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EFFECTIVENESS AND EFFICIENCY OF GAMMA RAYS ON SESAME (*Sesamum indicum* L.) GENOTYPES

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ABSTRACT

To evaluate the mutagenic effectiveness and efficiency of gamma rays on different biological parameters in two genotypes of sesame, Rama and Tillotoma; seeds were irradiated with five doses of gamma rays: (250, 300, 350, 400, 450 Gy) from Bhaba Atomic Research Centre (BARC), Trombay and grown (along with control) during pre-*kharif* 2015 in split plot design as M_1 and during pre-*kharif* 2016 with plant to progeny rows as M_2 generation at experimental farm of Visva-Bharati. The effectiveness and efficiency of the mutagen used was assessed with regard to biological damage *viz*. pollen sterility (%) and plant lethality (%) in the M_1 generation. In M_2 generation, mutation frequency was assessed with chlorophyll mutation. Three types of chlorophyll mutations viz., albina, xantha and chlorina were observed with maximum chlorina (66.1%) types. All the studied parameters were decreased with increase dose of gamma rays, whereas, mutagenic effectiveness and efficiency increased in lower doses. For both genotypes, lower dose 300 Gy (0.42 effectiveness and 12.57 efficiency) was found to be most effective and efficient dose. The present findings concluded that lower doses were more effective and efficient among the used doses and can be recommended for exploiting variability with isolating promising mutants.

INTRODUCTION

Sesame is known to be the most ancient oil seed crop dating back to 3050-3500 B.C (Bedigian and Harlan, 1986). Sesame belongs to the order Tubiflorae, family Pedaliaceae. It is grown in tropical and subtropical areas of the world (Ashri, 1998), can set seed and yield well under fairly high temperature and also can grow in stored soil moisture without rainfall and irrigation. But continuous flooding or severe drought adversely affect sesame plants and resulted in low yield (Mensah *et al.*, 2009). It had good crop rotation; capable to grown on pure or mixed stands and grown with low inputs (Brar and Ahuja, 1979). In India, it is the fifth most important edible oilseed crop after groundnut, rapeseed-mustard, sunflower, and soybean, although the productivity of sesame stands alarmingly poor comparing to other oil seed crops. The main fact behind this, due to narrow genetic pool, it is not possible to restructure the sesame crop and also the desired genetic variation is often lacking. However, radiation can be used to induce mutations and thereby generate genetic variation from which desired mutants may be selected. Ionizing radiation has been widely used to generate genetic variability for breeding and genetic studies. Moreover, the technology is simple, relatively cheap to perform and equally usable on a small and large scale (Siddiqui *et al.*, 2008). Mutation means a sudden heritable change in the genetic material at the gene on chromosome level (Chahal and Gosal, 2002). They may be caused by error during cell division or by exposure to the DNA-damaging agents or mutagens in the environment. A wide range of characters which have been improved through induced mutation breeding include plant architecture, yield, flowering and maturity duration, quality and tolerance to biotic and abiotic stresses. In general mutation breeding has been playing a key role in self-pollinated crop with limit variability. Mutation breeding has been reported by many workers, in cowpea (Dhanavel *et al.*, 2008), in black gram (Thilagavathi and Mullainathan, 2009) and soybean (Pavadai *et al.*, 2010) developed and improve plant varieties by mutation breeding. According to IAEA (2015), about 89% of mutant varieties have been developed using physical mutagens such as X-rays, gamma rays, thermal and fast neutrons where as with gamma rays alone accounting for the development of 60% of the mutant varieties. Therefore, the usefulness of a mutagens in 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 (Solanki and Sharma, 1994; Girija and Dhanavel, 2009). Keeping above aspects in mind, the present investigation was undertaken to study the spectrum and frequency of chlorophyll mutation along with effectiveness and efficiency of different doses of gamma rays.

MATERIALS AND METHODS

Site of Experiment

The study was carried out at Agriculture Farm of Palli Siksha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan (23°29′ N latitude and 87°42′ E longitudes and at an altitude of 58.9 m above the mean sea level under sub-humid, sub-tropical, lateritic belt of West Bengal) in pre-*kharif* season of 2015 and 2016.

Plant materials

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

Gamma irradiation

Dry, uniform and healthy seeds of these two genotypes of sesame were irradiated using ^{60}Co (Cobalt 60) gamma source (Gamma Chamber 900) with different doses (250, 300, 350, 400 and 450 Gy) of gamma rays at the Bhabha Atomic Research Centre (BARC), Trombay, India.

Field Experiment

M_1 generation

Irradiated seeds (M_0) along with the controls (un-irradiated) were sown in the field (treatment and variety wise) in a split plot design with three replications in twelve rows plot of 5m length keeping plant to plant and row to row distance of 10 and 30 cm., respectively during pre-*kharif* (pre-monsoon) season of 2015. Data were taken in the field as well as in laboratory on germination (%), pollen sterility (%), seedling height (cm), root-shoot length (cm) and plant lethality (%). Four to five capsules of each M_1 plants against all the treatments were collected separately to rise the M_2 generation.

M_2 generation

Individual plant to progeny rows were grown in M_2 generation (pre-*kharif*, 2016) keeping row to row and plant to plant distance at 30 cm and 15 cm, respectively. Immediately after germination (upto 20 days), various types of chlorophyll mutation (albina, chlorina, xantha) were recorded to study the mutagenic effect of different doses. In the same time normal looking plant population was also counted and recorded dose wise in each genotype to estimate the chlorophyll frequency.

Mutation frequency (Mf)

On M_2 plant basis (% of chlorophyll mutated M_2 plants) mutation frequency was estimated as per cent of segregating M_1 plant progenies (Gaul, 1964).

Mutagenic effectiveness and efficiency

The mutagenic effectiveness and efficiency were calculated by the following formula (Konzak *et al.*, 1965):

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency in M2 (Mf)}}{\text{Dose of mutagen (Gy)}}$$

Where, Gy = Grey

$$\text{Mutagenic Efficiency} = \frac{\text{Mutation frequency in } M_2}{\text{\% Bilogical damage in } M_2}$$

Biological damage indicates % pollen sterility or % plant lethality, sterility and lethality was calculated as percentage of pollen fertility and survivality reduction, respectively.

RESULTS AND DISCUSSION

Effect of gamma irradiation on seed germination, pollen sterility (S) and plant lethality (L) in M_1 generation

Data on seed germination (%), pollen sterility (S) and plant lethality (L) for both genotypes are presented in Table 2. In M_1 generation it was observed that, germination (%) was decreased with the increase of gamma radiation as well as all biological damages like pollen sterility (S) and plant lethality (L) increase with the increase of gamma radiation doses. Noticeable variations were observed in germination percentage after gamma irradiation in germination test. Maximum seed germination (%) was found tobe in Rama (70.2%) and in Tillotoma (70.35%) at 250 Gy. Acceleration of seed germination by low doses of ionizing radiation has been attributed to many factors. Maherchandani (1975) attributed the promotion of *Avena fatua* L. seed germination to the increase in oxygen uptake following irradiation with low doses of gamma rays, which resulted in the production of organic and inorganic peroxy radicals, which led to breaking seed dormancy. Also, the stimulating causes of gamma ray on germination may be certified to the activation of RNA or protein synthesis, which occurred during the early stage of germination after seeds irradiated (Abdel-Hady *et al.*, 2008). On the other hand, the inhibition of seed germination at high doses could be due to the damage in seed tissue, chromosomes and subsequent mitotic retardation and the severity of the damage depend on the doses used (Datta, 2009). Similar results have reported by Cheema and Atta (2003), Harding *et al.* (2012), Talebi and Talebi (2012).

As compared to control, maximum pollen sterility (S) was recorded in Rama (23.35%) and in Tillotoma (28.18%) at 450 Gy, while minimum pollen sterility was recorded in Rama (8.52%) and in Tillotoma (10.00%) at lowest dose 250 Gy. Chromosomal aberrations, changes involving DNA and RNA synthesis, meiotic abnormalities might be the causes of reduction in pollen fertility owing to radiation. Positive and highly significant correlation between chromosomal abnormality and pollen sterility has been reported in mung bean (Ignacimuthu and Babu, 1989) and in chrysanthemum (Kumari *et al.,* 2013). These trends were also observed in case of plant lethality. Maximum plant reduction was observed at 450 Gy in Rama (61.60%) and in Tillotoma (80.15%), while minimum reduction was recorded at dose 250 Gy in Rama (29.85%) and in Tillotoma (47.74%) (Table 2). The results obtained by Kiong *et al.* (2008) indicated that survival of plants to maturity depends on the nature and extent of chromosomal damage. Increasing frequency of chromosomal damage with radiation dose may be responsible for less germinability and reduction in plant growth and survival. These results are in agreement with those obtained by Park *et al.* (2008) on *Hosta*

Table 1: Some basic features of two sesame genotypes

Name of Cultivars	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 to110	3.1

Table 2 : Estimation of pollen sterility (%), plant lethality (%) and spectrum of chlorophyll mutation

Variety	Mutagen Treatment (Gy)	Total no. of M_2 seedling scored	Seed germin -ation (%)	Pollen sterility (%) (S)	Plant lethality (%) (L)	No. of mutant seedling	Mutation frequency (Mf %)	*Albina* (%)	*Chlorina* (%)	*Xantha* (%)
Rama	Control	-	97.85	-	-	-	-	-	-	-
	250	14299	70.2	8.52	29.85	138	0.97	34.78	41.3	23.91
	300	10926	67.33	10.12	35.79	139	1.27	40.29	35.25	24.46
	350	8697	60.53	15.32	48.74	120	1.38	21.67	54.17	24.17
	400	8226	56.27	19.6	57.85	100	1.22	21	52	27
	450	5518	47.88	23.35	61.6	59	1.07	-	66.1	33.9
Tillotoma	Control	-	98.52	-	-	-	-	-	-	-
	250	17645	70.37	10	47.74	153	0.87	32.03	45.75	22.22
	300	12425	62.11	13.18	57.85	152	1.22	34.21	40.13	25.66
	350	8383	55.51	18.59	67.89	116	1.38	17.24	43.97	38.79
	400	8057	45.05	23.78	77.52	105	1.3	30.48	55.24	14.29
	450	7958	38.11	28.18	80.15	74	0.93	13.51	45.95	40.54

Table 3 : Assessment of mutagenic effectiveness and efficiency in two genotypes of sesame (*Sesamum indicum* L.)

Dose (Gy)	Mutagenic effectiveness (Mf x 100/Dose)		Mutagenic efficiency			
	Rama	Tillotoma	Mf x 100/S		Mf x 100/L	
			Rama	Tillotoma	Rama	Tillotoma
250	0.39	0.35	11.33	8.67	3.23	1.82
300	0.42	0.41	12.57	9.28	3.55	2.11
350	0.39	0.4	9.01	7.44	2.83	2.04
400	0.3	0.33	6.2	5.48	2.1	1.68
450	0.24	0.21	4.58	3.3	1.77	1.16

plantaginea, Fardous *et al.* (2013) on *Moluccella laevis* L., Golubinova and Gecheff (2011) on Sudan grass and Ahirwar *et al.* (2014) on Lentil.

Spectrum of chlorophyll mutants and estimation of mutation frequency

Generally the chlorophyll mutants those are the common occurrence have been used as a measure of mutagenic action in the mutation breeding experiments. Mohan Rao (1972) and Rao and Rao (1983) suggested that the estimation of mutation frequency on the basis of M_2 plants gives the best estimate of actual frequency. Chlorophyll mutation was recorded in M_2 seedling basis. Among the treatments, the 400Gy (in Rama 52.00%, in Tillotoma 55.24%) and 450Gy (in Rama 66.10%, in Tillotoma 45.95%) gamma rays produced high frequency of chlorina (Table 2). Next common chlorophyll mutant observed was xantha in both varieties whereas albina mutants were less frequent compare to others. The frequency of chlorophyll mutations varied with the genotype as well as mutagen doses in M_1 generation. Total frequency of chlorophyll mutations was relatively higher in Tillotoma than Rama. The differential response of genotypes to induction of chlorophyll mutations was possible due to differences in the genetic makeup of the varieties used for mutagenesis. During the present study, chlorine occurred in higher frequency than xantha or albina. Several workers also reported a higher frequency of chlorine mutant in irradiated population in rice (Chakravarti *et al.*, 2013), in kodo millet (Cheema and Atta, 2003; Subramanian *et al.*, 2011) and in chickpea (Bara *et al.*, 2017).

Mutagenic effectiveness and efficiency

For any mutation breeding programme, selection of effective and efficient mutagen(s) is very useful to produce high frequency of desirable mutations. The data presented in Table 3 indicated that the effectiveness and efficiency of various doses and the response of genotypes varied. In between two genotypes, it was also noticed that, mutagenic effectiveness decreased with increase instrength of gamma beyond the dose of 300 Gy. This indicates that the genetic background of the material undergoing mutagenic treatment. It was also observed that, the mutagenic efficiency was lowest at higher dose 400 Gy and 450 Gy dose in both variety, because maximum pollen sterility and plant lethality was observed in 450 Gy dose followed by 400 Gy dose which resulted in decrease in the mutagenic efficiency in 450 Gy and 400 Gy doses. Maximum mutagenic efficiency was recorded in 300 Gy dose in Rama (12.57) and in Tillotoma (9.28) followed by 250 Gy dose in Rama (11.33) and in Tillotoma (8.67). Maximum effectiveness was also observed in 300 Gy in Rama (0.42) and in Tillotoma (0.41). Similar observations of general decrease in effectiveness with increasing doses of gamma rays irradiation was reported in finger millet by Muduli and Misra (2007), in mung bean by Solanki and Sharma (1994) and in finger millet by Ambavane *et al.* (2015). The results obtained on the basis of pollen sterility and plant lethality indicated that, for both verities the most efficient mutagen was 300 Gy. The above study revealed that, lower or intermediate dose proved to be more effective and efficient. Greater effectiveness and efficiency at lower or intermediated treatment of physical mutagen has also been reported earlier (Dhanavel *et al.*, 2008; Girija and Dhanavel, 2009; Singh and Singh, 2007; Khan *et al.*, 2005). The main reasons behind that, the effectiveness and efficiency of lower dose is due to the fact that injury, lethality and sterility increase with an increase in the mutagen doses. Moreover, lower or intermediated dose cause relatively less damage enabling the organism to express the induced mutation.

The present study revealed that, in M_1 generation, reduction of seedling survival (lethality) and pollen fertility reduction (sterility) were increased with increasing doses of mutagens. The maximum frequency of chlorophyll mutants were observed at 350Gy for both varieties and chlorina and xantha type mutants were more frequent than albina. 300 Gy followed by 350 and 250 Gy exhibited more effective and efficient than other mutagenic doses for Rama and Tillotoma.

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