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RESEARCH ARTICLE

STUDIES ON CYTOLOGICAL ABNORMALITIES INDUCED BY GAMMA RAYS, EMS AND THEIR COMBINATION IN SESAME (*SESAMUM INDICUM* L.) VAR.TMV3

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Abstract

The present study deals with the cytological abnormalities induced by gamma rays, EMS and their combination in sesame (Sesamum indicum L.) var.TMV3. The seeds of sesame var.TMV3 were treated with different doses of gamma rays (30, 40, 50, 60 and 70KR), various concentrations of EMS (0.6, 0.8, 1.0, 1.2 and 1.4mM) and their combinations (30KR+0.6mM, 40KR+0.8mM, 50KR+1.0mM, 60KR+1.2mM and 70KR+1.4mM). Through root tip squash technique and mitotic studies, various cytological abnormalities induced by different mutagens and treatments as said above were studied in the crop used. The results showed the dose dependent influence in the induction of chromosomal/ cytological abnormalities and it was more pronounced as the doses /concentrations of mutagens increased. The main chromosomal aberrations like stickiness, precocious movements, laggards, bridges and ring chromosomes were observed. Though the abnormalities increased with increasing doses or concentrations of mutagens, the mitotic index was gradually declined with increasing doses or concentrations of mutagens.

Key words: Sesame, Gamma rays, EMS, Chromosomal aberrations, Mitotic index

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Introduction

Sesame (*Sesamum indicum* L.), belonging to the family Pedaliaceae, is the most important oil seed crop in the world. Sesame is regarded as the "Queen of oil seeds" by virtue of its excellent oil quality and it is grown mainly for its seeds and the oil that is extracted from them. Sesame seeds are

rich in proteins and oil and the proportion of oil in the seed is 35-63% (Ashri 1998), (Baydar *et al.*,1999). The total fat content of the sesame seed is 12.5% and this is lower than soya bean, which has 14.5% of fat. Sesame oil is very stable due to the presence of antioxidants like sesamin, sesamolin and sesamol (Suja *et al*.,2004). Recent studies have shown that the sesame oil lowers the cholesterol level and hypertension in human beings (Lemcke-Norojarvi *et al*., 2001) and it reduces the incidence of cancer (Hipasami *et al*.,(2000), (Miyahara *et al*., (2000). Sesame oil is also used for manufacturing of soaps, cosmetics, hair oils, perfumes, insecticides and pharmaceutical products. Apart from edible purposes and others, the oil is also used for anointing the body and as fuel for lamps. The seeds are used for making sweetmeats. The oil cake is used as fodder. The utilization of sesame cake from dehulled seeds as a source of protein for human consumption is an important development.

Mutation is a sudden change in the amount, arrangement or structure of a DNA of an organism. This produces a change in the genotype which may be inherited by cells derived by mitosis or meiosis from the mutant cell. A mutation may result in the change in appearance of characteristics in a populationChanges happening in gamete cells are acquired, though those happening in substantial cells must be acquired by little girl cells delivered by mitosis. The last are known as substantial transformations.

A transformation coming about because of an adjustment in the sum or plan of DNA is known as chromosomal change or chromosomal variation. Increasingly, the term mutation is being used only when describing a change in the structure of a DNA at a single locus and this is known as a gene mutation or point mutation. The concept of mutation as the cause of sudden appearance of a new characteristic was first proposed by the Dutch Botanist Hugo de Veries in 1901, following his work on inheritance in the evening primrose *Oenothera lamarckiana*.

Gamma rays are non particulate ionizing radiations, having high energy and composed of photons. Gamma rays are produced by the decay of some radio isotopes e.g., C^{14}, Co^{60} etc, and are highly penetrating in biological tissues and are sparsely ionizing. The chemical and biological effects of radiations are produced primarily due to ionization. The genetic effects of radiations are resulted from their effect on DNA and these effects include change in a base (e.g., deamination), loss of a base, disruption of hydrogen bonds between complementary strands of DNA, single or double strands breaks in DNA and cross linkage of chromosomal proteins.

Ethyl methane sulphonate (EMS) ($CH_3SO_3C_2H_5$), is a mutagenic, teratogenic and possibly carcinogenic organic compound. It produces irregular transformations in hereditary material by nucleotide replacement; especially by guanine alkylation. This ordinarily creates just point transformations. It can actuate transformations at a pace of 5x10-4 to 5x10-2 for every quality without significant slaughtering. The ethyl gathering of EMS responds with guanine in DNA, framing the anomalous base O-6-ethylguanine. During DNA replication, DNA polymerases that catalyze the cycle every now and again place thymine, rather than cytosine, inverse O-6-ethylguanine. Following ensuing rounds of replication, the first G:C base pair can turn into an A:T pair. This changes in the genetic information, is often harmful to cell and can result in disease.

Materials and methods

Five sets of hundred, well matured and healthy dried seeds of sesame genotype TMV3 were packed in a paper cover and exposed to different doses of gamma rays, such as 30, 40, 50, 60 and 70KR (Kilo Rad) and it was done at “The Sugarcane Breeding Institute (ICAR), Tamilnadu Agricultural University, Coimbatore, Tamilnadu. Another five sets of hundred, well filled healthy seeds were soaked in distilled water for two hours and immediately transferred to freshly prepared different concentrations of EMS, like 0.6, 0.8, 1.0, 1.2 and 1.4mM for four hours with regular shaking. The treated seeds were taken from EMS solution and thoroughly washed in tap water for two to four times. For combined treatments, the seeds were treated with the following ways, i.e., 30KR+0.6mM, 40KR+0.8mM, 50KR+1.0mM,

60KR+1.2mM and 70KR +1.4mM. Untreated seeds were soaked in distilled water for six hours and kept as control. The treated and controlled seeds were sown in plastic cups for germination and from the seedlings the root tips were collected.

Root tip squashes were readied following the strategy for iron alum haematoxylin squash procedure (Marimuthu and Subramaniam, 2000). The iron alum haematoxylin staining method was found to be more satisfactory in counting the number and studying the morphological characters of chromosomes.

The collected root tips were thoroughly washed with water and fixed in 3:1 acetic alcohol for 3 hours or overnight. After fixation, the root tips were thoroughly washed with water and they were hydrolyzed in 0.1N HCL for 10 – 15 minutes at 60°C. The hydrolyzed root tips were again thoroughly washed with water and transferred to 4% iron alum for 3to 5minutes. The root tips were washed with water and transferred to diluted haematoxylin staining and kept for 2-3 hours. After completion of staining, the root tips were washed with water and treated for a few minutes in 45% acetic acid till the root tips became softened. One or two root tips were placed in a few drops of 45% acetic acid on a clean slide and a clean cover slip was placed over it. The root tips were squashed by applying gentle pressure and sealed with Canada balsam and kept for few minutes. The slides were observed under a microscope and photographs were taken. Based on the observations, the mitotic index was calculated using the following formula

$$\text{Mitotic index (\%)} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells studied}} \times 100$$

Results and discussion

The present study revealed that the mitotic divisions and chromosomal behaviours were normal in control root cells, whereas the root cells of treated plants showed high abnormal behaviours of chromosomes and low number of cell division. When increasing the doses or concentrations of mutagenic treatment, the number of dividing cells and mitotic index were gradually decreased when compared to control and the maximum reduction was noted in 70KR+1.4mM of combined treatment. But in case of abnormal cells, chromosomal aberrations were increased with increasing doses or concentrations of mutagens and the highest observation was made at 70KR+1.4mM of combined treatment (Table -1) and such a observations were also reported earlier (Kumar and Srivastava, 2010) in safflower. In the present study, five major types of chromosomal aberrations were noted and they were stickiness, precocious movements, bridges, laggards and ring chromosomes. Among these, the stickiness was more prononced when compared to other chromosomal aberrations.

Wide range of chromosomal aberrations was induced by all the mutagenic treatments, but the higher proportion has been attributed to stickiness of chromosomes, which might have been arisen either due to depolymerization of nucleic corrosive brought about by mutagenic medicines or because of fractional separation of the nucleoproteins and adjustments in their example of association (Kumar et al., 2003). It may also arise due to improper clustering of chromosomes at any phase of cell cycle, which makes the chromatids connected by subchromatid bridges (Mc Gill *et al*.,(1974). The behavior of laggard chromosome is characteristic in that they generally lead to micronuclei formation(Kumar and Ravi 2006). Micronuclei also arise either laggard or non-oriented chromosomes fail to reach the two opposite poles in time to be in main telophase nucleus (Utsunomiya et al (2002). (Shreekrishna, 2006) reported that chromosomal bridges might have arisen through breaks in two chromosomes followed by union of the centric fragments or due to stickiness of chromosome at metaphase and their failure to separate at anaphase or due to breakage and reunion of chromosome (Badr, 1988).(Pagliarini, 1990) reported that the laggard formation might to be late chiasma terminalization.

Conclusion

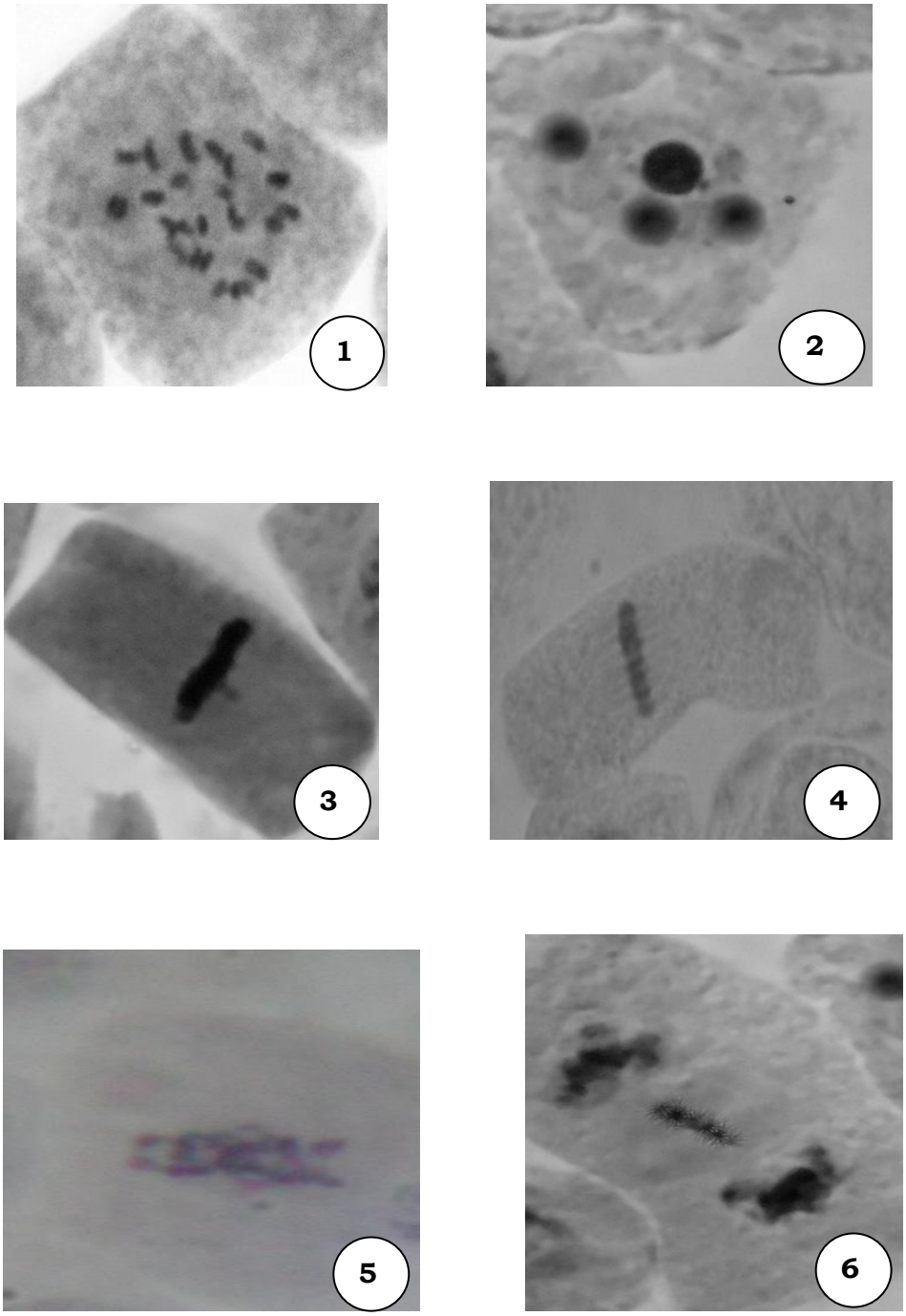
On the basis of the present findings, it can be concluded that the mutagens gamma rays, EMS and their combinations induced chromosomal aberrations in sesame and the combined treatment showed more effective in the induction of chromosomal aberrations in general and stickiness of chromosomes as predominant.

Table - 1

Effect of various doses /concentrations of gamma rays, EMS and their combinations on mitotic indices and abnormalities of chromosomes in root tip cells of sesame var.TMV3

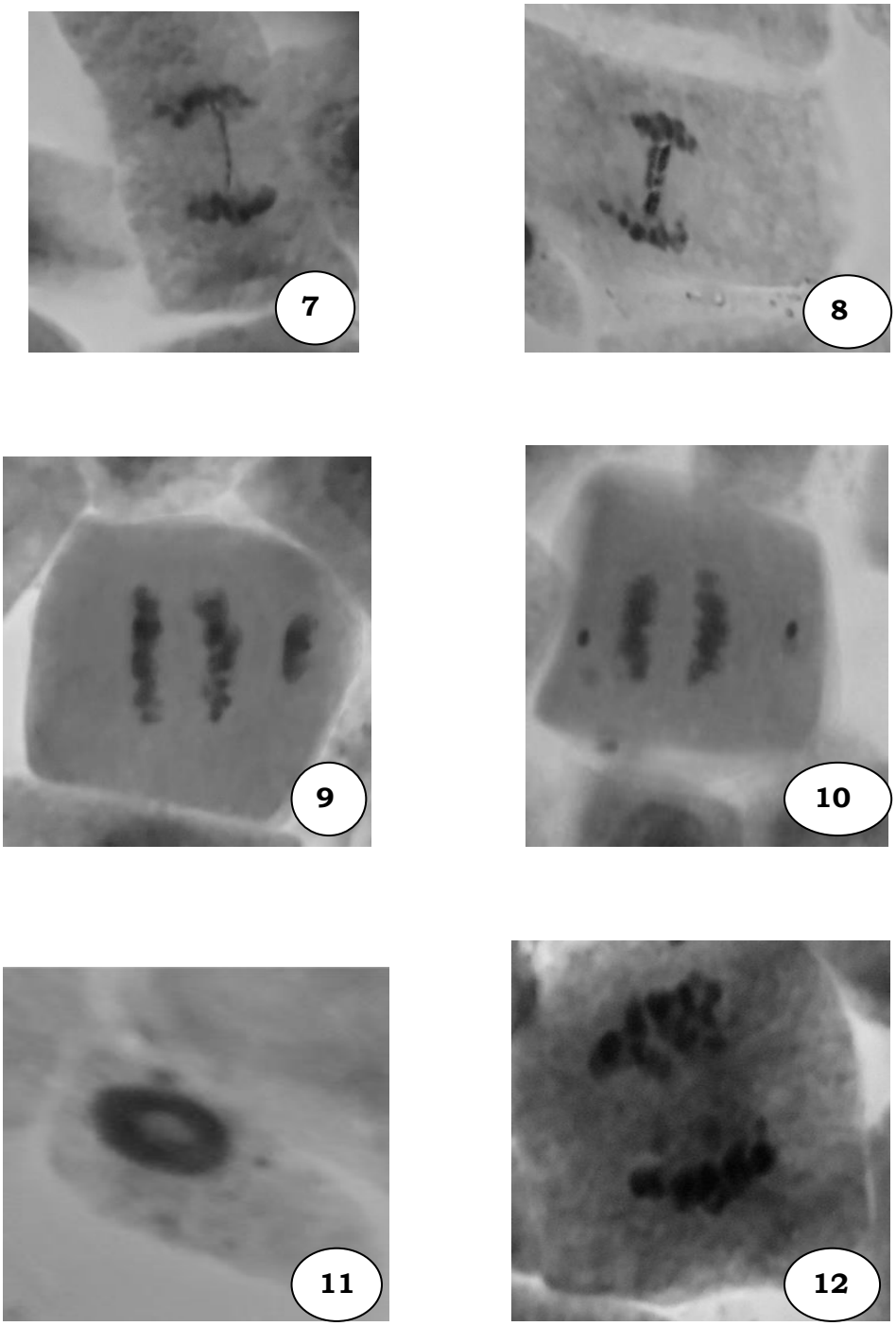
Treatment (Doses / cons.)		No. of cells observed	No. of dividing cells	No. of non dividing cells	Mitotic index	No. of abnormal cells	Chromosomal aberrations in percentage				
							Stickiness	Precocious movement	Laggards	Bridges	Ring Chromosomes
Gamma rays (KR)	**Control**	208	76	132	36.53	-	-	-	-	-	-
	30	205	68	137	33.17	5	2(0.975)	-	1(0.487)	2(0.975)	-
	40	201	63	138	31.34	9	5(2.487)	3(1.492)	1(0.497)	-	-
	50	219	60	159	27.39	11	6(2.739)	3(1.369)	2(0.913)	-	-
	60	211	52	159	24.64	16	7(3.317)	2(0.947)	3(1.421)	3(1.421)	1(0.473)
	70	213	47	166	22.06	19	9(4.225)	2(0.938)	3(1.408)	4(1.877)	1(0.469)
EMS (mM)	**0.6**	217	57	160	26.26	8	3(1.382)	1(0.460)	2(0.921)	2(0.921)	-
	0.8	208	51	157	24.57	11	5(2.403)	2(0.961)	2(0.961)	2(0.961)	-
	1.0	215	46	169	21.39	15	7(3.255)	3(1.395)	3(0.395)	2(0.930)	-
	1.2	204	37	167	18.13	19	9(4.411)	3(1.470)	4(1.960)	2(0.980)	1(0.490)
	1.4	201	24	177	11.94	21	10(4.975)	5(2.487)	3(1.492)	2(0.995)	1(0.497)
Combined (Gamma rays + EMS)	**30+0.6**	203	45	158	22.16	11	6(2.955)	2(0.985)	1(0.492)	2(0.985)	-
	40+0.8	225	41	184	18.22	16	9(4.000)	2(0.888)	3(1.333)	1(0.444)	1(0.444)
	50+1.0	223	39	184	17.48	20	10(4.484)	3(1.345)	4(1.793)	2(0.896)	1(0.448)
	60+1.2	229	32	197	13.97	23	12((5.240)	5(2.183)	3(1.310)	1(0.436)	2(0.873)
	70+1.4	242	27	215	11.15	24	14(5.785)	5(2.066)	2(0.826)	3(1.239)	-

PLATE -1
Chromosomal aberrations



Figs. 1-(Control- metaphase chromosomes), 2-Multi nucleus(50KR of gamma rays), 3- Stickiness of chromosomes (70KR of gamma rays), 4- Stickiness of chromosomes (1.2mM of EMS), 5-Clumping of chromosmes (40KR+0.8mM of combined treatment) and 6-Chromosomal bridge (70KR+1.4mM of combined treatment)

PLATE -2
Chromosomal aberrations



Figs. 7-Chromosomosomal bridges (50KR of gamma rays), 8- Chromosomosomal bridges (1.0mM of EMS), 9-Precocious movement of chromosomes (50KR +1.0mM of combined treatment), 10- Precocious movement of chromosomes (1.2mM of EMS), 11-Ring chromosmes (60KR+1.2mM of combined treatment) and 12-Laggard chromosomomes (50KR+1.0mM of combined treatment)

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