A Comparative Study of 80r and 240r Gamma Rays On Male Meiotic Chromosomes of Grasshopper Spathosternum Praciniferum

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Abstract: Adult healthy males of short horned Indian grasshopper, Spathosternum praciniferum were irradiated with gamma rays with a dose of 80r and 240r while the untreated specimens served as control. The testes material of treated and untreated specimens fixed at different time intervals were subjected to squash-flame-drying-iron-alum-haematoxylin technique. The control specimens showed very negligible frequency of aberrations in some meiotic stages while the treated specimens except only the spermatogonial metaphase, the Diplotene, Diakinesis, Metaphase – I, Anaphase –I, Metaphase –II, and Anaphase – II, yielded various types of aberrations. The comparative analysis of the data showed that maximum effect was at 16 hrs. i.e. somewhat time – dependent and the marker X-chromosome was more sensitive than autosomes and that Treatment – 2 (240r) showed more aberrations (18.74%) than Treatment – 1(80r) (16.20%).

Key Words: Grasshopper, chromosome aberrations, gamma rays.

I. Introduction

The profound genotoxic effect of ionizing radiations on living tissues had been demonstrated first by Muller (1927) in Drosophila melanogaster. This paved the path to succeeding workers towards cytogenetic study of radiation induced chromosomal aberration in insects, as Carlson (1938, 1940, 1941a, 1941b, 1949), Carlson & Harrington (1955), Ray-Choudhuri (1961), Manna and Majumdar (1962,1967,1968), Majumdar and Manna (1967), Kumaraswammy & Rajasekarasetty (1975, 1976), Rajasekarasetty & Kumarwaswamy (1979), Sur and Manna (1987), Sur (1998), Sur and Pandey (2004). Very recently, Song et al. (2008) mapped the chromosomal locations of two single-copy genes, Ser-1 and CI-13, in silkworm (Bombyx mori) by fluorescence in situ hybridization. Even, Dutrillauxet al. (2006) had reported a method for obtaining chromosome banding, from pachytene stage of the meiotic prophase in Coleopteran species, which will be of help in further cytogenetic studies by other workers.

In our previous studies **Sur, Das, Thakur and Roy (2010)**, we assessed the effect of γ -rays in two species of grasshoppers, Oxya velox and Gesonula punctifrons, with a dose of 160r **Sur; Das & Das (2011)** also studied the effect of X-ray (80r) on Oxya velox & G. punctifrons. The present study is a similar investigation, but with a dose of 80r and 240r on a single grasshopper species S. praciniferum. The effect was studied for four different time intervals, i.e. 0.25 hr (15 min), 1 hr, 16 hr and 48 hr. Comparison with untreated species which served as control had also been evaluated here in.

II. Materials And Method

Indian species grasshoppers Spathosternum praciniferum were exposed to whole body γ -irradiation (80r) and (240r). Only healthy, adult males were considered in the study. After irradiation, the testes lobes of the species were dissected out in normal saline and tissues fixed in freshly prepared aceto-alcohol (acetic acid : alcohol – 1:3 (v/v). The fixed testes were squashed in 45% acetic acid on albuminized slides, which were later kept overnight at 50% alcohol. The cover slips detached automatically and the slides were stained with iron-alum-hematoxylin. The slides were then studied under microscope. Data were scored by using 45 cells from spermatogonial metaphase, 450 cells each from diplotene, diakinesis, metaphase I, and 90 cells each from anaphase I, metaphase II and anaphase II stages (Table 3). A total of 136 individuals of species 1 (Table 1) and 135 individuals of species 2 (Table 2), were incorporated in the whole study, while the same number of individuals served as control. Various standard statistical tools as Standard Error (S.E.); Critical Difference (C.D.) at 5% and 1% levels; students' t-Test; chi-square (χ 2) analysis; Correlation coefficient (r-value); Analysis of Variance (ANOVA) or F-value of both Treatment 1 and Treatment 2; (Table 4) were used to analyze the data.

III. Results

Spathosternum praciniferum (80r)

Control Series: A total of 6660 cells were studied for this control series. This series showed minimum frequency of aberrations, highest being 1.11% in Metaphase II and lowest being 0.00% for both Spermatogonial Metaphase and Anaphase II. The total percentage of aberrations for this series was 0.08% (Table 3).

Treatment1 Series:Similarly, 6660 cells were studied in this series. Some aberrations observed in this series are 19.33% in autosomes, 4.66% in sex chromosomes of Diplotene stage at 16 hr., 11.56% in autosomes and 5 in sex chromosomes of Metaphase I stage at 16 hr., 19.11% in autosomes and 1.11% in sex chromosomes of Metaphase I at 48 hr., etc. In all, there were 877 aberrations involving autosomes and 204 involving X chromosomes (Table 3) in all the meiotic stages studied. The total frequency of aberrations in this series is 16.23% (Table 3). Among all the meiotic stages scored, the highest aberration was obtained in Diplotene stage of Prophase I i.e. 22.06% followed by 19.72% of Anaphase I, 19.17% of Anaphase II, 16.94% of Metaphase II, 14.61% of Diakinesis of Prophase I, 11.67% of Spermatogonial Metaphase and 11.06% of Metaphase I (Table 3, Fig 1, Fig 3: PM 1-4). With respect to time, the frequency of aberration was 2.28% at 0.25 hr (15 min), 7.09% at 1 hr, 30.57% at 16 hr and 24.98% at 48 hr. Therefore the aberration increased from 0.25 hr to 16 hr, was highest at 16thhr and decreased thereafter (Table 3, Fig 2). Moreover, the X chromosome was found to be more affected than the autosomes.

Statistical analysis:

Chi square value, T value etc. are given in table 4a. The ANOVA (F value) is 9.56* for this treatment species, which were significant at 5% level (Table 4a).

Spathosternum praciniferum (240r)

Control Series:

In the same way, 6660 cells were studied in this series. Negligible aberrations were encountered here too. The maximum aberration observed in this series was 0.16% in Metaphase 1 and minimum was 0.00% for Spermatogonial Metaphase, Anaphase I and Anaphase II. Total percentage of aberrations was 0.12% (Table 3).

Treated Series:

Again, a total of 6660 meiotic cells were studied in this series. Some aberrations observed in this series are 11.33% in autosomes, 1.56% in sex chromosomes of Diakinesis stage at 16 hr., 15.33% in autosomes and 2.88% in sex chromosomes of Diplotene stage at 48 hr., 20.67% in autosomes and 6% in sex chromosomes of Diplotene at 16 hr., etc. Here 1026 aberrations were observed with autosomes and 226 aberrations observed with X chromosomes (Table 3). Total aberration percentage was 18.80%. Among all the meiotic stages recorded, the maximum frequency of aberration was 26.06%, observed with Diplotene stage of Prophase I, followed by Anaphase I (23.06%), Anaphase II (22.78%), Metaphase II (21.67%), Diakinesis of Prophase I (15.44%), Metaphase I (13.17%) and Spermatogonial Metaphase (11.67%) (Table 3, Fig 1, Fig 4: PM 5-8).

Moreover, frequency of aberrations varied with respect to time. The maximum aberration was recorded at 16 hr (35.14%) post exposure. The other frequencies were 4.56% at 0.25 hr, 9.67% at 1 hr and 25.83% at 48 hr. Therefore the aberrations increased from 0.25 hr to 16 hr, were highest at 16thhr and decreased thereafter (Table 3, Fig 2). Further the X chromosome was found to be more affected than the autosomes.

Statistical analysis:

The Chi square value. Tvalue are all highly significant and are given in table 4 b . The ANOVA (F value) for this species are 11.38* and 9.56* respectively, which were significant at 5% level.

 Table 1: Number of male Spthosternum praciniferum (Treatment- 1) exposed to γ-rays (80r) and their testes fixed at different time intervals

Fixation Time	Number of males irradiated	Number died	Number survived
0.25 hr (15 min)	35	2	33
1 hr	38	1	37
16 hr	31	2	29
48 hr	32	6	26

Table 2: Number of male Spthosternum praciniferum (Treatment-2) exposed to γ-rays (240r) and their testes fixed at different time intervals

Fixation Time	Number of males irradiated	Number died	Number survived
0.25 hr (15 min)	34	1	33
1 hr	36	3	33
16 hr	35	4	31
48 hr	30	7	23

Fixation Time		SP. MTH	þ			PROPH	ASE -I			META	PHASE	-I	A	NAPHAS	E - I	ME	TAPHAS	E - II	AN	APHASE	- II
_	1	2011 St. 41. 20 . 10 . 10		DIPLO	DTENE		DIAKI	NESIS		50 V00 14 M			100000		water w	50 00000			-034-30		204702
	NC	BR	OT	NC	BR	OT	NC	BR	OT	NC	BR	OT	NC	BR	OT	NC	BR	OT	NC	BR	OT
CON-1	45	0(0)	0(0)	450	0(0)	0(0)	450	0(0)	0(0)	450	0(0)	0(0)	90	1(0)	0(0)	90	1(0)	0(0)	90	0(0)	0(0)
TRT-1	45	1(0)	0(0)	450	4(1)	1(0)	450	1(1)	2(0)	450	3(1)	7(3)	90	3(01)	2(1)	90	1(0)	1(1)	90	1(1)	1(0)
0.25 Hr		5 038W	5 - CEVER	5	to statt Si			50 89	56 20005			00000	10 - 10 20 - 20	00-00			1080			80 80 85	- 55 (6
CON-2	45	0(0)	0(0)	450	1(0)	0(0)	450	0(0)	0(0)	450	1(0)	0(0)	90	0(0)	0(0)	90	1(0)	0(0)	90	0(0)	0(0)
TRT-2	45	2(0)	0(0)	450	7(2)	3(1)	450	1(0)	5(1)	450	6(1)	8(2)	90	7(2)	9(3)	90	2(1)	2(0)	90	4(1)	5(1)
CON-1	45	0(0)	0(0)	450	1(0)	0(0)	450	2(0)	1(0)	4 50	5(2)	1(0)	90	0(0)	0(0)	90	1(0)	0(0)	90	0(0)	0(0)
TRT-1	45	5(0)	0(0)	450	15(4)	5(1)	450	9(2)	1(1)	450	8(2)	11(3)	90	9(2)	11(3)	90	3(2)	7(1)	90	3(2)	5(3)
1 Hr				2. 2	2) 20			3) — — — — — — — — — — — — — — — — — — —	2				3					8		2)	-3
CON-2	45	0(0)	0(0)	450	0(0)	0(0)	450	0(0)	0(0)	450	0(0)	0(0)	90	0(0)	0(0)	90	0(0)	0(0)	90	0(0)	0(0)
TRT-2	45	4(0)	1(0)	450	22(7)	9(2)	450	10(1)	15(3)	450	17(3)	8(2)	90	2(4)	5(2)	90	4(1)	8(5)	90	9(6)	8(3)
CON-1	45	0(0)	0(0)	450	0(0)	0(0)	450	0(0)	0(0)	450	1(0)	0(0)	90	0(0)	0(0)	90	1(0)	0(0)	90	0(0)	0(0)
TRT-1	45	4(1)	2(0)	450	87(21)	93(25)	450	42(4)	51(13)	450	52(5)	18(3)	90	7(2)	9(5)	90	10(5)	9(5)	90	12(6)	10(6)
16 Hr									_		-	_					-				
CON-2	45	0(0)	0(0)	450	1(0)	0(0)	450	1(0)	0(0)	450	1(0)	0(0)	90	0(0)	0(0)	90	1(0)	0(0)	90	0(0)	0(0)
TRT-2	45	7(0)	1(0)	450	93(27)	103(29)	450	51(7)	69(7)	450	68(3)	22(5)	90	15(4)	10(3)	90	22(2)	5(7)	90	8(4)	5(4)
CON-1	45	0(0)	0(0)	450	1(0)	0(0)	450	1(0)	0(0)	450	1(0)	0(0)	90	0(0)	0(0)	90	1(0)	0(0)	90	0(0)	0(0)
TRT-1	45	7(0)	1(0)	450	61(9)	51(19)	450	79(6)	43(8)	450	59(5)	15(4)	90	6 (2)	5(3)	90	4(2)	5(3)	90	8(3)	6(2)
48 Hr							_				8 - 12 8 - 16		13								22 - 59 10 - 40
CON-2	45	0(0)	0(0)	450	0(0)	0(0)	450	0(0)	0(0)	450	1(0)	0(0)	90	0(0)	0(0)	90	0(0)	0(0)	90	0(0)	0(0)
TRT-2	45	5(0)	1(0)	450	69(13)	65(17)	450	39(0)	58(11)	450	69(7)	13(03)	90	3(3)	7(4)	90	5(1)	10(3)	90	9(4)	7(4)
			-		<i>11</i>				17				- 22						1	7	-

 Table 3: A comparative study of the frequency of aberrations in autosomes and sex chromosomes (in parentheses) induced by Gamma –ray dose of 80r(Trt-1) and 240r (Trt-2) in different meiotic stages of control and treated male grasshopper, Spathosternum praciniferum.

Grand Total Control 1 = 21/6660 = 0.31%Grand Total Treatment 1 = 1079/6660 = 16.2%Grand Total Control 2 = 8/6660 = 0.12%Grand Total Treatment 2 = 1248/6660 = 18.73%N.B.: Hr. = Hours, NC = No. of cells, BR = Break , OT = Other types, CON = Control, TRT = Treatment

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Aberration	SP. MTP	DIPLOTENE	DIAKINESI	METAPHA	ANAPHASE I	METAPHASE	ANAPHASE	POOLE
Statistics			S	SE I		II	II	D
Control	1.788	1.643	1.816	3.271	1.732	1.342	1.789	4.321
S.E +/-								
Treatment -1	1.821	24.981	16.124	9.142	2.394	2.61	3.18	26.421
S.E. +/-								
Treatment -1	1.853	97.924	63.205	35.836	9.384	10.245	12.446	81.325
C.D. 5%								
Treatment-1	2.304	128.693	83.065	47.096	12.333	13.47	16.36	96.568
C.D. 1%								
Treatment-1	3.506	195.851**	126.412**	71.673**	18.769**	20.491**	24.893**	85.269*
t – values								*
Treatment-1	8.561	69.451**	54.124**	37.376**	13.125*	16.392**	18.933**	59.587*
Chi-Square								*
values								
Treatment -1	0.32	0.64*	0.84**	0.79**	0.54*	0.65*	0.71**	0.91**
r- values								

Overall Treatment – 1F- Value = 9.56**

Table – 4(b Statistical analysis of data of Spathosternum praciniferum treated with 240r gamma ray (Treat-2))

Aberration	SP. MTP	DIPLOTENE	DIAKINESIS	METAPHAS	ANAPHASE I	METAPHAS	ANAPHASE	POOLE
Statistics				ΕI		ΕII	Π	D
Control	1.447	1.411	1.433	2.379	1.047	1.456	1.647	2.031
S.E +/-								
Treatment -2	1.786	27.226	15.115	10.938	2.577	3.259	2.341	16.324
S.E. +/-								
Treatment -2	1.753	106.723	59.249	42.876	10.938	12.751	9.117	52.364
C.D. 5%								
Treatment-2	2.819	140.257	77.866	56.348	13.278	16.758	12.061	69.527
C.D. 1%								

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A	Com	parative	Study	Of a	80r A	And	240r	Gamma	Ravs	On	Male	Meiotic	Ch	romosomes	0f	Grassho	nn	ver
	Comp		Sinay	$\mathcal{O}_{\mathcal{J}}$	001 1	mu	2101	Gunnina	Intrys	$\mathcal{O}\mathcal{H}$	munu	meione	$\mathcal{O}_{\mathcal{H}}$	omosomes	$\mathcal{O}_{\mathcal{J}}$	Orabbilo	PP	C.

Treatment-2	4.383*	213.449**	118.499**	85.753**	20.208**	25.503**	18.355**	92.358*
t – values								*
Treatment-2	11.361*	112.682**	43.752**	39.981**	14.562**	15.369**	12.381*	71.892*
Chi-Square								*
values								
Treatment -2	0.43	0.67**	0.87**	0.81**	0.62**	0.71**	0.80**	0.93**
r- values								

Overall Treatment – 2F- Value = 11.38**



Sp metaphase = spermatogonial metaphase, Treatment 1= Spathosternum praciniferum (Treated with 80r), Treatment 2= Spathosternum praciniferum (Treated with 240r).

Fig. 1: Bar diagram showing a comparative study between Treatments I (80r) and Treatment II (240r) against γray dose w.r.t. of different meiotic stages.



Treatment 1= Spathosternum praciniferum Treated with gamma (80r), Treatment 2= Spathosternum praciniferum Treated with gamma rays with (240r)

Fig. 2: Line-Diagram depicting chromosomal aberrations in Spathosternum praciniferum induced by γ-ray (80r) and (240r) w.r.t. time

IV. Discussion

In series of experiments with grasshoppers by different workers, it was reported that the X chromosome was more affected to γ -irradiations than the autosomes (Manna and Mazumdar, 1962, 1967; Sur & Manna 1987; Sur 1998, Sur and Pandey 2004, Sur, Das, Thakur and Roy 2010). Both spontaneous (Giles 1940) as well as γ -irradiated (Sax and Mather 1939) aberrations occur more frequently near the centromere than in the distal parts of the chromosome arms. Recently, experiments of Yoshimura et al. (2006) reported the results of zoo-blot hybridization of the genomic DNA from four species of grasshopper, Oxya hyla intricata, Oxya japonica japonica, Oxya chinensis formosana and Oxya. yezoensis. The results suggest that the two satellite DNA families found in their studies are species specific for O. hyla intricata. This result may also be helpful in localizing hot spots in the genome of Oxya against radiations. Also, Zainullin (1997) described the genotoxic effect of low intensity nuclear radiations in Drosophila melanogaster after the Chernobyl nuclear disaster in 1986 at Ukraine.

In our previous study, (Suret al 2010), reported that the most affected time interval 16 hr (26.61%) in case of Oxya velox and(32.91%) in case of Gesonula punctifrons).Sur et al. (2012 a & b) 2012, (2015 a& b) studied effects of different doses of gamma rays & X-rays on fish, Oreochromis mossambicus, mice Mus musculus & house musk shrew Suncus murinus. Sur (2012) had also reported some radio-protective phytomolecules extracted from Barlaria lupulina and subsequently patented with Govt. of India (2012). In the present study, similar results were obtained i.e. most affected time interval was 16 hr (30.40% in case of Treatment II (240r)(Table 3, Fig 2)). Moreover, previously, the most

affected meiotic stage was Anaphase I (19.72%) for Oxya velox and Metaphase II (24.72%) for Gesonula punctifrons, where as in this study the most affected stage was Diplotene for both the species (11.23% for Treatment I and 13.34% for Treatment II(Table 3, Fig 1)). Further we reported previously that Gesonula punctifrons was more sensitive than Oxya velox and the X chromosome was more affected to γ -irradiations than the autosomes . In the present study , in treatment 1 (80r) series aberration percent was 16.2 and in the treatment 2 series (240r) aberration percent was 18.73 and the sex chromosome was more sensitive than the autosomes.

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A Comparative Study Of 80r And 240r Gamma Rays On Male Meiotic Chromosomes Of Grasshopper



Photomicrographs (PM) showing different types of chromosomal aberrations in Spathosternum praciniferum in 80r gamma ray.



Photomicrographs (PM) showing different types of chromosomal aberrations in Spathosternum praciniferum in 240 r gamma ray.