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Research Article

Radiation sensitivity of sesame (*Sesamum indicum* L.) genotypes in M_1 generation

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Abstract
The present study investigated the gamma ray sensitivity of two sesame (*Sesamum indicum* L.) varieties *viz.,* SVPR1 and TMV7 irradiated with five different doses from 250 Gy to 450 Gy at 50 Gy intervals. Germination percentage, root and shoot length, pollen fertility (%) decreased gradually and observed a dose dependent relationship with an increase in dosage of gamma rays. Survival percentage recorded irregular decreasing trend with upsurge in survival rate at certain doses. SVPR1 showed a pronounced maximum reduction in germination percentage (37.5%) than TMV7 (40.05) at 450 Gy, whereas survival percentage of TMV7 (20.02%) and SVPR1 (19.5%) was drastically reduced at 250 Gy itself. At a higher dose of 450 Gy, root (3.08) and shoot (3.03) length of seedlings of SVPR1 were greatly inhibited. Pollen fertility percentage showed a linear reduction irrespective of the genotypes, and maximum reduction was rear at 450 Gy in SVPR1 (62.76%) was recorded. LD_{50} values that showed 50% reduction in biological parameters differed between TMV7 and SVPR1. Based on all the biological parameters studied, the mutagen sensitivity of SVPR1 is higher than TMV7. The overall considerations on M1 generation effects showed that SVPR1 were highly sensitive to gamma rays.

Key words
Sesamum indicum L, Gamma rays, mutagen sensitivity

INTRODUCTION

Sesame (*Sesamum indicum* L, 2n = 26) or gingelly is one of the ancient oil seed crop, belongs to the family Pedaliaceae. Sesamum mainly grown for the oil extraction and edible purpose holds 48 to 55 % and 20 to 28 % of oil and protein content (Pathak *et al.,*2014). Sesame seed oil has phenomenal antioxidant function due to the presence of sesamin, sesaminol, sesamolinol which resist oxidation and increases the shelf life period. Sesame seeds are highly recommended in traditional medicines due to its nutritive and curative properties. It has antioxidant and anti-carcinogenic agents that drastically increased its health benefits which assist in cardiovascular disease, liver protection and tumor prevention (Cheng *et al.,* 2006). However, India has low productivity in sesame due to lack of high yielding varieties and availability of narrow germplasm. Development of superior desirable genotypes foster higher productivity and it can be achieved easily through induced mutagenesis. Mutation is a sudden heritable change in the nucleotide sequence of a gene (Chahal *et al.,* 2002). Mutation breeding provides scope for the creation of variability and followed by the selection of desirable genotypes. Physical mutagens such as X-rays, gamma rays, thermal neutrons and fast neutrons accounts for 89 % of mutant varieties whereas gamma rays alone holds 60 % of the varieties (Kharkwal, 2000). Mutation breeding helps in the reconstruction of plant ideotype with incorporation of one or two desirable traits in well adopted varieties and induction of disease resistant traits (Swaminathan, 1961).

Use of gamma rays, ionizing radiation highly penetrate biological tissues and react with water molecules to produce free radicals which in turn disrupts the H-bond between complementary base pair in double helix DNA. Mutagen sensitivity is estimated through percentage of seed germination, seedling injury, survival percentage, pollen fertility and chromosomal aberrations (Kivi, 1965). Radiation tolerance or mutagen sensitivity also differ between species and even among genotypes within a species (Sparrow, 1966). It is inevitable to determine the appropriate doses for each genotype to induce artificial mutagenesis with least biological damage. Therefore, the present study investigated the mutagen sensitivity of Gamma rays in two popular sesame varieties TMV7 and SVPR1 in the M_1 generation.

MATERIALS AND METHODS

The experiment was carried out during Kharif season (June – Sept) of 2019-20 at 'D-block' farm, Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Killikulam, Vallanad Tuticorin Dt, Tamil Nadu. The soil is red lateritic with pH: 6.8 and EC: 0.05 dSm^{-1} , and sub-tropical monsoon climate with relative humidity of 60-80%.

Plant Materials

Seeds of two commercial growing sesamum cultivars TMV7 and **SVPR1** were obtained from Oilseeds Research Station, Tindivanam and Cotton Research Station, Srivilliputtur, Tamil Nadu Agricultural University

Gamma Irradiation

A quantity of 2g per dosage of uniform, healthy and dry seeds with 9 % moisture content of two varieties of sesame *viz.,* TMV7 and SVPR1 were exposed to gamma irradiation at different doses (250,300, 350, 400, 450 Gy) using Cobalt-60 (^{60}Co) gamma source installed at Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Chennai, Tamil Nadu. The irradiated seeds were sown in the field along with control (non - treated) seeds within 24 hours of treatment.

Field Study

Irradiated seeds (M_0) along with the controls (non-irradiated) were sown in the field in a randomized block design with three replications keeping plant to plant and row to row distance of 10 and 30 cm, respectively during Kharif 2019. Four to five capsules of each M_1 plants against all the treatments were collected separately at maturity to raise the M_2 generation. All the agronomic practices and plant protection measures were taken throughout the entire experiment as per the crop production guidelines. In the M_1 generation various biological parameters and several other quantitative traits were measured to assess the effect of gamma rays.

Studies on Biological Injury:

Mutagen sensitivity was estimated with the help of biological injury and measured in terms of germination percentage on 15 DAS, survival percentage followed by root and shoot length measurement on 30 DAS whereas pollen fertility was measured at the flowering stage.

Germination percentage

Germination index observed from radicle emergence to 15 DAS by counting the number of seeds germinated and the percentage of germination was calculated as follows:-

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

Root and Shoot Length

Shoot length measured from cotyledonary node to the shoot tip and root length from cotyledonary node to primary root tip on 30DAS and expressed in cm.

Survival Percentage

The plant survival rate was calculated as the percentage of plants survived at the time of harvesting. Survival percentage is the reflection of lethality of each dose of mutagenic treatment.

$$\text{Plant Survival (\%)} = \frac{\text{No. of plants survived}}{\text{Total no. of seeds sown}} \times 100$$

Pollen Fertility

Randomly selected flowers from 10 plants per treatment were collected and flowers were dissected with the help of needle and forceps. Pollen grains were smeared in the glass slide and mounted on a compound microscope after staining using 0.5 % potassium iodide (KI) solution (Baker and Baker 1979) and counted for five microscopic fields. Regularly shaped and stained pollen grains were considered as fertile, whereas the unstained and empty ones as sterile.

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

RESULTS AND DISCUSSIONS

The term radio sensitivity implies a quantity of dose, that produces recognizable effects of radiation on the biological materials exposed (Van Harten, 1998). Therefore, the biological effects and relative mutagen sensitivity in the context of similarities and dissimilarities of the sesame genotypes TMV7 and SVPR1 in regard to radiation sensitivity were investigated.

The germination percentage of mutated seeds ranged from 80.0% (250Gy) to 40.0% (450 Gy) for TMV7 and from 72.5% (250Gy) to 37.5% (450 Gy) for SVPR 1. Significant reduction in germination per cent was exhibited by both TMV7 (86.49 to 43.24 %) and SVPR1 (87.88 to 45.45 %). However, the highest percentage of reduction in seed germination was observed at 450 Gy (56.76%)

of TMV7 and 54.55% for SVPR1 respectively (**Table 1**). Seed germination percentage decreased linearly with an increase in dose of gamma rays in both the genotypes. At 400 Gy greater than 50% reduction in germination was observed for SVPR1 (51.52%) was in TMV7 at 450 Gy. Response of the genotypes for the dose which causes 50% reduction in seed germination percentage was different because of their genetic background (**Fig 1**). The works of Emrani *et al.,* 2011; Anbarasan *et al.,* 2013; Anbarasan *et al.,* 2015, Kumari *et al.,* 2016 in various sesame genotypes observed a dose dependent reduction in germination percentage. Gamma rays interfere with metabolic process of the mutated seeds causing physiological changes coupled with chromosomal damage leads to inhibitory effect on seed germination (SJODIN, 1962 and Sinha and Godward,1972). The results are in agreement with Sangle *et al.,* (2011) in pigeon pea; Bhat *et al.,* (2011) in chick pea; Gowthami *et al.,* (2017) in rice; Ramesh *et al.,*(2019) in barnyard millet and Datir *et al.,* (2007) in horse gram.

Table.1 Effect of gamma rays on seed germination percentage, root length and shoot length, pollen fertility percentage and survival percentage expressed as percent reduction.

Variety	Dose	Germination % (15 DAS)		Root length (cm)		Shoot length (cm)		Pollen fertility %		Survival % (30 DAS)	
		Mean ± SE (%)	Per cent over control	Mean ± SE	Per cent over control	Mean ± SE	Per cent over control	Mean ± SE (%)	Per cent over control	Mean ± SE (%)	Per cent over control
TMV7	**Control**	92.5±0.3	0.00	12.69±0.65	0.00	10.79±0.28	0.00	96.96±0.28	0.00	92.5±0.3	0.00
	250 Gy	80.0±3.7	13.51	8.30±0.28	34.59	9.81±0.37	9.08	84.49±6.60	5.91	20.0±2.2	78.38
	300 Gy	77.5±2.5	16.22	7.70±0.24	39.32	8.83±0.425	18.21	71.88±2.23	25.87	27.5±3.7	70.27
	350 Gy	65.0±4.8	29.73	6.22±0.083	51.01	6.98±0.26	35.28	61.4±2.95	36.67	27.0±1.0	70.81
	400 Gy	47.5±1.7	48.65	5.10±0.55	59.81	5.53±0.315	48.80	60.33±2.33	37.78	21.0±1.8	77.30
	450 Gy	40.0±5.0	56.76	4.40±0.41	65.33	5.78±0.5	46.48	44.3±1.60	54.31	10.5±2.1	88.65
SVPR1	**Control**	82.5±0.1	0.00	9.76±0.67	0.00	9.11±0.3	0.00	96.6±0.49	0.00	82.5±0.1	0.00
	250 Gy	72.5±3.5	12.12	5.61±0.16	42.52	8.66±0.24	4.94	65.72±8.12	31.97	19.5±1.5	76.36
	300 Gy	57.5±2.4	30.30	5.29±0.162	45.82	7.20±0.415	20.97	46.19±5.82	52.18	16.0±1.2	80.61
	350 Gy	42.5±2.5	48.48	3.82±0.343	60.89	6.12±0.52	32.86	43.09±6.13	55.39	26.5±0.9	67.88
	400 Gy	40.0±4.8	51.52	4.08±0.37	58.25	5.88±0.285	35.51	41.55±9.38	56.99	10.0±1.0	87.88
	450 Gy	37.5±2.5	54.55	3.08±0.313	68.49	3.03±0.027	66.79	35.97±7.39	62.76	4.00±1.6	95.15

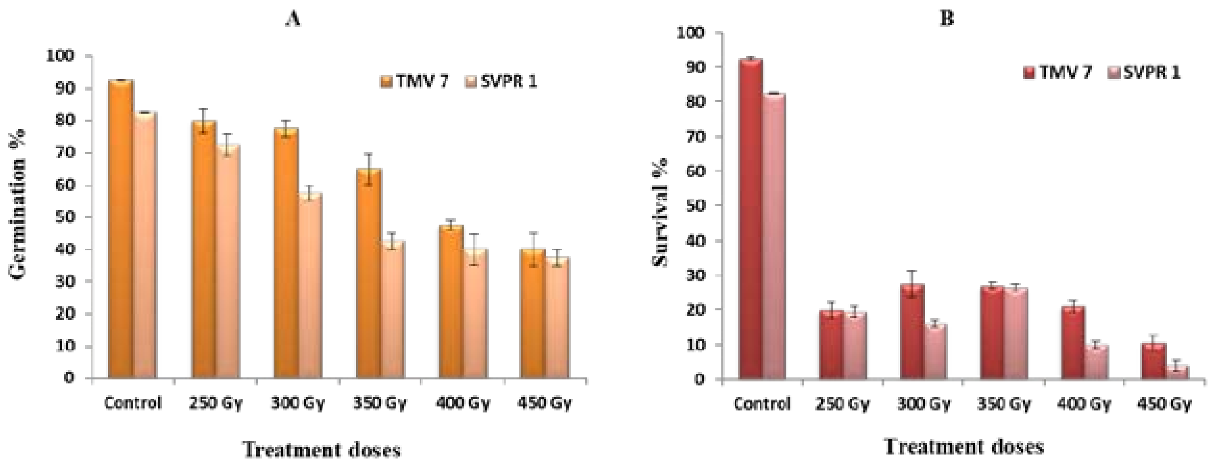

Fig.1 Effect of gamma rays on seed germination (A) and Survival percentage (B

The length of the root decreased gradually from 8.30 cm (250Gy) to 4.40 cm (450 Gy) for TMV7 and from 5.61cm (250 Gy) to 3.08 cm (450 Gy) for SVPR1. Similarly, shoot length decreased from 9.81 cm to 5.78 cm and 8.66 cm to 3.03 cm for TMV7 and SVPR1 respectively (**Table 1**). Effect of gamma rays on root and shoot length showed a negative dose dependent relationship. Root length of SVPR1 observed a drastic reduction at 250 Gy (5.61 cm) whereas TMV7 showed gradual reduction at 250 Gy (8.30 cm.). However, at 400 Gy SVPR1 (4.08) observed uptrend in root length whereas TMV7 showed a dose dependent reduction. At 350 Gy, TMV7 recorded 51.01% reduction whereas SVPR1 observed 45.82% and 60.89 % reduction in root length at 300 and 350 Gy respectively. In case of shoot length a gradual reduction in both the genotypes was observed and at 450 Gy significant reduction of 66.79% (SVPR1) and 46.78 % (TMV7) was observed (**Fig 2**).

A) TMV 7

B) SVPR 1

Fig.2 Effect of Gamma rays on root and shoot length at 30 days old seedlings of TMV7 (A) and SVPR1 (B)

The dose that causes 50% reduction in root length was lower than the dose required for 50% reduction in shoot length. This indicate that root region of both the genotypes was prone to more biological damage than the shoot., Significant differences between varieties were found in both root and shoot length due to their difference in radio sensitivity. SVPR1 observed significant root and shoot length reduction than TMV7 (**Fig 3**). Similar results were reported by Pradhan *et al.,* (2019); Raja Ramadoss *et al.,* (2014) and Anbarasan *et al.,* (2015) for a dose dependent relationship for root length, shoot length and seedling height of m_1 generation.

Percentage of pollen fertility decreased linearly from 84.49 (250Gy) to 44.3 % (450 Gy) for TMV7 and in case of SVPR1 it decreased from 65.72 % to 35.97%. Per cent reduction of fertile pollen grains was highest at 450 Gy of SVPR1 (62.76%) than TMV7 (54.31%) (**Table1**). As like that of germination percentage, fertility percentage of pollen grains decreased gradually and recorded dose dependent reduction in both the varieties. The dose required to cause 50 % reduction of pollen fertility was completely different for the two genotypes. At 300 Gy SVPR1 showed 52.18% reduction in pollen fertility whereas TMV7 recorded 54.31% reduction at 450 Gy (**Fig 3**). The results are in agreement with the works done by Kumar *et al.,* 2013; Kumari *et al.,* 2016 and Pradhan and Paul, 2019. Irrespective of the mutagen dose TMV7 produced more fertile grians showing least effect of gamma rays whereas SVPR1 recorded less pollen fertility and stand as radio sensitive genotype.

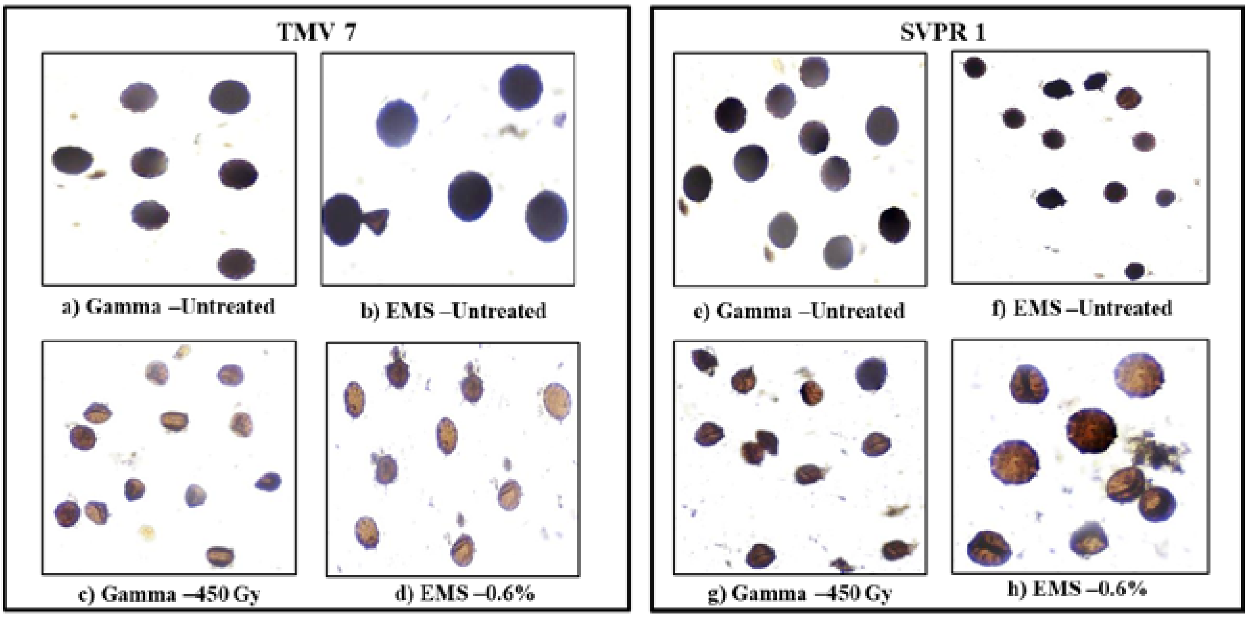

Fig. 3 Effect of different doses of gamma rays on pollen grains

Note: Reduction in pollen fertility (normal and stained round pollen to the shrivelled and unstained irregular shaped pollen grains.

Survival percentage decreased significantly at 450 Gy (10.5%) for TMV7 and 4.0% for SVPR1. At 350 Gy of SVPR1 (26.5 %) and 300 Gy of TMV7 (27.5 %) showed an uptrend in reduction of plant survival than the remaining doses. Highest percentage of reduction in seedling survival was recorded at 450 Gy of SVPR1 (95.15 %) and TMV7 (88.65 %) (**Table 1, Fig 1**). The effect of gamma rays highly pronounced in plant survival rate for both the genotypes under study. The lowest reduction in survival percentage was observed at 300 Gy for TMV7 (70.27 %) and 350 Gy for SVPR1 (67.88 %). Contrasting to that of germination percentage, root and shoot length, and pollen fertiliy %, survival percentage observed non linear reduction agaisnt the dose of gamma rays. The above results were contrary to the earlier works done by Pradhan and Paul, 2019; Kumari *et al.,* 2016 and Boureima *et al.,* 2009 in sesame in which they observed a steady decrease in survival rate with an increasing dose of mutagen. In terms of survival rate SVPR1 was found to be more radiosensitive than TMV7.

From the study, variety TMV7 showed high germination percentage, survival (%) pollen fertility (%), root and shoot length in all the treatments than SVPR1. Root length reduced drastically than shoot length irrespective of the genotypes. Root system appeared to be more sensitive than shoot. The pollen fertility and plant growth showed a linear fashion of reduction with increase in dosage of mutagens, in both the varieties. Gamma rays has proven to be a possible and more efficient mutagen to induce essential economic mutations in TMV 7. The results also suggest that the lower doses or concentrations of mutagen are more effective and efficient than the higher doses to exploit desirable mutants.

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