J. Phytol. Res. 18(2): 183-185, 2005

EFFECT OF GAMMA – RAYS AND EMS ON MEIOTIC CHROMOSOME BEHAVIOUR OF *WITHANIA SOMNIFERA* (L.) DUN.

MOHSINA IQBAL and ANIMESH K. DATTA*
Department of Botany, Genetics and Plant Breeding Section, Kalyani University, Kalyani – 741235, India.
*e-mail : dattaanimesh@gmail.com

Meiotic chromosome behaviour (bivalent configurations, chaismata per cell, metaphase I chromosome associations and anaphase I cells) and pollen fertility were assessed (during the months of September to November) from M_1 plants following gamma irradiation (2.5, 5, 10, 20, 30 and 40kR) and EMS (0.25% and 0.5% for 2h and 4h durations) treatments to dry seeds of *Withania somnifera* (L.) Dun. (2n = 48, family Solanaceae) to ascertain responsiveness of the species to mutagens. The results obtained have been discussed.

Keywords : EMS; Gamma rays; Meiosis; *Withania somnifera*.

Introduction

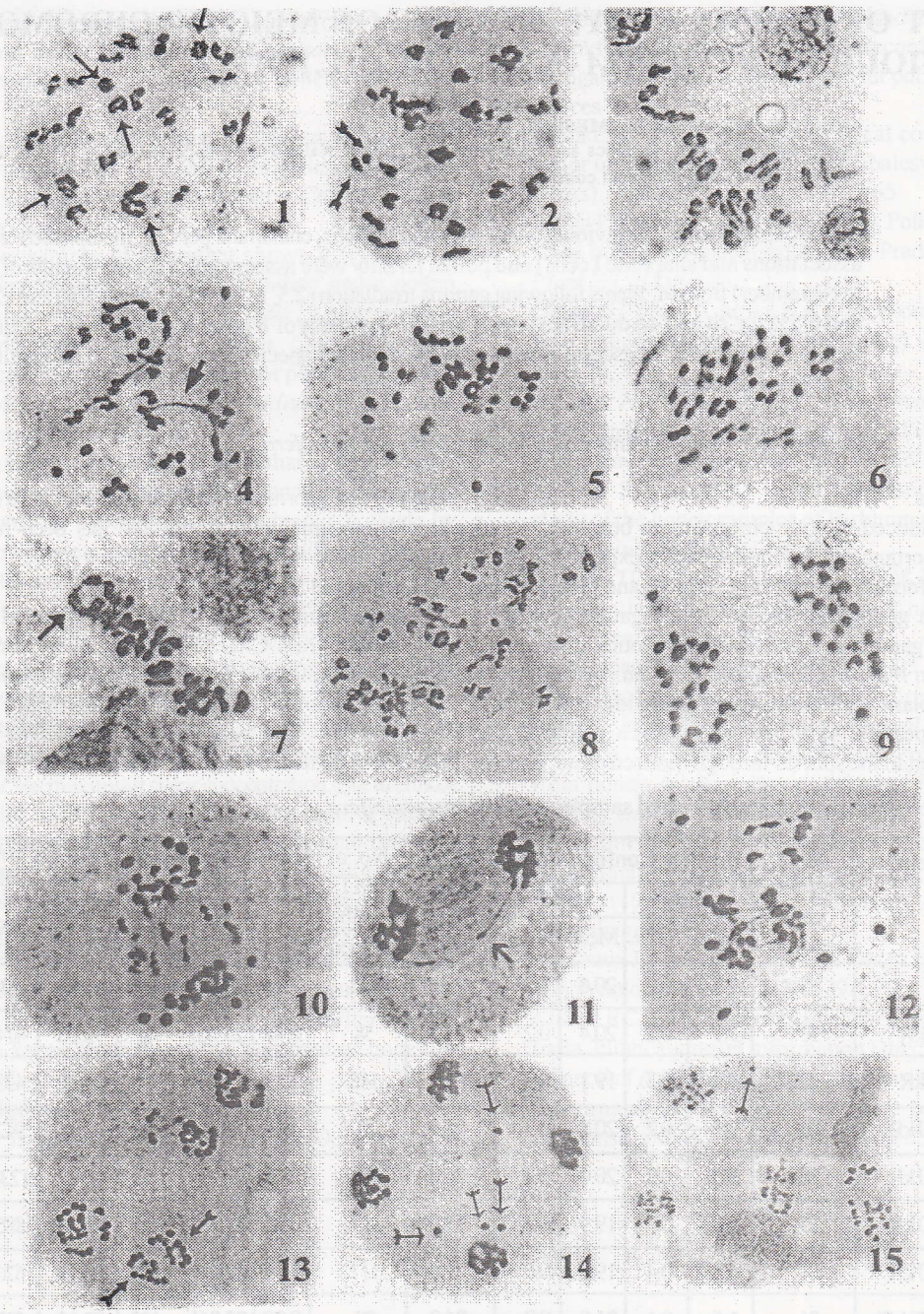
Mutagen induced meiotic chromosome behaviour is of utmost importance in any mutagenesis experiment as it provides information regarding the role and effect of the mutagen on genotypes. Present investigation describes the effect of gamma-rays and EMS on meiotic chromosome behaviour in *Withania somnifera* (L.) Dun., an important medicinal plant with anticancer[1], antioxidant[2] and anti-stress[3] properties, as a part of research initiated for improvement in the species through induced mutagenesis. Cytological information in *Withania somnifera* is restricted to chromosome counts only and has been found to be variable as 2n = 24[4], 2n = 48[5] and 2n = 72[6].

Materials and Methods

Mutagens (gamma-rays – 2.5, 5, 10, 20, 30 and 40kR from ^{60}Co source from CRIJAF, Nilganj, West Bengal; EMS: 0.25% and 0.50% for 2h and 4h durations, concentrations of EMS prepared in 0.2M phosphate buffer at pH 6.8) induced M_1 plant population of *Withania somnifera* (Neemuch cultivar: MPST NO.- NHII/XVIII/946, CST NO.-

Table 1. Meiosis in control and in treated samples of *Withania somnifera*.

Treatment		No of Diplotene cells scored	Bivalent Configurations				No. of chais-mata per cell	No. of M I cells scored	Mean/Cell		No. of A I cells scored	Cytologically balanced (24/24) AI cells (%)	Total no. of pollen obser-ved	Pollen fertil-ity (%)
			Ring		Rod				I	II				
			Mean	S.E.±	Mean	S.E.±								
0		33	2.9	0.2	20.6	0.2	26.5	164	0.95	23.52	166	63.85	1939	70.25
Gamma-rays	2.5 kR	30	3.7	0.2	20.4	0.2	27.7	58	0.48	23.76	61	95.08	474	47.27
	5.0 kR	22	4.5	0.3	19.1	0.3	28.1	118	1.13	23.43	74	74.32	435	57.44
	10.0 kR	26	2.5	0.2	20.8	0.3	25.9	41	3.46	21.78	85	71.76	942	55.42
	20.0 kR	30	3.0	0.2	20.0	0.2	26.0	86	2.02	22.99	171	80.12	1231	52.14
	30.0 kR	25	3.6	0.3	19.9	0.3	27.0	40	0.55	23.73	36	100.00	886	35.63
	40.0 kR	21	4.2	0.4	19.0	0.6	27.4	117	1.76	22.91	108	85.18	676	24.93
EMS	0.25%,2h	34	1.5	0.1	21.8	0.2	24.9	29	0.28	23.86	123	72.13	597	68.51
	0.25%,4h	28	2.6	0.3	20.4	0.3	25.7	32	1.75	23.13	54	94.44	702	36.90
	0.50%,2h	38	3.8	0.2	19.6	0.3	27.3	39	0.97	23.51	17	88.24	443	56.43
	0.50%.4h	21	2.2	0.3	19.8	0.4	24.4	29	1.38	23.31	25	100.00	535	72.15
CD at 5% level			0.7		0.6		0.9							



Figs. 1 - 15. Meiosis (2n = 48) in mutagen treated materials of *Withania somnifera*. 1. 24II with five rings (⟶) at late diplotene. 2. 23II + 2I (⟶) with rings and rods. 3. 24II at MI. 4. MI showing differential condensation (⟶) of chromosomes. 5. 14II + 20I at MI. 6. 17II + 14I at MI. 7. 1 IV (ring ⟶) + 22II at MI. 8. Polyploid PMC. 9. AI with 24/24 chromosome separation. 10. AI with irregular separation of chromosomes. 11. Bridge with an attached fragment (⟶). 12. Multiple bridges with irregularly distributed chromosomes at AI. 13. AII showing tendency of five group formation (⟶). 14 - 15. AII with laggards (⟷).

NHII/XVIII/596; moisture content: 3.89%) were meiotically assessed in relation to control (3 to 5 randomly selected plants from each dose of treatment including control). For the purpose, flower buds were fixed during the months of September to November (total rainfall – 210.3mm; humidity – max :98.0%; min : 65.7%, range: 51.0- 79.0%; temperature – max : 31.7 °C, range : 30.5- 32.7°C; min : 21.1 °C, range : 16.7-24.8 °C) in Carnoy's fluid between 5:30 to 6:30 AM and preserved in 70% alcohol. PMCs and pollens were stained in propionocarmine solution. Fully stained pollen grains were considered fertile. Photomicrographs were made from temporary squash preparations and subsequently magnified. Cytological data has been pooled over the plants from each treatment (including control plants) and statistically analyzed.

Result and Discussion

Meiosis in control and treated samples has been presented in Table 1. Results indicated that mean chaisma per cell was correlated significantly with ring ($r = 0.97$, p value < 0.001 at 10 DF) and rod ($r = -0.59$, p value < 0.05 at 10 DF) configuration of bivalents (Figs. 1-2) and it has either increased (2.5, 5.0 and 40kR gamma-rays) or decreased (0.25%, 2h and 0.50%, 4h EMS) significantly with respect to control in most treatments; however, the response was not dose dependent. Enhancement in chaisma formation may be the outcome of increase in crossover frequency; while, decrease in chaisma formation has been attributed to delay in DNA synthesis resulting in non-synchronization of nuclear processes[7]. Thus, mutagenic treatments have brought about recombinational changes and is expected to induce genetic variations.

Control plants had 24II (2n = 48, Fig. 3) in 70.7% cells, while the rest showed 23II+2I (20.1%), 22II + 4I (5.5%), 21II + 6I (0.6%), 20II + 8I (1.2%), 19II + 10I (1.2%) and 18II + 12I (0.6%) with an average of 23.52II + 0.95I / cell at metaphase I. In treatments average chromosome association at MI per cell varied from 21.78II + 3.46I (10kR) to 23.76II + 0.48I (2.5kR) in gamma irradiation and 23.13II + 1.75I (0.25%, 4h) to 23.86II + 0.28I (0.25%, 2h) in EMS. Univalents arising due to pairing defects (non-random distribution of univalents in treatments as evidenced from χ^2 test of heterogeneity, $p < 0.001$) bear no significant correlation with mean chaismata per cell ($r = -0.18$ at 10 DF) thereby indicating that other bivalents might be compensating. In EMS, high duration (4h) of treatments seems to induce enhanced univalent frequency per cell, while such response was not distinctive in gamma irradiated materials. Few PMCs of gamma irradiated samples had a high frequency of univalents per cell (20kR : 17II + 14I - 1.2%, Fig. 6; 10kR : 14II + 20I – 9.8%, Fig. 5). Univalents formed were mostly found to lie at close proximity to one another (Figs. 5-6). Only 40kR gamma irradiation formed quadrivalents (0.10 per cell, Fig. 7) and a polyploid PMC (Fig. 8). Occasionally few meiocytes of treated cells also showed differential condensation of chromosomes (Fig. 4).

About 63.9% of AI cells were cytologically balanced (24/24, Fig. 9) in control and in treatments it varied from 71.76% to 100%. Rest of the cells (both in control and treatments) showed irregular separation of chromosomes, bridges with or without fragments and laggards (Figs. 10-12). Laggards (1 –2; 27.7%), occasionally formed bridges (2.40%) and irregular separation of chromosomes (6.02%) were noted in control but in treatments, bridges with or without fragments and irregular separation of chromosomes were predominant along with cells with laggards (1–7). Rarely, gamma irradiated AII cells formed laggards (Fig. 14-15) and showed tendency of multipolarity (Fig. 13). Pollen fertility was reduced in mutagen treated samples (24.9% to 68.5%) than control (70.3%). Balanced AI cells showed no relationship with univalent frequency per cell ($r = -0.21$ at 10 DF) and pollen fertility ($r = -0.43$ at 10 DF) thus indicating that the univalents were randomly distributed to either of the poles and all the cytologically balanced cells were not genetically balanced. Reduced pollen fertility noted in control plants may be attributed to environmental factors as well as to intra-chromosomal variations. Present investigation therefore reveals that gamma irradiation and EMS have produced both chromosomal and genic changes in the species as evidenced from MI meiotic chromosome behaviour and is expected to cause variations in M_1 plant progenies which will be helpful for screening desirable mutations.

References

1. Leyon P V and Kuttan G 2004, Effect of *Withania somnifera* on B16F-10 melanoma induced metastasis in mice. *Phytother. Res.* **18** 118-122.
2. Bhattacharya A, Ghosal S and Bhattacharya S K 2001, Anti-oxidant effect of *Withania somnifera* glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *J. Ethnopharmacol.* **74** 1-6.
3. Bhattacharya S K, Goel R K, Kaur R and Ghosal S 1987, Anti-stress activity of sitoinosides VII and VIII, new acylsteryl glucosides from *Withania somnifera*. *Phytother. Res.* **1** 32-37.
4. Mohan Ram H Y and Kamini I 1964, Embryology and fruit development in *Withania somnifera* Dunal. *Phytomorphology* **14** 574-587.
5. Baquar S R 1967, Cytomorphological studies in the family Solanaceae from West Pakistan. *Genetica*, **38** 388-397.
6. Bir S S and Neelam 1980, *In*: Chromosome number reports LXIX. *Taxon* **29** 703-730.
7. Lawrence C W 1961, The effect of radiation on chaisma formation in *Tradescantia*. *Radiation Botany* **1** 92-96.