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EFFECT OF GAMMA-RAYS, EMS AND dES ON CHIASMA FREQUENCY AND CHROMOSOME ASSOCIATIONS IN *OCIMUM BASILICUM* L.

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The effect of physical (gamma-rays) and chemical (EMS and dES) mutagens on chiasma frequency and chromosome associations were studied (months of September and November) in the M_1 plants of *Ocimum basilicum* L. (family : Lamiaceae; 2n = 72, n = 36, x = 12) raised from the treated seeds in comparison to control. The mutagens have induced differential responses in relation to chiasma formation as the mean frequency of chiasmata per nucleus and per bivalent have either decreased or increased significantly in most treatments than control; however, the response was not dose dependent. Diethyl sulphate has induced mostly relatively higher frequency of chiasma per cell than EMS when compared on equimolar concentration. Ring bivalent per cell enhanced significantly in treatments than control and the increase was mostly not found to be related with an increase in chiasmata per nucleus. Univalent frequency per cell was higher in mutagenic treatments than control and was not correlated significantly with anaphase I abnormalities, and their random distribution within meiocytes have possibly enhanced the frequency of cells having more than 18 group classes. Pollen fertility was found to increase as well as decrease in treatments than control and the variation in fertility has been attributed to recombinational changes induced through mutagen treatments.

Keywords : Chiasma; Chromosome associations; Mutagenic treatments; *Ocimum basilicum*; Pollen fertility.

Introduction

Ocimum basilicum L. (Sweet basil; family : Lamiaceae) is an aromatic herb yielding essential oil of commerce[1] and the oil extracted from leaves, inflorescences and entire plant through steam distillation possesses anticancerous[2] and antioxidant[3] properties. Present authors have initiated a research programme for raising 'plant type' mutation with high essential oil yield in *O. basilicum* and have been exploring the genetic and cytogenetic consequences resulting through induced mutagenesis. This paper is a part of it and describes the effects of gamma irradiations, ethyl methane sulfonate (EMS) and diethyl sulphate (dES) on chiasma frequency and chromosome associations from male meiosis. Further, interrelationship between pairing behaviour and fertility has also been ascertained in the species.

Materials and Methods

Control (moisture content – 8.33%; seed stock obtained from Medicinal Plant Garden, Narendrapur, West Bengal) and mutagen treated (gamma-rays – 5 kR, 10 kR, 20 kR and 40 kR doses given from ^{60}Co source; EMS : 0.25%, 0.50% and 1.00% doses for 3 and 6h durations, concentrations of EMS prepared in 0.2M phosphate buffer at pH 6.8; dES : 0.25%, 0.50% and 1.00% for 3 and 6h durations, aqueous solution used) seeds were sown in the Experimental plots of Kalyani University (50 seeds from each lot including control) to raise M_1 plant population.

For meiotic analysis suitable sized inflorescences (3 to 5 randomly selected plants from each treatment were assessed) were fixed in Carnoy's fluid (4 PM to 5 PM) and preserved in 70% alcohol. PMCs and pollen grains were stained in 2% propionocarmine solution. Fully stained pollen grains were considered fertile. Photomicrographs were taken from temporary squash preparations. The M_1 plants (including control) were cytologically assessed during the months of September to November and the data obtained have been statistically analyzed.

Results and Discussion

Results indicated that in comparison to control mean frequency of chiasmata per cell and per bivalent at diplotene have either increased or decreased significantly (excepting 10 kR gamma-rays, 1.0%,3h EMS and 0.5% and 1.0% 6h dES); however, the response was not dose dependent (Table 1). The mutagens therefore have induced differential responses in relation to chiasma formation and the potency of the mutagens assessed over control seems to be more or less similar; although, dES have induced relatively higher frequency of chiasmata than EMS compared on equimolar concentration (excepting 0.5%, 6h). Significant increase in chiasmata per cell over control following mutagenic treatments has been attributed either due to failure of terminalization or increase in crossing over[4]. On the contrary, reduction in chiasma may be the consequence of delay in DNA synthesis thereby resulting in asynchronization in

Table 1. Meiotic chromosome configurations and chiasma frequency in control and treated plant types of *O. basilicum*.

Treatments		No. of cells scored at diplotene	Configurations / cell								No. of chiasma per cell	Mean chiasma per bivalent
			Ring				Rod					
			Range	Mean	S.E.	C.V.	Range	Mean	S.E.	C.V.		
0		38	1-4	2.74	0.23	37.15	28-34	31.95	0.43	5.88	37.42±0.45	1.04
Gamma-rays	5kR	60	0-13	4.47	0.50	61.88	20-32	26.00	0.63	13.32	34.93±0.98	0.97
	10kR	28	1-7	4.21	0.50	44.16	22-33	29.29	0.77	9.78	37.71±0.56	1.05
	20kR	52	4-9	5.31	0.27	25.46	25-32	28.81	0.31	5.45	39.42±0.48	1.10
	40kR	36	2-9	4.78	0.43	37.91	26-34	29.33	0.47	6.82	38.89±0.54	1.08
EMS	0.25%,3h	32	2-6	3.50	0.50	40.40	26-32	29.25	0.75	7.20	36.38±0.79	1.01
	0.25%,6h	24	3-5	4.00	0.47	20.40	23-30	26.67	1.66	10.75	34.67±1.36	0.96
	0.50%,3h	34	0-8	4.41	0.46	43.36	23-32	27.88	0.62	9.11	36.76±0.83	1.02
	0.50%,6h	20	3-10	5.30	0.66	39.62	25-31	28.30	0.58	6.53	38.90±1.07	1.08
	1.00%,3h	32	0-7	3.38	0.42	50.10	25-34	30.25	0.59	7.80	37.00±0.73	1.03
	1.00%,6h	24	2-4	3.00	0.35	23.57	27-32	30.00	0.94	6.24	36.25±0.74	1.01
dES	0.25%,3h	28	2-5	4.07	0.24	21.72	26-34	30.00	0.57	7.13	38.14±0.45	1.06
	0.25%,6h	24	2-3	2.33	0.27	20.21	31-32	31.33	0.27	1.50	36.00±0.47	1.00
	0.50%,3h	20	4-8	6.00	1.41	33.33	24-29	26.50	1.77	9.43	38.50±1.06	1.07
	0.50%,6h	32	1-5	3.38	0.47	38.96	29-34	30.63	0.53	4.88	37.38±0.73	1.04
	1.00%,3h	20	2-7	4.00	0.42	33.55	27-33	30.20	0.53	5.50	38.20±0.85	1.06
	1.00%,6h	24	3-5	4.50	0.43	19.24	26-30	28.50	0.83	5.82	37.50±0.83	1.04
CD at 5% level				0.45				0.45			0.58	0.02

Table 2. Meiotic chromosome configurations and pollen fertility in control and treated plant types of *O. basilicum*.

Treatments		No. of MI cells analyzed	Frequency / cell		AI configuration			Pollens	
			I	II	No. of AI cells assessed	Frequency of 36/36 separation (%)	Abnormality (%)	No. of total pollens observed	Pollen fertility (%)
0		304	1.76	35.12	404	86.14	13.86	1966	46.85
Gamma-rays	5kR	31	8.00	32.00	36	61.11	38.89	2207	34.84
	10kR	89	4.61	33.70	67	73.13	26.87	802	35.66
	20kR	69	1.54	35.23	169	82.84	17.16	296	77.03
	40kR	49	2.04	34.98	56	91.07	8.93	198	65.57
EMS	0.25%,3h	22	5.27	33.36	44	81.82	18.18	363	35.26
	0.25%,6h	15	7.20	32.40	51	90.20	9.80	208	28.37
	0.50%,3h	19	3.68	34.16	42	83.33	16.67	323	37.77
	0.50%,6h	18	4.00	34.00	58	82.76	17.24	397	35.26
	1.00%,3h	25	5.60	33.20	50	80.00	20.00	254	45.28
	1.00%,6h	30	3.00	34.50	39	84.62	15.38	223	47.09
dES	0.25%,3h	73	2.60	34.70	42	88.10	11.90	809	46.35
	0.25%,6h	73	2.66	34.67	32	68.75	31.25	441	58.96
	0.50%,3h	34	3.06	34.47	46	91.30	8.70	403	41.44
	0.50%,6h	16	2.00	35.00	36	69.44	30.56	246	30.49
	1.00%,3h	27	5.33	33.33	54	44.44	55.56	285	63.86
	1.00%,6h	15	5.33	33.33	42	95.24	4.76	650	36.77
p value of χ^2-test heterogeneity			<0.001	<0.001					

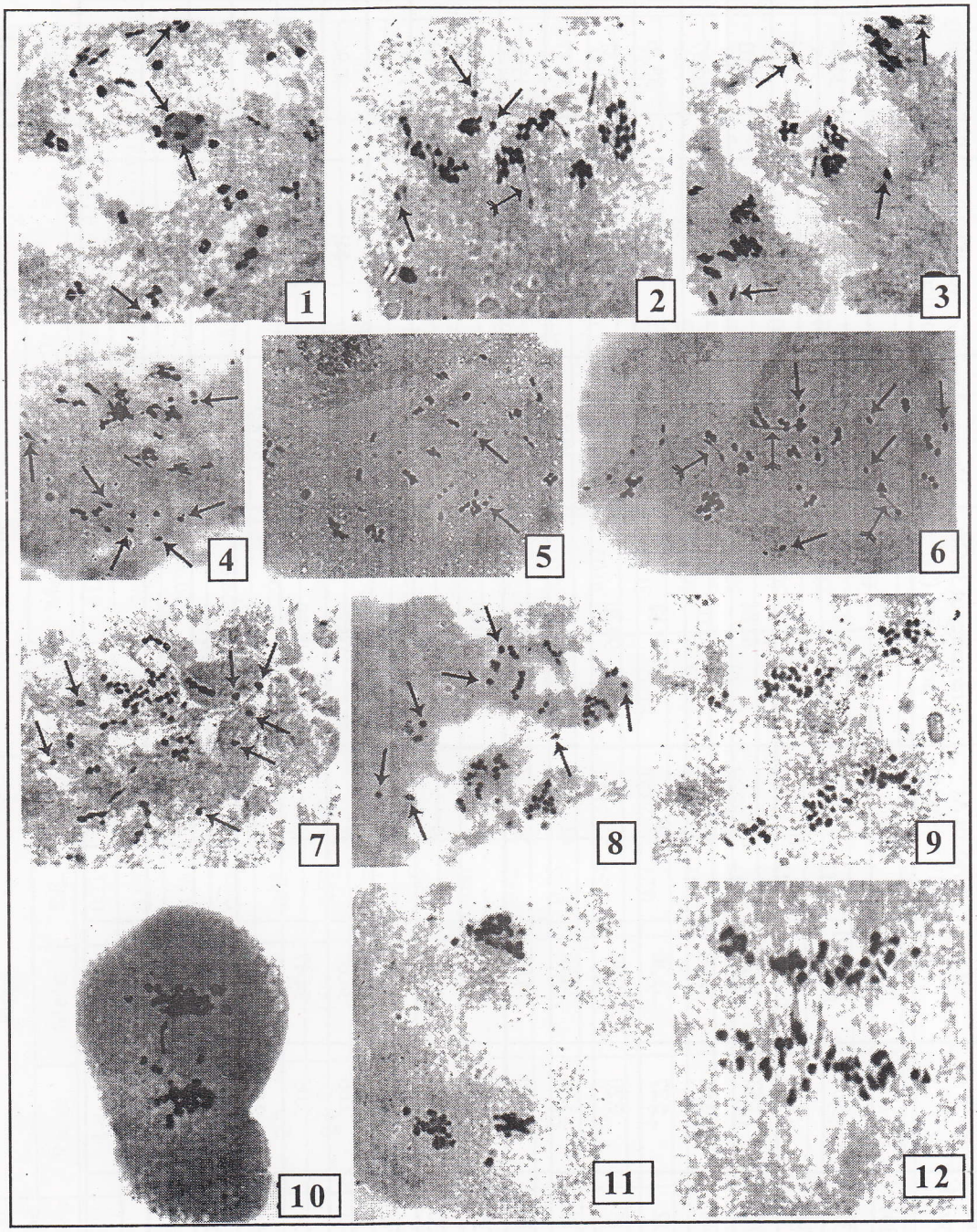

Fig. 1-12. Meiosis (2n = 72) in control (1) and in mutagen treated **(2-12)** materials of *Ocimum basilicum (***1**-diplotene, **2-8** metaphase I and **9-12** anaphase I plates). **1.** 34II + 41 (→); **2-8.** Bivalents and univalents (→) are associated in secondary groups (**2-3** : groups <18; **4-8** : group >18, cells with higher number of univalents). Differential condensation (↠) of bivalents (**2** and **6**); **9** Equal (36/36) separation of chromosomes; **10**. Laggards; **11**. Tripolar organization of chromosomes; **12.** Double bridge formation.

Table 3. Secondary chromosome groups in control and treated materials of *O. basilicum*.

Treatments	No. of PMCs assessed	PMCs with secondary groups (%)	Chromosomes in groups (%)						
			3	6	9	12	15	18	>18
Control	304	94.74	3.13	20.83	12.50	43.75	8.33	9.38	2.08
Gamma-rays	238	77.73	22.16	29.19	16.22	18.92	4.32	2.16	7.03
EMS	129	76.74	3.03	23.23	17.17	32.32	7.07	6.06	11.11
dES	238	78.15	6.45	11.83	9.68	36.02	15.59	4.30	16.13

nuclear processes or affecting coiling patterns of chromosomes[5,6]. However, Stern and Hotta[7] were of opinion that the effect of the mutagens may be through certain proteins which have specific role in chiasma formation.

Significantly higher frequency of ring bivalents per cell have been observed in treatments (excepting 1.0%, 6h EMS and 0.25%, 6h dES) than control (Table 1) and therefore expected to increase the number of chiasmata per cell, which however was not evidenced in most treatments possibly due to lack of pairing in other homologues subsequently resulting in univalent formation. As compared to control (2n = 72, Fig.1), univalent frequency per cell at metaphase I enhanced in mutagenic treatments (excepting 20 kR gamma-rays) thereby reducing bivalent frequency per cell (Table 2). Distribution of univalents and bivalents among treatments was non random as evidenced from χ^2-test of heterogeneity (p value < 0.001). Occurrence of higher frequency of univalents in treatments than control and their random distribution within meiocytes possibly enhanced the number of cells having more than 18 secondary group classes (Table 3). Control plants showed persistent occurrence (94.74%) of secondary chromosome associations in their meiocytes[8]. Mutagenic treatments (data pooled over the doses) have reduced the frequency of cells with secondary association of chromosomes. Preponderance of 12 group class among meiocytes has been noted in control and in chemical mutagen treatments; however, gamma irradiated samples showed 6 group class as the predominant one (Table 3).

Meiocytes of control plants had laggards (1-4) only; while, treated materials formed tripolar groups of chromosomes, laggards (1-5), unequal segregating groups (35/37, 34/38 and 33/39) and bridges (Figs.10-12) at anaphase I. Correlation analysis made between univalent frequency per cell and anaphase I abnormalities revealed non-significant (r = 0.27 at 16 DF) relationship between the attributes, thereby indicating that major proportion of AI abnormalities was not the outcome of pairing disturbances. However, all cytologically balanced cells (Fig. 9) were surely not genetically balanced and definitely included recombinational changes induced through mutagenic treatments as was reflected from non-significant negative correlation (r = - 0.17 at 16 DF) with pollen fertility. As compared to control, pollen fertility either reduced or enhanced in treatments (Table 2). Low pollen fertility in control plants also indicated the possible influence of environmental factor(s) associated to it.

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