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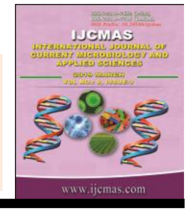

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Study on Radio Sensitivity of Gamma Rays on Different Genotypes of Sesame (*Sesamum indicum*)

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A B S T R A C T

In the present investigation dry and homogeneous seeds of two varieties of sesame were irradiated with different doses of gamma rays viz. 250, 300, 350 and 400 Gy. The effect of mutagenesis or radio sensitivity of gamma rays on different biological parameters like germination (%), pollen fertility (%), shoot length, root length, plant survival are being studied in M_1 generation. It has been found that germination (%) and plant survival (%) and shoot length decreased progressively with increasing doses of gamma rays, whereas in case of pollen fertility (%), shoot length, root length, there was gradual decrease with increase in doses in both the varieties of sesame. In the cases of biological parameters, where doses higher than 400 Gy were required to obtain 50% reduction, ID_{30}, ID_{25}, ID_{15} (Inhibitory dose) were calculated. It was clear that for germination (%) and plant survival, Tillotoma was more radiosensitive than Rama, whereas for pollen fertility (%), shoot length, root length, plant survival, the cultivar Rama was more radiosensitive than Tillotoma.

Introduction

Sesame (*Sesamum indicum L.*) is one of the world's important oil seed crops and belongs to the family Pedaliaceae. It is self-pollinated annual shrub grown in tropical, sub-tropical and southern temperate areas of the world, especially in India, China, South America and Africa. Sesame is commonly known as "Queen of the oil seeds", because among the oil crops, it contains highest oil content and protein. The oil and fatty acid compositions are determined by genetic and environmental factors and the oil content of sesame ranges from 34 to 63%. Sesame seed oil has high shelf life due to the presence of lignans (sesamin, sesaminol, sesamolinol), which have remarkable antioxidant function, resisting oxidation.

Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like, morphology, cytogenetics, biotechnology and molecular biology etc. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops (Lee *et al.,* 2002). These mutations provide beneficial variation for practical plant breeding purpose. During the past seven decades, more than 2252 mutant varieties have been officially released in the world (Maluszynski *et al.,* 2000). Mutation breeding is relatively a quicker method for improvement of crops and induced mutation serves as an effective method for development of economically high yielding mutants. Applications of appropriate doses of physical mutagen have brought about adequate mutations that could benefit sesame breeding programme.

Gamma rays, the physical mutagen is non-particulate ionizing radiations, having high energy and penetrable capacity in biological tissues and make changes in base, disruptions of hydrogen bonds between complementary stands of DNA. A great majority of mutant varieties (64%) were developed by the use of gamma rays (Ahlowalia *et al.,* 2004).

Materials and Methods

Dry, uniform, bold seeds of variety Rama and Tillotoma each weighing 150g were irradiated with 250,300,350,400 Gy doses of gamma rays (^{60}Co) at BARC, Trombay, Mumbai. The irradiated seed along with the control planted during post-kharif season, 2016 for raising M_1 generation.

In laboratory, one hundred and fifty seeds from each of the treatment combinations were placed on moist filter paper in three petridishes having fifty seeds in each to test germination. Three petridishes of each treatment were treated as three replications. Accordingly, the petridishes were arranged in a split-plot fashion. Germination of seeds was carefully observed every day and the emergence of radicle, which was taken as an index for germination was recorded by counting the number of seeds germinated after 7 days of sowing in each petridish and the percentage of germination was calculated as follows:-

$$Germination\ \% = \frac{No.of\ seeds\ germinated}{Total\ no.of\ seeds\ sown} \times 100$$

Length of the shoot from cotyledonary node to the tip of the shoot, root length from the cotyledonary node to tip of primary root were measured on 7th day after sowing in each petridish and were measured in cm.

Flower buds of 10 randomly selected plants were harvested. Petals of the flowers were removed by dissecting flower head with needle and then anthers were removed by forceps. Then pollen was collected from the anthers after being smashed using the tip of needle and slides were prepared by treating with 2% acetocarmine stain. Slides were placed under a compound microscope. Five microscopic fields were chosen for recording of stained or fertile pollens and total no. of pollens in a microscopic field was counted.

$$Fertile\ pollen\% = \frac{No.of\ stained\ pollen}{Total\ no.of\ pollen\ in\ a\ microscopic\ field} \times 100$$

The plant survival was computed as the percentage of plants surviving till maturity. It indicates lethality of each dose of mutagenic treatment.

$$Survival\ \% = \frac{Total\ no.of\ plants\ survived}{Total\ no.of\ seeds\ sown} \times 100$$

Results and Discussion

In order to induce variability for efficient plant breeding, systematic study of mutagen sensitivity of various crop plants and different cultivars within a crop are essential (Brock, 1971). Although studies have been made on the biological effects of radiations and the relative mutagen sensitivity in lentil (Sharma and Sharma, 1981), Mungbean (Jgnacimuthu and Babu, 1989), urd bean (Singh *et al.*, 1999), chick pea (Kharkwal, 1998) and field pea (Paul and Mondal, 2012), such reports are limited in sesame. Therefore, in the present investigations, an attempt has been made to study the mutagen sensitivity in two high yielding varieties of sesame. The similarities or differences between genotypes with regard to radio-sensitivity following gamma treatments to those sesame varieties were worked out.

Germination percentage

Significant reduction in germination percentage was observed in Rama at 400 Gy from control, 250 Gy, 300 Gy and 350 Gy. In case of Tillotoma significant reduction was recorded at 400 Gy from control, 250 Gy and 300 Gy. However, in both the varieties, there was significant reduction in germination percentage at each dose over the control. The effect of doses over the two genotypes was significantly different from each other except 350 Gy and 400 Gy and response of the two genotypes over all doses were significantly different from each other.

Emrani *et al.,* (2011) observed significant effect of mutagen doses on seed germination. Anabarasan *et al.,* (2013, 2015), Kumar and Srivastava (2013) and Kumari and Chaudhary (2016) while studying the effects of gamma rays on different varieties of sesame also found the gradual decrease in germination percentage with the increase in doses. Reduced germination might be due to higher physiological damage in seeds resulting from inhibition of auxin synthesis (Gordon, 1955), and catalase peroxidase and cytochrome oxidase (Kleinhots *et al.,* 1974).

Root length

Root length of gamma irradiated plants and control plants differ significantly in both the genotypes of sesame. The results revealed that the effects of three doses (250 Gy, 300 Gy and 350 Gy) over two genotypes were statistically at par with each other but were significantly different from control. Significant reduction in root length, however was observed in both Rama and Tillotoma, at 400 Gy from control and other three doses. The effect of genotypes pooled over doses revealed that the two genotypes were not significantly different from each other. Anabarasan *et al.,* (2015) reported that the root length decreased with increase in dose of gamma radiation.

The sensitivity of root length to mutagens may be due to simultaneous effect of miotic arrest and preponderance of cell in G_2 phase in the meristems because such phase of cell cycle has been found to be more sensitive as compared to G_1 phase of cell cycle to irradiation (Sharma and Sarbhoy, 1990).

Shoot length

Similar trend as in root length was observed in shoot length in both the varieties, although significant differences could be formed between two varieties over all the doses. Reduction in seedling growth has been attributed to inhibitory action of enzymes and changes in the enzyme activity due to gamma irradiation (Patil *et al.,* 1992).

Pollen fertility (%)

In Rama there was significant reduction in pollen fertility with the increase in dose of gamma irradiation up to 350 Gy. Similar trend was observed by Kumar and Srivastava (2013) and Kumari and Chaudhary (2016). The effect of doses on pollen fertility (%) in

Tillotoma was not significant. The results revealed that the effect of doses over two genotypes was significantly different from each other except between two genotypes over all the doses.

According to Blixt and Gottschalk (1975), chromosome aberrations, changes involving DNA and/or RNA synthesis, meiotic abnormalities might be the causes of pollen sterility owing to radiation. There was a positive and highly significant correlation between chromosomal abnormalities and pollen sterility (r = 0.82 – 0.98) (Ignacimulthu and Babn, 1989).

Survival (%)

Drastic reduction in survival (%) was observed in Rama and Tillotoma at 400 Gy, which was significantly different from control, 250 Gy and 300 Gy. The results revealed that effects 300 Gy and 400 Gy over the genotypes were not significantly different or statistically at par with each other but were significantly different from control. The effects of genotypes pooled over doses, however, revealed that the two genotypes were not significantly different from each other. Anabarasan *et al.,* (2015) and Kumari *et al.,* (2016) observed that mutagen treatment in sesame reduced seedling survival in M_1 generation. From the results of survival (%), it appears that survival (%) of seedlings cannot be considered as a reliable parameter to indicate biological effects of radiation.

In the present investigation, it has been found that germination (%) and plant survival (%) and shoot length decreased progressively with increasing doses of gamma rays in both the varieties of sesame under study. This is clearly understood from the steepness of the probit lines (Fig. 1). The germination percentage decreased significantly and followed a linear relationship in Rama (R^2 = 0.999, $p < 0.001$) as well as in Tillotoma (R^2 = 0.881, $p < 0.001$). Similarly, reduction (%) in plant survival showed highly significant differences among the lower and higher doses of irradiation and followed relationship in Rama (R^2 = 0.933, $p < 0.001$) as well as in Tillotoma (R^2 = 0.979, $p < 0.001$).

Similar percent reduction in length progressively increased with the increase in doses and followed a linear relationship in Rama (R^2 = 0.957. $p < 0.001$) as well as in Tillotoma (R^2 = 0.881, $p < 0.001$).

In case of characters like pollen fertility (%), shoot length, root length, although there was gradual decrease with increase in doses, the relationship, however, was not so sharp and definite (as compared to germination (%) and plant survival (%) and shoot length) which is indicative from less steepness or rather somewhat flatness of the probit lines (Fig. 1). Root length were more affected in Rama (R^2 = 0.910, $p < 0.001$) than in Tillotoma (R^2 = 0.832, $p < 0.001$ respectively). However, in case of pollen fertility (%), Tillotoma was more affected than Rama (R^2 = 0.919, R2 = 0.895, $p < 0.001$, respectively) (Table 1).

Perusal of Table 2 and 3 reveals the different ID_{50} along with their fiducial values for different biological parameters under study in M_1 generation. Regression equation, based on probit analysis, has been given in the tables. χ^2 values for hetero genicity test against each parameter in both the cultivars were non-significant which indicate that homogeneity in the population and that the regression line is well fitted. In the cases of biological parameters, where doses higher than 400 Gy were required to obtain 50% reduction, ID_{30}, ID_{25}, ID_{15} were calculated. Reduction in the 30% germination (ID_{30}) occurred at 353.61 Gy in Rama, whereas slightly lower dose (336.92 Gy) was needed for Tillotoma.

Table.1 Mean performance in respect of different characters studied in the laboratory
V_1 = Rama, V_2 = Tillotoma

Variety	Germination (%)			Root length (cm)			Shoot length (cm)			Pollen fertility (%)			Survival (%)		
	V_1	V_2	Avg	V_1	V_2	Avg	V_1	V2	Avg	V_1	V_2	Avg	V_1	V_2	Avg
Control	87.37 (69.19)	96.16 (78.96)	91.76 (74.07)^a	2.401	2.072	2.24^a	1.89	1.91	1.9^a	93.08 (74.80)	94.68 (76.66)	93.88 (75.73)^a	71.01 (57.74)	73.91 (59.36)	72.46 (58.40)^a
250 Gy	74.89 (59.93)	78.68 (62.61)	76.78 (61.26)^b	1.26	1.396	1.33^b	1.66	2.12	1.26^b	86.06(68 .37)	88.41 (70.18)	87.24 (69.27)^b	57.20 (49.14)	55.95 (44.59)	56.58 (46.86)^b
300 Gy	68.5 (55.87)	76.18 (60.79)	72.34 (58.33)^c	1.01	1.73	1.17^b	1.35	1.29	1.32^b	75.01 (60.07)	87.21 (69.20)	81.11 (64.63)^c	40.60 (39.57)	43.65 (39.33)	42.12 (39.45)^c
350 Gy	61.79 (51.82)	61.43 (51.62)	61.61 (51.71)^d	1.01	1.58	1.15^b	1.25	1.32	1.28^b	68.21 (57.15)	81.16 (64.30)	74.69 (60.72)^d	25.62 (30.49)	22.04 (27.93)	23.8 (29.21)^d
400 Gy	54.85 (47.79)	60.11 (50.84)	57.48 (49.31)^d	0.646	1.01	0.83^c	0.98	1.01	0.99^c	68.18 (55.66)	79.74 (63.58)	73.96 (59.62)^d	17.49 (24.73)	19.48 (26.18)	18.49 (25.45)^e
Avg	60.95^b	56.92^a		1.41^a	1.26^a		1.67^b	1.42^a		68.78^a	63.21^a		40.27^a	39.47^a	
LSD	8.85			0.32			0.65			7.07			9.97		

Any two means having a common letter in the row / column of Avg are not significantly different at 5% level of significance as per Duncan's multiple range test (DMRT) or mean separation in the row / column of Avg by DMRT at 5% level. Figures in the parenthesis are transformed values (Arc-sin)

Table.2 and 3 Sensitivity of Rama and Tillotoma against each biological parameter has been given below

Biological parameters	Radio sensitivity
Germination (%)	Tillotoma >Rama
Pollen fertility (%)	Rama >Tillotoma
Shoot length	Rama >Tillotoma
Root length	Rama >Tillotoma
Plant survival	Tillotoma >Rama

Table.4 Effect of ϒ-radiation on different biological parameters of sesame (var. Rama)

Biological parameter	% Reduction over control (Lower-Upper)	ID_{50} (Gray)	Fiducial limits at p=0.05 (Lower-Upper)	Regression equation (from probit)	χ^2 for heterogenicity
Germination	25.11-45.14	353.61[3]	324.49-405.49	Y= 3.623x-4.759	0.003[NS]
Pollen fertility	13.93-31.84	387.82[2]	335.78-439.22	Y= 3.640x-5.015	1.993[NS]
Survival	42.79-82.50	319.80	307.04-332.91	Y= 7.637x-14.131	0.526[NS]
Root length	47.08-72.92	266.26	204.95-295.67	Y= 2.983x-2.236	1.665[NS]
Shoot length	11.64-47.62	394.43	368.81-441.13	Y= 5.473x-9.209	1.764[NS]

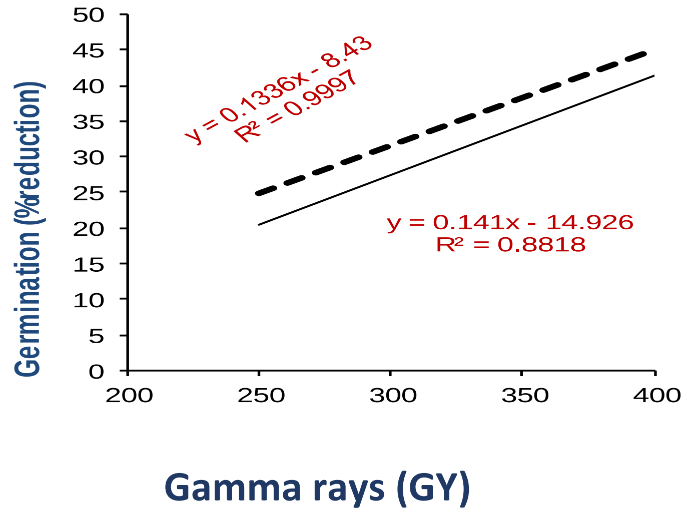
NS: Non-significant
2: ID_{25} value calculated; 3: ID_{30} value calculated

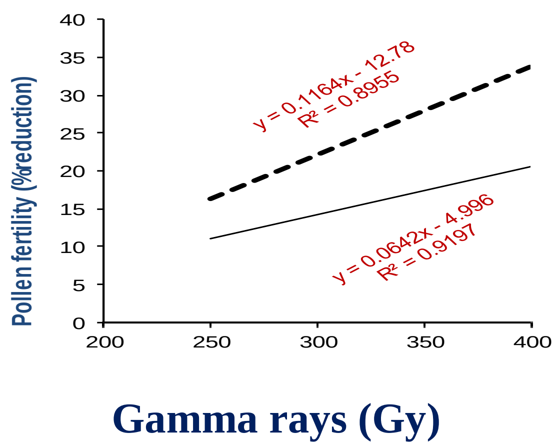
Table.5 Effect of ϒ-radiation on different biological parameters of sesame (var.Tilottoma)

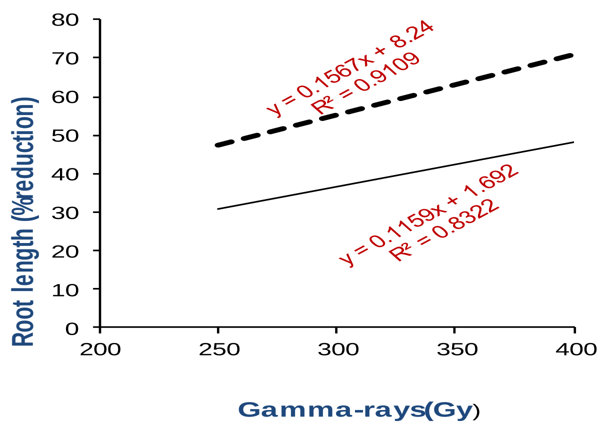
Biological parameter	% Reduction over control (Lower-Upper)	ID_{50} (Gray)	Fiducial limits at p=0.05 (Lower-Upper)	Regression equation (from probit	χ^2 for heterogenicity
Germination	21.31-39.89	336.92[3]	350.02-381.95	Y= 3.291x-3.844	1.611[NS]
Pollen fertility	11.58-20.26	382.07[1]	332.27-809.65	Y= 2.709x-3.033	0.504[NS]
Survival	50.71-80.52	300.93	282.68-316.86	Y= 5.690-9.103	2.393 [NS]
Root length	32.37-51.21	246.04[3]	102.55-287.79	Y= 2.187x-0.754	1.721 [NS]
Shoot length	1.56-46.35	368.77[3]	385.59-355.39	Y= 9.837x-20.773	2.631 [NS]

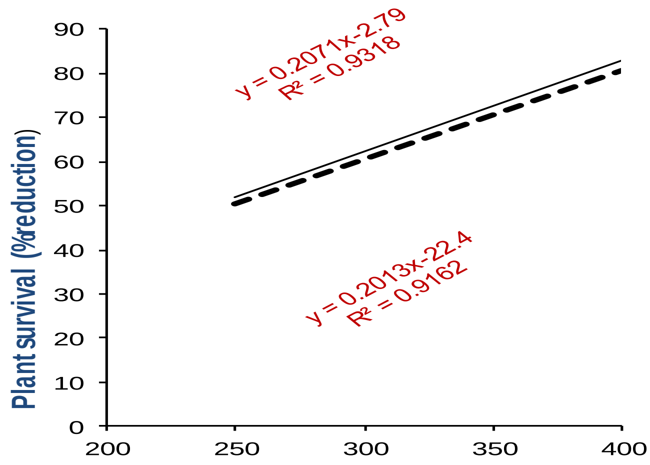
NS: Non-significant
1: ID_{15} value calculated; 3: ID_{30} value calculated

Fig.1 Effect of gamma-rays on different parameters in M_1 generation in sesame

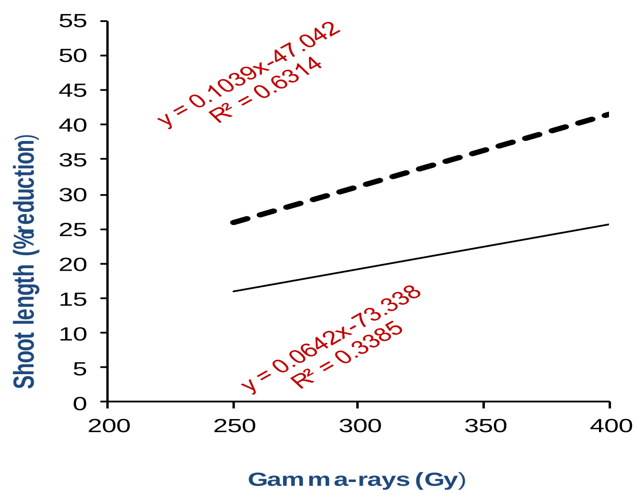








Gamma rays (Gy)



Another immediate effect of irradiation in the M_1 generation was also observed through reduction in pollen fertility for which ID_{25}being 387.Gy for Rama and ID_{15} being 382.07 Gy for Tillotoma.

The ID_{50} for plant survival was at 319.80 Gy in Rama and 300.93Gy in Tillotoma. In case of root length, doses higher than 250 Gy was required to obtain 50% production (ID_{50} being 266.26 Gy) in Rama, whereas in Tillotoma, doses lower than 250 Gy was required to obtain 30% reduction (ID_{30} being 246.04 Gy). The ID_{50} for shoot length in Rama was at 394.43 Gy, slightly less than the highest strength of dose (400 Gy) under study, whereas, it was beyond 400 Gy in Tillotoma, ID_{30} being 368.77 Gy.

In the present investigation, it was observed that retardation in the root length was more pronounced than found in the shoots. The root system appears to be relatively more sensitive to gamma rays (Table 4 and 5).

This can possibly be due to an inhibition of division in root cells by mutagen which exert less effect on the elongation of shoot cells.

The shoot growth is reported to be mainly due to the cell elongation while root growth is more dependent on cell division (Brock, 1971).

It was clear that for germination (%) and plant survival, Tillotoma was more radiosensitive than Rama, whereas for pollen fertility (%), shoot length, root length, plant survival, the cultivar Rama was more radiosensitive than Rama.

It appears from above results that mutagen sensitivity, at least for these cultivars is independent of the genotypic background as well as of biological parameters under investigation.

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