Biological Forum – An International Journal **14(2): 634-637(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Study of Biological Damage in M_1 Generation of Indian Mustard (*Brassica juncea* L. Czern and Coss)

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(Received 11 February 2022, Accepted 22 April, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The experiment was carried out during *rabi* 2018-19 in the experimental field of College of Agriculture, Central Agricultural University, Imphal. Seeds of two Indian mustard genotypes CAULC- 2 and NRCHB-101 were exposed to three doses of gamma rays (1000, 1100 and 1200 Gy), three concentrations of ethyl methanesulphonate (0.3, 0.5 and 0.7%) and their combination (1000Gy+0.5%, 1100Gy+0.5% and 1200Gy+0.5%). The treated seeds along with the control were laid out in Randomised Block Design with three replications to raise M_1 generation. To determine the biological damage in M_1 generation, parameters like seed germination, seedling height, plant survival and pollen fertility were taken into account. Reduction in germination and survival percentage, seedling height and pollen fertility was observed in the mutagen treated population. The reduction was more pronounced in combined treatments. The reduction in these traits is caused due to cytological and physiological changes in the cell of the plants.

Keywords: Biological damage, ethyl methanesulphonate, gamma ray, Indian mustard.

INTRODUCTION

In India, rapeseed-mustard is the second most important oilseed crop which contributes about one-third of the edible oil in the country (Pratap *et al*., 2014). The production of edible oil in the country is quite low and cannot meet its requirement. India is importing edible oil from other counties in order to fulfil its demand. During 2019-20, India imported a total of 13.35 million tonnes of vegetable oils worth Rs. 61,559 crore (Annual report, 2020-21, Department of Agriculture, Cooperation & Farmers' Welfare).The threat of growing population and increasing oil consumption demands for yield improvement on long term basis. In spite of achieving impressive productivity gains through development of many improved cultivars, still there is compelling need to further increase and stabilize the productivity of this crop (Meena *et al*., 2015).

Creation of genetic variation is an important tool to improve an existing cultivar. Genetic variation can be achieved effectively through induced mutagenesis, particularly for traits having low level of genetic variation (Szarejko and Forster, 2007). Induced mutagenesis is an effective method in crop improvement involving single or few economic traits within a very short period of time (Manjaya and Nandanwar, 2007). The present investigation was therefore, taken up to induce genetic variability and to isolate desirable mutants for their use in improvement of Indian mustard. However, the germination, plant survival, seedling height and pollen fertility are crucial in early generation as initial indicators.

MATERIALS AND METHOD

The uniform, disease-free and dry seeds of Indian mustard genotypes CAULC-2 (local cultivar) and NRCHB-101 were exposed to 1000 Gy, 1100 Gy and 1200 Gy doses of gamma rays (Source : ^{60}CO gamma chamber installed at Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal). For chemical treatment, seeds were initially soaked in distilled water for 6 hrs and treated with 0.3, 0.5 and 0.7 per cent EMS (ethyl methane sulphonate) prepared in phosphate buffer of pH 7 for 6 hours, and then, were washed thoroughly with running water. Gamma irradiated (1000 Gy, 1100 Gy and 1200 Gy) seeds were also soaked in freshly prepared 0.5 per cent EMS solution for 6 hours and thus combined treatment between gammas rays and EMS was prepared. The untreated seeds were used as control. For laboratory experiment, 100 seeds from each treatment of both the genotypes along with control were allowed to germinate in petridishes with moist germination paper in three replications to study the seedling behaviors viz. germination percentage, root length, shoot length and root/shoot ratio (recorded at 10th day of sowing). Regular watering was done to keep the paper moist. To raiseM_1 generation in field, the treated material along with untreated seeds of each genotype as control was sown in randomized block design with three replications in the experimental field of College of

Agriculture, Imphalduring *rabi* 2018-19. Survival percentage and seedling height was recorded on 30th day of sowing. Pollen fertility was determined from freshly opened buds. To test the biological effects of gamma ray, EMS and their combined treatment on different characters studied in M_1 generation, the recorded data were analysed statistically in accordance with the analysis of variance for two factors experiment in RBD (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The effects of different doses/concentrations of gamma rays, EMS and their combination treatments were evaluated with respect to seed germination, seedling height, plant survival and pollen fertility in M_1 generation of Indian mustard and presented in Table 2. Analyses of variance for these characters are presented in Table 1.

Table 1: Analysis of variance for germination, survival, pollen fertility, root length, shoot length, root/shoot ratio and seedling height in M_1 generation of Indian mustard genotypes CAULC-2 and NRCHB-101.

Source of variation	D.F.	Mean sum of squares						
		Germination percentage	Survival percentage on 30th day	Pollen fertility	Root length on 10th day	Shoot length on 10th day	Root/shoot ratio	Seedling height on 30th day
Replication	2	3.45^{NS}	9.84^{NS}	49.59^{NS}	3.11**	0.19^{NS}	0.14*	0.41^{NS}
Treatments (T)	19	180.19**	312.96**	161.75**	1.79**	0.59**	0.03^{NS}	6.50**
Variety (V)	1	463.93**	28.77*	1426.91**	2.16*	1.35*	0.003^{NS}	25.69**
Dose (D)	9	314.31**	617.13**	140.85**	3.28**	0.83**	0.04^{NS}	9.52**
V×D	9	14.54^{NS}	40.36**	42.08*	0.26^{NS}	0.27^{NS}	0.03^{NS}	1.35**
Error	38	6.91	6.72	19.12	0.34	0.22	0.03	0.35

**,* = Significant at 1% and 5% levels respectively, NS = not significant

Table 2: Biological effects of gamma ray, EMS and their combination in M_1 generationof two Indian mustard genotypes CAULC-2 and NRCHB-101.

Treatments	Germination %		Survival % on 30th day		Pollen fertility %		Root length (cm) on 10th day		Shoot length (cm) on 10th day		Root/shoot ratio		Seedling height on 30th day (cm)	
	CAU LC-2	NRC HB-101	CAU LC-2	NRC HB-101	CAU LC-2	NRC HB-101	CAU LC-2	NRC HB-101	CAU LC-2	NRC HB-101	CAU LC-2	NRC HB-101	CAU LC-2	NRC HB-101
Control	87.62	84.34	98.02	96.29	92.62	72.62	7.46	7.41	5.06	4.67	1.48	1.59	13.33	14.44
1000 Gy	74.80	71.72	95.88	95.2	72.37	70.1	6.16	6.22	4.17	4.55	1.49	1.38	11.00	9.45
1100 Gy	74.74	66.86	95.79	92.33	75.96	68.98	5.49	5.9	3.85	4.09	1.43	1.46	10.95	9.53
1200 Gy	71.18	59.52	81.68	87.28	74.75	69.64	5.4	5.6	3.54	4.31	1.53	1.31	11.29	8.91
0.3% EMS	85.35	81.06	94.34	90.34	74.66	63.71	5.02	5.44	4.3	4.36	1.16	1.25	11.81	10.53
0.5% EMS	83.99	81.71	78.58	86.98	74.50	61.3	5.61	5.71	4.34	3.91	1.29	1.47	12.04	10.08
0.7% EMS	80.76	76.93	73.78	85.05	74.22	60.84	4.33	5.46	3.24	4	1.33	1.37	11.17	9.24
1000Gy+0.5%EMS	72.07	68.83	74.59	73.93	70.69	63.49	4.91	5.93	3.88	4.42	1.27	1.35	10.76	9.56
1100Gy+0.5%EMS	73.13	64.7	74.06	73.34	70.36	64.93	4.97	4.97	3.53	4.04	1.42	1.23	9.97	9.04
1200Gy+0.5%EMS	72.84	65.21	69.06	68.88	72.52	59.5	4.98	5.48	3.41	3.97	1.46	1.4	10.45	8.86
Mean	77.65	72.08	83.58	84.96	75.26	65.51	5.43	5.81	3.93	4.15	1.38	1.41	11.28	9.96
C.V. (%)	3.51		3.09		6.21		10.35		11.61		12.71		5.58	
$C.D._{0.05}$ (V)	1.37		1.36		2.29		0.34		0.25		NS		0.30	
$C.D._{0.05}$ (D)	3.07		3.04		5.11		0.68		0.56		NS		0.69	
$C.D._{0.05}$ (V×D)	NS		4.30		7.23		NS		NS		NS		0.97	

A. Germination

The analysis of variance for seed germination revealed that the variance ratios due to mutagen doses and varieties were significant. However, the variance due to variety × doses interaction was not significant showing that the genotypes were not differentially sensitive to mutagenic treatments.

The reduction in the germination percentage was not high with EMS as compared to gamma rays, although the germination percentage has shown linear decrease with increased dose of EMS. The damage in germination percentage was more pronounced in gamma rays treatment in both the genotypes. Lowest germination was observed at 1200 Gy in both the genotypes. The seed germination percentage was found to reduce progressively with increasing dose of mutagens and a more pronounced reduction was observed at higher dose in both the genotypes. Such a dose dependent reduction in germination has been reported earlier in oilseed Brassica by Kumar (1992); Chauhan *et al*. (2001)' Yassein and Aly (2014); Sathawane (2016). The reduction in germination of mutagen treated seed might be attributed to the disturbances at cellular level (physiological level or physical level) including chromosomal damages or the combined effect of both (Khan and Tyagi, 2010). The different varieties also responded differentially to mutagen treatment. The variety NRCHB-101 was observed to be more sensitive to mutagenic treatments than CAULC-2. Such a differential response of the varieties might be due to the genotypic background of the cultivars/varieties and degree of selection pressure obtained by the genotypes during their evolution. Reduction in seed germination, according to Chrispeeds

and Varner(1967), is due to delay or inhibition in physiological and biological processes necessary for seed germination, or due to hormonal imbalance (Ananthaswamy *et al*., 1971) or inhibition of mitotic process (Sato and Gaul, 1967).

B. Survival percentage on 30th of sowing

A negative linear relationship was observed between dose of mutagen and survival in both the genotypes studied. The survival percentage was highest at the lowest treatment whereas it was lowest at the highest treatment in all the mutagens. Such a dose dependent reduction in survival was also reported by Swaminathan and Gupta (1967) in *Brassica campestris* and Kumar (1992) in *Brassica juncea*. The various factors responsible for seedling mortality include i) inhibition of auxin as suggested by Skoog (1935), ii) inhibition of mitosis (Gunkel and Sparrow, 1961), iii) structural changes (Caldecott, 1954), iv) chromosomal breakage (Sato and Gaul, 1967) and v) inhibition of DNA synthesis (Mikaelson, 1968).The rate of reduction in survival was higher in EMS (chemical treatment) as compared to gamma ray (physical treatment).Reduction in survival was found to be more pronounced in case of combination treatment. The results of the present investigation also got support from the results of the studies carried out by Nallamthambi and Guruswamy Raja (1983). The genotypes also responded differentially to the mutagen treatments and the genotype CAULC-2 was more sensitive to mutagenic treatment as compared to NRCHB-101, indicating that effect of the mutagens on survival in Indian mustard depends mostly on the genetic background of the plant receiving the treatment.

C. Pollen fertility

From the analysis of variance for pollen fertility, it was revealed that the variance ratios due to variety, mutagen doses and variety × doses interaction were highly significant. For the genotype CAULC-2, the lowest pollen fertility (70.36%) was recorded at 1100Gy+0.5% EMS, whereas in case of NRCHB-101, the minimum pollen fertility (59.5%) was observed at 1200Gy+0.5%EMS. Thus, the combination treatment was found to be more effective in reducing pollen fertility. From the pooled mean, NRCHB-101 showed lower pollen fertility than CAULC-2, indicating that NRCHB-101 was more sensitive to mutagenic treatment. The reduction in pollen fertility percentage after gamma rays, EMS and combination treatment was reported earlier by Kumar and Das (1972) in *Brassica campestris* L., Burghate *et al*. (2013); Sikder *et al*. (2013). "The pollen fertility reduction might be attributed to cumulative effects of various aberrant meiotic stages as well as physiological and genetic damages that induced probably by the breakage of chromosome through formation of an anti-metabolic agent in the cell or might be due to irregular disjunction of chromosomes at anaphase" (Larik, 1975). Decrease in pollen fertility might be mainly due to chromosomal interchange, chromosomal aberrations and gene mutation (Gautam *et al*., 1992).

D. Root length, shoot length and their ratio

From the analysis of variance for root length and shoot length, it was revealed that the variance ratios due to mutagen doses and varieties were significant whereas the variance ratio due to variety × doses interaction was not significant. Seedling shoot length is commonly used as an indicator in determining the biological damage in M_1 generation caused by various physical and chemical mutagens (Konzak *et al*., 1972). A gradual decrease in root and shoot length with increasing dose/concentration of mutagens was observed in both the genotypes. The minimum mean root length of 4.90 cm was recorded at 0.7% EMS. Also, the minimum mean shoot length of 3.62 cm was observed at 0.7% EMS. Thus, the mutagen EMS of 0.7% concentration caused the maximum significant reduction in both root length and shoot length. The varieties also responded differentially to mutagen treatments for both root length and shoot length. The mean root length and shoot length recorded from CAULC-2 was significantly lower than that of NRCHB-101.

The root/shoot length ratio for the genotypes in different treatment was found increasing with the increase in the dose/concentration of the mutagens. From the data of root length and shoot length, the possible reason for the increase in the root/shoot ratio was higher rate of reduction in the root length than that of the shoot length. It was evident that root length was highly sensitive to the mutagen treatment as compared to shoot length which at lower concentration showed a stimulatory effect irrespective of the genotypic background.

E. Seedling height on 30th day of sowing

From the result of analysis of variance for the seedling height at each treatment, it was discovered that the component of variance due to mutagen doses, variety and variety x dose interaction were highly significant. There was no clear cut trend whether increase or decrease in seedling height with increase or decrease of the dose/concentration of mutagens. Both the mutagens and their combination treatment were found to be effective in reducing the seedling height. The minimum seedling height i.e. 8.86 cm was observed in NRCHB-101 at 1200Gy+0.5% EMS combination treatment. The maximum reduction in combination treatments was also reported by Sikder *et al*. (2013) in tomato. Among the varieties, the mean seedling height of NRCHB-101 was significantly lower than CAULC-2. The reduction in seedling growth was due to slow rate of cell division, reduced activity of amylase and increased activity of peroxidase. The mutagen has known to cause cytological, morphological and physiological changes in the cell, resulting in growth aberrations (Chaudhuri and Das, 1956).

CONCLUSION

From the present investigation, it was concluded that biological damage increased with increasing dose or concentration of mutagens in M_1 generation of Indian mustard. Seed germination, survival, pollen fertility and seedling height were higher at lower dose or

concentration of the mutagens, which was due to the fact that biological damage (in terms of lethality, injury and sterility) increase with increasing dose or concentration of mutagens. Gamma rays and EMS, alone as well as in combination, are able to induce mutations in Indian mustard.

Acknowledgement. The authors are highly thankful to B.C.K.V., Mohanpur, West Bengal for irradiating the experimental materials, the Dean, the H.O.D. of Genetics and Plant Breeding, and the authorities of College of Agriculture, Central Agricultural University, Imphal for providing all the necessary facilities for carrying out the present investigation.
Conflict of Interest. None.

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How to cite this article: T. Julia, E.V.D.Sastry, N. Brajendra Singh, Th. Renuka and N. Reetisana (2022). Study of Biological Damage in M_1 Generation of Indian Mustard (*Brassica juncea* L. Czern and Coss). *Biological Forum – An International Journal*, *14*(2): 634-637.