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Mutagenic effects of chemical and physical mutagens on mitotic chromosomes in *Brassica juncea* (L.) cv. Bio-902

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Abstract

The present study reveals the mitotic index frequency and mitotic abnormality frequency of various mutagenic treatments. True to type pre-soaked seeds of *Brassica juncea* (L.) cv. Bio-902 were treated with different concentrations of chemical mutagens; sodium azide (SA), ethyl methane sulphonate (EMS) and physical mutagen; gamma radiations. Mutagen doses were determined based on LD_{50}. Doses for gamma rays' treatments were 800Gy, 1000Gy and 1200Gy. The concentrations for the dry seed treatments of SA and EMS were 0.003%, 0.006% and 0.009% for each mutagen. The concentrations of pre-soaked treatments for 12 hrs and 18 hrs of both chemical mutagens were 0.04%, 0.08% and 0.12%. Various mitotic abnormalities like clumping of prophase and metaphase, stickiness, laggards, single, double and multiple bridges, distorted anaphase, multi-spindle anaphase, fragment, diagonal arrangement, and unequal separation were observed. Mitotic index and abnormality frequency were computed by scoring the somatic cell. Mitotic study provides idea about the effectivity of mutagens and confirms about mutation. The study helps researcher to confirm about the doses determined and predict the direction of mutation.

Keywords: *Brassica juncea, gamma rays, mitotic abnormalities, mitotic index, sodium azide*

Introduction

Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is one of the most economically important oilseed crops in India. It is considered as one of the most important vegetable oil and protein meal crops in the Indian subcontinent. *Brassica juncea* (2n=36) is an amphidiploid species derived from an interspecific cross between *Brassica nigra* (2n=16) and *B. rapa* (2n=20). To meet the breeding objective like yield and adaptability of crop to non-traditional areas, mutation breeding is the most successfully used tool for the improvement of crop plants. Various chemical and physical mutagens like sodium azide, EMS and gamma rays are employed to artificially induce the mutation in plants. The study of chromosome biology is an important source of information regarding the genotoxic and mitotoxic behaviour of any mutagen. Cytological studies provide important information for plant breeding program for induction of mutation in genetic composition and to select beneficial one. Mutation can also be helpful to find out particular action of a particular gene.

The effect of mutagens for the creation of variation in a crop like *B. juncea* is an important criterion in the contemporary world where climate change and food insecurity are alarming at the doors of various nations. Chromosomal abnormalities induced by mutagenic agents in plants are an indicator of genetic changes. Analysis of chromosomal behaviour of various mitotic stages is the most dependable indices for estimation of the potency of any mutagen (Mendhulkar *et. al*., 2015). The cytological analysis clarifies the specific response of different genotypes to a specific mutagen and provides significant evidence for the selection of desirable traits (Dhulgande *et. al.*, 2015). It also helps to manipulate chromosome segments or whole individual chromosome or sets of chromosomes to solve a particular problem.

Materials and Methods

Treatment details

Physiologically similar seeds of *B. juncea* cv. Bio-902 were treated with different concentrations of sodium azide, ethyl methane sulphonate and gamma radiations. Mutagen doses were determined based on LD_{50}. In the present study, dry and pre-soaked seeds were used with different doses of physical mutagen (gamma rays) treated at BARC, Trombay, Mumbai and chemical mutagens sodium azide (SA) and ethyl methane sulphonate (EMS) treatments given at cytology and genetics laboratory, Dept. of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati. Doses for gamma rays' treatments were 800Gy, 1000Gy and 1200Gy. The concentrations for

the 18hrs dry seed treatments of SA and EMS were 0.003%, 0.006% and 0.009%. The concentrations of pre-soaked treatments for 12 hrs and 18 hrs of both chemical mutagens were 0.04%, 0.08% and 0.12%. All the above treatments were in triplicates. The treated and control seeds were kept for germination in petri plates. On the emergence of radical the root tips were fixed in a Carnoy's fixative I (3:1, absolute alcohol: acetic acid) in their respective bottles for 24h and then stored in 70% ethyl alcohol in refrigerator and were used for cytological analysis.

Cytological studies

For slide preparation control and treated fixed root tips of Bio- 902 was subjected for squash preparation by hydrolysing the root tip material in 1N HCl at 60°C for 7 minutes in mini-incubator. The hydrolysed root tips were transferred to orcein stain. The stained root tips were teased with the help of a needle and cover slip is placed on the tissue. It is spread evenly by tapping with blunt end of needle, sealed with wax and various stages of mitosis were studied under the compound microscope. Photomicrographs were taken by "Canon Powershot G12" equipped with Carl Zeiss lenses. The total number of cells, number of dividing cells, number of abnormal cells with different phases were scored. Mitotic index (MI) frequency and mitotic abnormality frequency were calculated by following formulae:

Mitotic Index Frequency = (No. of dividing cells/ Total no. of cells scored) × 100

Mitotic abnormal cell frequency = (No. of abnormal cells/ Total no. of cells scored) × 100

Results and Discussion

The observations on mutagenic effects of various EMS treatments such as 18 hrs dry EMS, 12 hrs PSW + 6 hrs EMS and 18 hrs PSW + 6 hrs EMS with different concentrations on mitotic index frequency and mitotic abnormality frequency in *B. juncea* (L.) cv. Bio-902 are tabulated in Table 1. In control, MIF was recorded at 8.47% and there was no mitotic abnormality found in it whereas in EMS treatments 18 hrs. Dry EMS treatment concentrations 0.003%, 0.006% and 0.009% MIF were recorded as 6.21%, 7.06% and 7.67% while MAF were recorded as 1.08%, 0.83% and 1.17% respectively. In 12 hrs. PSW + 6 hrs EMS treatment, concentrations 0.04%, 0.08% and 0.12% MIF 7.08%, 7.07% and 7.59% while MAF were recorded as 0.99%, 1.08% and 1.15% respectively. In 18 hrs PSW + 6 hrs EMS treatment, concentrations 0.04%, 0.08% and 0.12% MIF were recorded as 8.06%, 10.47% and 8.53% while MAF were recorded as 1.58%, 1.09% and 1.43% respectively. Gandhi *et al.* (2014), Verma *et al*. (2012) and Bharti *et al*. (2015) have also been found an increase in mitotic index and mitotic abnormality with the increase in concentration of EMS.

The observations on mutagenic effects of various SA treatments such as 18 hrs dry SA, 12 hrs PSW + 6 hrs SA and 18 hrs PSW + 6 hrs SA with different concentrations on mitotic index frequency and mitotic abnormality frequency in *B. juncea* (L.) cv. Bio-902 are tabulated in Table 2. In control, MIF was recorded at 10.47% and there was no mitotic abnormality found in it whereas in SA treatments 18hrs. Dry SA treatment concentrations 0.003%, 0.006% and 0.009% MIF were recorded as 4.30%, 4.28% and 5.36% while MAF were recorded as 0.08%, 0.15% and 0.29% respectively. In 12 hrs PSW + 6 hrs SA treatment, concentrations 0.04%, 0.08% and 0.12% MIF 5.02%, 4.47% and 3.35% while MAF were recorded as 0.21%, 0.23% and 0.21% respectively. In 18 hrs PSW + 6 hrs SA treatment, concentrations 0.04%, 0.08% and 0.12% MIF were recorded as 4.77%, 5.83% and 8.28% while MAF were recorded as 0.69%, 0.42% and 0.40% respectively. The values of mitotic abnormalities in SA treatment have been reported as increasing in horse gram (Shirsat *et al*., 2010) whereas decrease in mitotic index with increase in SA treatments was reported by Bhat *et al*. (2007) in *Vicia faba*, Kumar and Choudhary (2015) in *Phaseolus vulgaris*, Kumar and Dwivedi (2013) in *Brassica rapa* and Kamble and Petkar (2014) in *Cicer reticulatum.*

The observations on mutagenic effects of various gamma irradiation doses such as 800Gyr., 1000Gyr. and 1200Gyr. on mitotic index frequency and mitotic abnormality frequency in *B. juncea* (L.) cv. Bio-902. are tabulated in Table 3. In control, MIF was recorded at 10.72% and there was no mitotic abnormality found in it whereas in gamma irradiation treatment doses 800Gyr., 1000Gyr. and 1200Gyr. MIF were recorded as 11.19%, 8.25% and 7.56% while MAF were recorded as 1.66%, 0.94% and 1.33% respectively. Kumar and Srivastava (2010) observed a gradual decrease in MI with the increase in Gamma rays doses. The chromosome aberration increased with increased in gamma irradiation doses in *Vigna unguiculata* (Dhanavel *et al*., 2012), *Phlox drummondii* (Ahirwar and Verma, 2015) and Groundnut (Hassan and Anes, 2015).

Various mitotic abnormalities like clumping of prophase and metaphase, stickiness, laggard, single, double and multiple bridges, distorted anaphase, multi-spindle anaphase, fragment, diagonal arrangement, unequal separation were observed (Fig. 1). Bhat *et.al.* (2006) have reported different types of chromosomal aberrations

Table 1: Effect of EMS treatments 18 hrs dry, 12 hrs PSW + 6 hrs EMS and 18 hrs PSW + 6 hrs EMS on mitotic chromosomes in *B. juncea* cv. Bio-902

Treatment	Total	Dividing cells				TDC	MIF	Abnormalities													TAC	MAF
	no. of cells	P	M	A	T		(%)	Cl P	Cl M	Cl A	SB	DB	MB	L	DA	UA	F	D	US	MSA		(%)
Control	3059	196	28	30	5	259	8.47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18h Dry EMS																						
0.003%	3975	160	39	42	6	247	6.21	14	21	0	3	0	2	3	0	0	0	0	0	0	43	1.08
0.006%	3257	154	31	39	6	230	7.06	6	13	0	1	0	1	3	1	0	0	0	2	0	27	0.83
0.009%	3235	159	37	32	20	248	7.67	11	20	0	1	2	1	1	0	0	0	0	0	2	38	1.17
12h PSW +6h EMS																						
0.04%	3727	193	21	39	11	264	7.08	8	23	3	1	0	0	1	1	0	0	0	0	0	37	0.99
0.08%	3692	172	42	38	9	261	7.07	16	23	0	0	0	0	0	0	0	0	0	0	1	40	1.08
0.12%	3216	185	27	27	5	244	7.59	10	20	0	1	0	0	0	0	3	2	0	0	1	37	1.15
18h PSW + 6h EMS																						
0.04%	2915	186	14	32	3	235	8.06	9	27	1	2	1	1		3	1	0	0	1	0	46	1.58
0.08%	2942	239	24	27	18	308	10.47	6	17	0	0	0	1	1	1	2	0	1	2	1	32	1.09
0.12%	2859	195	20	19	10	244	8.53	12	23	0	2	1	2	0	0	0	1	0	0	0	41	1.43

TDC – total dividing cells, TAC – total abnormal cell, MIF – mitotic index frequency, MAF – mitotic abnormality frequency, PSW – pre-soaked in water, P – prophase, M – metaphase, A – anaphase, T – telophase, Cl P – clump prophase, Cl M – clump metaphase, Cl A – clump anaphase, SB – single bridge, DB – double bridge, MB – multiple bridge, L – laggard, DA – distorted anaphase, UA – un-oriented anaphase, F – fragment, D – diagonal arrangement, US – unequal separation, MSA – multi spindle anaphase

Table 2: Effect of SA treatments 18 hrs dry, 12 hrs PSW + 6 hrs SA and 18 hrs PSW + 6 hrs SA on mitotic chromosomes in *B. juncea* cv. Bio-902

Treatment	Total	Dividing cells				TDC	MIF	Abnormalities													TAC	MAF
	no. of cells	P	M	A	T		(%)	Cl P	Cl M	Cl A	SB	DB	MB	L	DA	UA	F	D	US	MSA		(%)
Control	2589	221	31	18	1	271	10.47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18h Dry SA																						
0.003%	3981	95	43	29	4	171	4.30	0	2	0	1	0	0	0	0	0	0	0	0	0	3	0.08
0.006%	4065	94	43	33	4	174	4.28	0	5	0	0	0	1	0	0	0	0	0	0	0	6	0.15
0.009%	3469	102	42	36	6	186	5.36	0	6	0	1	0	1	1	1	0	0	0	0	0	10	0.29
12h PSW + 6h SA																						
0.04%	4285	130	58	26	1	215	5.02	0	6	0	2	1	0	0	0	0	0	0	0	0	9	0.21
0.08%	3981	115	34	27	2	178	4.47	0	8	0	0	0	0	1	0	0	0	0	0	0	9	0.23
0.12%	4702	84	49	29	5	167	3.55	0	8	0	0	0	1	1	0	0	0	0	0	0	10	0.21
18h PSW + 6h SA																						
0.04%	3793	106	31	37	7	181	4.77	0	12	0	4	0	2	1	7	0	0	0	0	0	26	0.69
0.08%	3310	115	38	30	10	193	5.83	0	8	0	4	1	0	1	0	0	0	0	0	0	14	0.42
0.12%	4036	237	48	45	4	334	8.28	5	7	1	1	0	1	1	0	0	0	0	0	0	16	0.40

Table 3: Effect of gamma rays' treatments 800Gy, 1000Gy and 1200Gy on mitotic chromosomes in *B. juncea* cv. Bio-902

Treatment	Total	Dividing cells				TDC	MIF	Abnormalities													TAC	MAF
	no. of cells	P	M	A	T		(%)	Cl P	Cl M	Cl A	SB	DB	MB	L	DA	UA	F	D	US	MSA		(%)
Control	3805	298	57	40	13	408	10.72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
800Gy	3987	391	23	21	11	446	11.19	10	33	0	5	1	1	0	0	7	4	2	1	2	66	1.66
1000Gy	3709	251	20	28	7	306	8.25	6	20	0	9	0	0	0	0	0	0	0	0	0	35	0.94
1200Gy	3692	209	32	30	8	279	7.56	11	25	0	6	1	1		2	0	2	0	0	1	49	1.33

TDC – total dividing cells, TAC – total abnormal cell, MIF – mitotic index frequency, MAF – mitotic abnormality frequency, PSW – pre-soaked in water, P – prophase, M – metaphase, A – anaphase, T – telophase, Cl P – clump prophase, Cl M – clump metaphase, Cl A – clump anaphase, SB – single bridge, DB – double bridge, MB – multiple bridge, L – laggard, DA – distorted anaphase, UA – un-oriented anaphase, F – fragment, D – diagonal arrangement, US – unequal separation, MSA – multi spindle anaphase

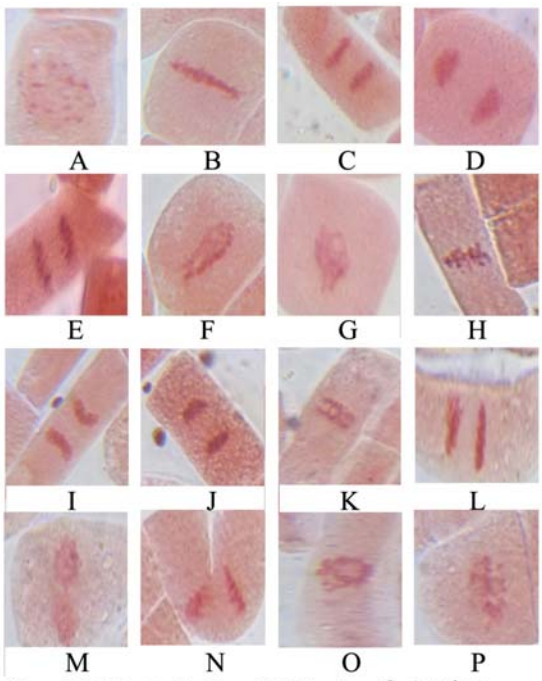

Figure 1 (A-P) : A- Prophase, B- Metaphase, C- Anaphase
D- Telophase, E- Laggard, F- Clump metaphase, G- Clump prophase
H- Fragment, I- Arrested telophase, J- Multiple chromosomal bridge
K- Stickiness, L- Unoriented anaphase, M- Telophasic bridge
N- Unequal chromosomal separation in late anaphase
O- Ring formation in prophase, P- Unorganized metaphase

because of physical and chemical mutagens. Chromosomal bridge formation may be due to stickiness or occurrence of dicentric chromosomes caused by breakage and reunion (Dempong and maxwell, 1973) and (El-Khodary *et.al.*, 1990). The formation of bridges and breaks may lead to loss of genetic material (Salam *et. al.*,1993). Stickiness is a common chromosomal abnormality reported by Mitra and Bhowmick (1996) in *Nigella sativa* treated with EMS. Laggard formation occurs because of errors at the time of separation of chromosomes during anaphase (Soheir *et al.*, 1989). Kesarwani *et al.* (2003) reported the formation of ring chromosome as a result of broken chromosomal ends and small fragments formation are due to chromosomal breakage.

Conclusion

From the present investigation, it can be concluded that the mutagens (Sodium azide, EMS, Gamma rays) induce various types of mitotic abnormalities in *Brassica juncea* cv. Bio-902. Mitotic index and mitotic abnormality show the induction of variability in the chromosomal behaviour and division of cells. The magnitude of induced variation is found to depend upon the mutagen used, the character under study and the genotypic background of the mutant. These promising mutant lines need to be further utilized in the next generations to derive distinct lines with improved agronomic traits.

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