub-tropical monsoon climate and lateritic soil.

Plant materials

Two popular sesame cultivars, from West Bengal, Rama and Tillotoma were taken for the present study (Table 1).

Gamma irradiation

10,000 dry, uniform and healthy seeds of these two genotypes of sesame were irradiated using ⁶⁰Co (Cobalt 60) gamma source with different doses (250, 300, 350, 400, 450 Gy) of gamma rays at the Bhabha Atomic Research Centre (BARC), Trombay, India.

Field Experiment

Irradiated seeds (M₀) along with the controls (un-irradiated) were sown in the field in a split plot design with three replications keeping plant to plant and row to row distance of 10 and 30 cm, respectively during pre-*kharif* season of 2015. Four to five capsules of

each M₁ plants against all the treatments were collected separately to rise the M₂ generation. Individual plant to progeny rows were grown in M₂ generation (pre-*kharif* 2016) keeping row to row and plant to plant distance at 30 cm and 10 cm, respectively. Immediately after germination, various types of chlorophyll mutation (albina, chlorina, xantha) were recorded to study the mutagenic effect of different doses. Throughout the experiment, all putative mutants were tagged and selected. The characteristics of the desirable mutants were: early matured, shatter resistant, determinate growth habit, more number of capsules/plant, multi locules capsule, capsule length, multi capsules/leaf axil or cluster type, more seed weight, dwarf and monostem types. Potential mutants selected from two cultivars in 2016 seasons were grown in M₃ generation to confirm breeding behavior during pre- *kharif* 2017 in progeny rows keeping row to 30 cm and plant to plant 10 cm. In every generation, other agronomic package, practices and plant protection measures were taken throughout the experiment.

The mutation frequencies for progeny were estimated by dividing the total number of mutants confirmed by the total number of M₂ plants (Gaul, 1964).

Results and Discussion

Mutation spectrum and frequency over cultivars and doses

In total 103134 M₂ plants were grown under experiment. M₂ population sizes for each cultivar are given in Table 2. Differences were observed between cultivars for mutation frequency in each dose. The viable mutation frequency indicated a maximum rate for Rama at 300 Gy (8.90×10^{-3}) followed by Tillotoma at 350Gy (1.28×10^{-2}). Between two cultivars, maximum desirable mutants were selected from Rama (455) with highest

frequency (8.64×10^{-3}). In M₁ generation, radio sensitivity test confirmed that Tillotoma was more sensitive than Rama. The reduction of plant population in M₂ generation with increasing of doses and it differed between two cultivars also confirmed this finding. Though, high mutation frequency was observed in both cultivars, Rama showed greater frequency in respect to all characters than Tillotoma. In this experiment maximum viable mutant types were lies in moderate dose (250 to 350 Gy) which indicate the selection efficiency of these doses. Several researchers (Cagirgan, 2007; Van-Zanten, 2001; Diouf, 2010) also stated that 300 to 400 Gy of gamma rays is effective enough to induced mutation from any sesame cultivar. The present findings are closely agreed to the previous research work in sesame.

Selection of individual macro mutants

Shattering resistant types

52 mutants were found with shatter resistance for both cultivars. The highest mutation frequency for this category was observed in Rama (5.13×10^{-4}) with 27 mutants followed by in Tillotoma (4.95×10^{-4}) with 25 mutants (Table 3) (Fig. 2b). Reduction of seed loss at maturity and harvest by developing cultivars with shattering resistant capsules is the key to successful cultivation of sesame. A spontaneous indehiscent “id” allele was discovered by Langham (1946) in Venezuela. Later on, a peer of scientists (Wongyai *et al.*, 1997; Diouf, 2010), found delayed, closed capsule and semi shattering mutants similar to our cases.

Early maturing types

Earliness has been an important objective of breeding in several crops under different ecological conditions. The early types have the possibilities to permit multiple cropping systems, escape from late season pests. The

leaf shapes of early matured plant were narrow (Fig. 2a) though the un-irradiated plant had broad leaves (Fig. 1). 37 early matured mutants were selected in both cultivars from all doses, from which the maximum mutation frequency obtained in Rama (4.56×10^{-4}) (Table 3). Early maturing and high-yielding sesame have been developed by using NaN_3 and colchicines, and Mensah *et al.*, (2007) found that 0.0625% NaN_3 and 0.125% colchicine were the most efficient concentration for inducing mutations in sesame.

Determinate types

19 determinate mutants were isolated, there of 10 were in Rama having highest mutation frequency (1.96×10^{-3}) and 9 were in

Tillotoma background (Table 3) (Fig. 2c). After the invention of “dt-45” by (Ashri, 1981), Cagirgan 2006, confirmed 6 true botanical mutant with determinate growth habit in sesame, though the breeding value and affinity of confirmed mutants are under study.

Dwarf types

To start a breeding program of any crop wind should be taken into consideration due to possibility of lodging. Generally, tall plants bearing high capsules are prone to lodging, whereas, dwarf plants are more suitable in these conditions. 99 dwarf type mutants were selected and confirmed in M_2 and M_3 generation respectively, whereas 53 from Rama (1.01×10^{-3}) and 46 from Tillotoma (9.11×10^{-4}) counterparts (Table 3).

Table.1 Some properties of two sesame cultivars

Name of Cultivar	Selected from	Seed coat colours and surface conditions	Size	Days to maturity	1000 Seed weight (g)
Tillotoma	West Bengal	Brown, rough and dull	Medium, bold	90 to 100	3.0
Rama	West Bengal	Brown, rough and glossy	Medium, bold	100 to 110	3.1

Table.2 Total spectrum and frequencies of induced mutants in two sesame cultivars

Cultivar	Dose (Gy)	No. of M_2 seedling studied	No. of mutants	Mutation frequency
Rama	250	14299	51	3.57×10^{-3}
	300	12926	115	8.90×10^{-3}
	350	9697	154	1.59×10^{-2}
	400	8226	99	1.20×10^{-2}
	450	7518	36	4.79×10^{-3}
	Total		52666	455
Tillotoma	250	15645	47	3.00×10^{-3}
	300	10425	60	5.76×10^{-3}
	350	8383	107	1.28×10^{-2}
	400	8057	60	7.45×10^{-3}
	450	7958	45	5.65×10^{-3}
	Total		50468	317

Table.3 Spectrum and frequency of some promising macro mutants in Rama and Tillotoma

Cultivar	Dose (Gy)	No. of M ₂ seedling studied	Shattering resistant types	Early maturing types	Determinate types	Dwarf types	Monostem types	More no. capsules/plant types
Rama	250	14299	4.20 x 10 ⁻⁴ (6)	5.59 x 10 ⁻⁴ (8)	2.80x 10 ⁻⁴ (4)	6.99 x10 ⁻⁴ (10)	6.29x10 ⁻⁴ (9)	--
	300	12926	8.51 x 10 ⁻⁴ (11)	3.09 x 10 ⁻⁴ (4)	3.09x 10 ⁻⁴ (4)	8.51x10 ⁻⁴ (11)	7.74x10 ⁻⁴ (10)	2.32 x 10 ⁻³ (30)
	350	9697	6.19 x 10 ⁻⁴ (6)	8.25x 10 ⁻⁴ (8)	2.06x10 ⁻⁴ (2)	1.55x 10 ⁻³ (15)	8.25x10 ⁻⁴ (8)	3.61 x 10 ⁻³ (35)
	400	8226	2.43 x 10 ⁻⁴ (2)	2.43 x 10 ⁻⁴ (2)	--	1.09x 10 ⁻³ (9)	-	3.16 x 10 ⁻³ (26)
	450	7518	2.66 x 10 ⁻⁴ (2)	2.66 x 10 ⁻⁴ (2)	--	1.06x10 ⁻³ (8)	-	1.60 x 10 ⁻³ (12)
	Total	52666	5.13 x 10⁻⁴(27)	4.56 x 10⁻⁴(24)	1.90x10⁻⁴(10)	1.01x10⁻³(53)	5.13x10⁻⁴(27)	1.96 x 10⁻³(103)
Tillotoma	250	15645	3.84 x 10 ⁻⁴ (6)	1.28 x 10 ⁻⁴ (2)	1.28x 10 ⁻⁴ (2)	5.75 x10 ⁻⁴ (9)	5.75x10 ⁻⁴ (9)	5.75 x 10 ⁻⁴ (9)
	300	10425	5.76 x 10 ⁻⁴ (6)	2.88 x 10 ⁻⁴ (3)	1.92x 10 ⁻⁴ (2)	7.67x10 ⁻⁴ (8)	--	1.15 x10 ⁻³ (12)
	350	8383	7.16 x 10 ⁻⁴ (6)	4.77 x 10 ⁻⁴ (4)	4.77x10 ⁻⁴ (4)	1.43x10 ⁻³ (12)	1.43x10 ⁻³ (12)	1.19 x 10 ⁻³ (10)
	400	8057	2.48 x 10 ⁻⁴ (2)	2.48 x 10 ⁻⁴ (2)	1.24x 10 ⁻⁴ (1)	1.37x10 ⁻³ (11)	7.45x 10 ⁻⁴ (6)	2.48 x 10 ⁻⁴ (2)
	450	7958	6.28 x 10 ⁻⁴ (5)	2.51x 10 ⁻⁴ (2)	--	7.54x10 ⁻³ (6)	--	--
	Total	50468	4.95 x 10⁻⁴(25)	2.58 x 10⁻⁴(13)	1.78x 10⁻⁴(9)	9.11x 10⁻⁴(46)	5.35 x10⁻⁴(27)	6.54 x10⁻⁴(33)

Values in parentheses are no. of macromutants selected

Table.4 Spectrum and frequency of no. of locules per capsule, capsule length, cluster capsule and 1000 seed weight (gm) in Rama and Tillotoma

Cultivar	Dose (Gy)	No. of M ₂ seedling studied	Multi locule types	Long capsule types	Clustering types	More seed wt. (1000 seeds) types
Rama	250	14299	4.20 x 10 ⁻⁴ (6)	--	--	5.59 x10 ⁻⁴ (8)
	300	12926	1.08 x10 ⁻³ (14)	7.74x 10 ⁻⁴ (10)	8.51x 10 ⁻⁴ (11)	7.74x10 ⁻⁴ (10)
	350	9697	2.27 x 10 ⁻³ (22)	2.17x 10 ⁻³ (21)	9.28x10 ⁻⁴ (9)	2.89x10 ⁻³ (28)
	400	8226	2.19 x 10 ⁻³ (18)	1.22x 10 ⁻³ (10)	9.73x10 ⁻⁴ (8)	2.92x10 ⁻³ (24)
	450	7518	--	--	--	2.93x 10 ⁻³ (22)
	Total	52666	1.14 x 10⁻³(60)	7.78x 10⁻⁴(41)	5.32x10⁻⁴(28)	1.75x 10⁻³(92)
Tillotoma	250	15645	6.39 x 10 ⁻⁴ (10)	--	--	--
	300	10425	--	--	5.76x 10 ⁻⁴ (6)	2.21x 10 ⁻³ (23)
	350	8383	1.31 x 10 ⁻³ (11)	2.62 x 10 ⁻³ (22)	4.77x 10 ⁻⁴ (4)	2.62x10 ⁻³ (22)
	400	8057	--	1.49x 10 ⁻³ (12)	1.12x 10 ⁻³ (9)	1.86x10 ⁻³ (15)
	450	7958	1.51 x 10 ⁻³ (12)	--	7.54x 10 ⁻⁴ (6)	1.76x10 ⁻³ (14)
	Total	50468	6.54 x 10⁻⁴(33)	6.74x 10⁻⁴(34)	4.95x10⁻⁴(25)	1.47 x10⁻³(74)

Values in parentheses are no. of macromutants selected

Monostem types

54 mutants having single stem were confirmed. The frequency of monostem types was higher in Tillotoma (5.35×10^{-4}) than in Rama (5.13×10^{-4}) (Table 3) (Fig. 2d). Monostem habit is preferred for mechanized harvest and also much suitable for dense stand under scarce of land for cultivation of sesame. Cagirgan (2001) reported monostem or unbranched with heavy capsule bearing types could be suited to sowing in closely spaced-row under intensive management condition.

More number of capsules per plant

136 mutants were confirmed from the study of their segregating pattern. The highest frequency was observed in Rama (1.96×10^{-3}) followed by Tillotoma (6.54×10^{-4}) (Table 3). Many authors for example, Asthana and Raj (1970), Ganesh and Sakila (1999) stated that number of capsules on main stem and number of capsules on branches showed a high positive direct effect on single plant seed yield in sesame.

Multi locules types

93 mutants with capsules having six to ten locules were confirmed (Fig. 2e and 2f) whereas 60 mutants from Rama and 33 mutants from Tillotoma background (Table 4). Some of the adult plants from both cultivars were erect and slightly twisted at the top. The stem was flattened like ribbon or phylloclade. Flowers and capsules were born directly on the flat stem without any distinct pedicel. Capsules were mostly 8 to 10 locules (Fig. 2g). In this category cultivar Rama had the highest mutation frequency (1.14×10^{-3}). Our results confirmed multi locules per capsule could be induced by irradiation which has sound contribution to increasing yield. However, Langham (2007) reported that by converting lines from 2 locules to 4 locules,

the seed weight per 4 locule capsules was greater although it didn't double.

Long capsule types

75 mutants were confirmed under this category with Rama having the highest mutant frequency (7.78×10^{-4}) followed by Tillotoma (6.74×10^{-4}) (Table 4) (Fig. 2h). The contribution of this character in plant seed yield may be investigated.

Cluster capsule types

In this category 53 mutants with up to 3 capsules per leaf axil were selected (Fig. 2i and 2j) whereas, the source of parents have only one capsule per leaf axil. Rama had the highest frequency for cluster type mutants (5.32×10^{-3}) (Table 4). Baydar (2005) screening for the ideal plant type in sesame also found that lines with quadricapsules, tricapsules per leaf axil.

Since it is a recessive character, it is obtained easily by mutagenesis (Cagirgan, 2001). To obtain high-yielding varieties in sesame, multi-capsules per leaf axil has been a good choice as ideal plant type.

More seed weight (1000 seeds)

1000 seed weight of any crop to be considered as an important yield component. 166 mutants were confirmed from this category having highest frequency in Rama (1.75×10^{-3}) (Table 4). Maximum mutant types were selected and confirmed for greater seed weight and higher number of capsules per plant which indicate the higher yield of a new plant type and might be increasing the oil quality. The possible cause of aforesaid morphological mutations or macro mutations may be chromosomal aberration, small deficiencies or duplications and obviously gene mutations.

This study confirmed that, viable mutations induced gross morphological changes in stem structure, phenology, growth habit, capsules shape and sizes and plant architecture. In the present investigation, all mutants were confirmed to breed true in M₃ generation. As revealed by the M₂ family analysis and the segregation of heterozygous M₃ plants, the true mutant traits were: i) randomly found in population ii) recessive and monogenically inherited, which are the basic features of mutation breeding. Although sesame seeds were known to be resistant to gamma radiation, it is not necessary to apply higher doses since medium doses (250 to 350Gy) could be effective to generate viable mutations.

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