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Gamma rays and EMS induced morphological mutation in Indian mustard (*Brassica juncea* L. Czern and Coss)

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Abstract
The present investigation was carried out in the experimental field of College of Agriculture, Central Agricultural University, Imphal during *rabi* 2019-20 and rabi 2020-21. Seeds of Indian mustard genotypes CAULC-2 (local cultivar) and NRCHB-101 were exposed to three doses of gamma rays (1000Gy, 1100Gy and 1200Gy), three concentrations of ethyl methanesulphonate (0.3, 0.5 and 0.7%) and in various combinations (1000Gy + 0.5%, 1100Gy + 0.5% and 1200Gy + 0.5%). Different types of morphological mutations affecting plant growth habit, plant height, maturity, foliage, seed and siliqua characteristics were isolated. In general, maximum frequency of morphological mutants was observed in combination treatment of gamma ray and EMS in both the genotypes CAULC-2 and NRCHB-101. For the genotype CAULC-2, the trend of induction of morphological mutations was in the order of combination treatment > gamma rays > EMS, whereas for the genotype NRCHB-101, it was combination treatment > EMS > gamma rays. The genotypes used in the study showed differential response with regard to viable mutations with NRCHB-101 being more sensitive for the production of viable mutants.

Keywords: Gamma rays, ethyl methanesulphonate, morphological mutation, Indian mustard

Introduction
Mutation is an important breeding method for creating variability in crop species. The utilization of induced mutations for the improvement of crop plants has yield several mutants which have been used directly as new cultivars (Gottschalk and Wolf, 1983) [1]. Induced mutants constitute a valuable resource for research aimed at understanding the process in governing plant development (Cove, 1993) [2]. However, in mutagenic experiments most of the mutations are deleterious and have no direct practical value. Moreover, majority of the agricultural crops exhibit a high percentage of morphological mutations in mutagenesis experiments. The mutagenic effect is being reflected in the form of segregation of morphological mutants and it serves as a good indicator to forecast the spectrum of genetic variability that can arise from the mutated population. The probability of producing desirable mutations and genetic variability by artificial means is theoretically higher in self-pollinated crops (Welsh, 1981) [3] like mustard. The frequency of morphological mutation is being used as a convenient guide for the effectiveness of different mutagen dose. The present paper deals with the frequency and spectrum of morphological mutations in M_2 and M_3 generations of two genotypes of Indian mustard *viz*. CAULC-2 and NRCHB-101 induced by different doses of gamma rays and EMS.

Materials and Methods
The uniform, healthy 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 pre-soaked in distilled water for 6 hrs and treated with 0.3, 0.5 and 0.7 per cent EMS (ethyl methanesulphonate) prepared in phosphate buffer (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 gamma rays and EMS was prepared. The untreated seeds were used as control. The treated material along with untreated seeds of each variety as control was sown in randomized block design with three replications in the experimental field of Department of Genetics and Plant Breeding, College of Agriculture, Central Agricultural University, Imphal, during *rabi* 2018-19, 2019-20 and 2020-21. Seeds of M_1 plants were harvested separately and the individually harvested M_1 plants were sown following plant to progeny method during *rabi* 2019-20 to raise M_2 generation. 252 plants (126 from each genotype) from M_2 population were selected and the progenies of the selected families were again laid out in field following plant to progeny rows during rabi 2020-21 to raise M_3 generation The treated and control material were carefully screened for the frequency of morphological mutation throughout the life period of the plant in M_2 and M_3 generations. All visible changes in comparison to the control were classified for deviation from the normal and the most conspicuous characters taken into consideration were general architecture of the plant, duration, leaf, seed and siliqua characters etc. The spectrum and frequency of viable mutations was calculated using the following formula.

$$Morphological\ mutation\ frequency\ (\%) = \frac{Total\ no.of\ morphological\ mutants}{\text{Total number of M2 or M3 plants}} \times 100$$

Results and Discussion

Careful screening of control as well as treated populations was undertaken to identify the response of Indian mustard to different mutagenic treatments. Various types of morphological mutations were observed at different stages of growth in the M_2 as well as M_3 generation. The morphological mutations identified were grouped on the basis of the trait affected as listed below.

Mutations affecting plant growth habit

Profuse basal branches ii) High number of branches (Fig 1).

Mutations affecting plant height:

1. Tall: The tall mutants were vigorous and tall, attained the height of 160-175 cm while the parents attained the height of 110-120 cm in CAULC-2 and 100-110 cm in NRCHB-101.
2. Dwarf: The dwarf mutants attained the height of only 60-75 cm in CAULC-2 and 35-45 cm in NRCHB-101. (Fig. 2).

Mutations affecting maturity

1. Early flowering: The early flowering mutants flowered 11-18 days and 7-15 days earlier than the control in the genotypes CAULC-2 and NRCHB-101 respectively.
2. Late flowering: The late flowering mutants flowered 10-20 days late in both the genotypes.
3. Early maturity: The early maturing mutants matured 8-17 days and 10-20 days earlier than the control in the genotypes CAULC-2 and NRCHB-101 respectively. (Fig. 3).

Mutations affecting foliage

1. Narrow leaf
2. Broad leaf
3. Round leaf
4. Ovate leaf
5. Purple leaf (Fig. 4).

Mutations affecting seed size and colour

1. Bold seeded
2. Small seeded
3. Orange seeded (Fig. 5).

Mutations affecting siliqua characteristics

1. Long siliqua
2. Short siliqua
3. Sterile siliqua
4. Partially sterile siliqua
5. Appressed siliqua
6. Purple siliqua (Fig. 6).

Data on spectrum and frequency of morphological mutations in M_2 generation induced by gamma rays, EMS and their combination treatment in CAULC-2 and NRCHB-101 are presented in Table 1 and Table 2 respectively. The analysis of the frequency of mutations induced by physical and chemical mutagens as well their combination treatment had shown variations in the mutation spectrum. All types of morphological mutants were observed in CAULC-2 and NRCHB-101, except that the orange seeded mutant wasn't observed in NRCHB-101. Out of different morphological mutants observed in CAULC-2, appressed silqua, profuse basal branching and short siliqua mutants were observed in maximum frequencies. However, in NRCHB-101, early flowering, early maturing and appressed siliqua mutants occurred in maximum frequencies. None of the treatments was observed to give all the morphological mutants in both the genotypes.

The maximum frequency of morphological mutation (6.94%) in CAULC-2 was observed at 1200 Gy+0.5% EMS, whereas the lowest frequency (2.03%) was observed at 0.5% EMS. In general, combination treatment induced the maximum morphological mutation (5.69%) followed by gamma rays (3.36%) and EMS (3.16%).

For the genotype NRCHB-101, the morphological mutation frequency ranged from 4.01% in 1000 Gy to 7.05% in 1200Gy+0.5% EMS. The trend of induction of morphological mutations with different mutagens was in the order of combination treatment (6.24%) > EMS (5.61%) > gamma rays (4.57%). The comparison of the two genotypes revealed that NRCHB-101 (5.48%) produced greater morphological mutation frequency than CAULC-2 (3.99%).

The plants in M_3 generation were also screened visually for the viable/morphological mutations throughout the growing season. Several morphological mutants were isolated. Most of these resembled those already reported in M_2 generation. However, two new mutants (not isolated in M_2 generation) were observed in M_3 generation. These are the mutants affecting flower morphology *viz*. white flower and malformed flower, and are presented in Fig 7. They were isolated from both the genotypes.

Various workers have identified the occurrence of different types of morphological mutations induced by different mutagens in different crops including oilseed Brassica. The response of plant genotypes to different physical or chemical mutagens appears as various manifestations of morphological variations. For plant species, these morphological variations may be beneficial or detrimental to

cause lethality to the organism. In the present study, the spectrum of mutation was observed to be dependent on the type of mutagen used. Different types of morphological mutants were induced. These include profuse basal branches, high number of branches, tall, dwarf, early flowering, late flowering, white flower, malformed flower, early maturity, narrow leaf, broad leaf, round leaf, ovate leaf, purple leaf, bold seeded, small seeded, orange seeded, long siliqua, short siliqua, sterile siliqua, partially sterile siliqua, appressed siliqua and purple siliqua. The differential spectrum of viable mutants observed in the present investigation was also reported earlier by several workers in Brassica species *viz*. Verma (1973) [4], Verma and Rai (1980) [5], Anand and Mishra (1985) [6], Chauhan and Kumar (1986a) [7], Shah *et al.* (1990) [8], Rahman and Das (1993) [9], Roy *et al.* (1997) [10], Das *et al.* (1999) [11], Singh and Sareen (2004) [12], Wang *et al.* (2004) [13], Patel *et al.* (2006) [14], Barve *et al.* (2009) [15], Landge *et al.* (2009) [16], Hassan and Abl-El-Haleem (2014) [17], Kumar *et al.* (2018) [18], Malek *et al.* (2014) [19]. The possible cause of these macro-mutations may be chromosomal aberrations, small deficiencies or duplications and most probably gene mutation (Singh *et al.*, 1980) [21]. Several workers have reported that these viable mutations were monogenic and recessive in nature controlled by one or more recessive genes (Singh and Yadav, 1982).

Mutation frequency was highest for combination treatments. Similar results were obtained by Yadav (1992) [20] in *Brassica juncea*. Combined treatment of physical and chemical mutagen may cause greater mutations because of higher frequency of both chromosome and factor mutations.

Table 1: Spectrum and frequency of morphological mutation in M_2 generation of CAULC-2

Type of mutation		Mutagenic treatment										Mutagen		
	Control	1000 Gy	1100 Gy	1200 Gy	0.3% EMS	0.5% EMS	0.7% EMS	1000Gy+ 0.5%EMS	1100Gy+ 0.5%EMS	1200Gy+ 0.5%EMS	Total	Gy	EMS	Gy+EMS
No. of M_2 plants studied	500	575	562	587	604	591	578	477	512	504	4990	1724	1773	1493
Tall	-	-	1	1	2	1	1	-	1	1	8	2	4	2
Dwarf	-	1	-	1	2	1	1	-	1	1	8	2	4	2
Profuse basal branches	-	3	2	2	2	2	2	2	5	3	23	7	6	10
High no. of branch	-	1	1	2	2	-	1	1	-	3	11	4	3	4
Early flowering	-	1	3	2	4	-	1	-	2	2	15	6	5	4
Late flowering	-	1	-	-	-	1		1	-	2	5	1	1	3
Early maturing	-	1	1	2	-	1	1	2	2	1	11	4	2	5
Narrow leaf	-	-	1	2	2	-	2	2	-	2	11	3	4	4
Broad leaf	-	2	-	1	-	1	-	1	1	1	7	3	1	3
Rounded leaf	-	-	-	-	-	1	-	-	1	1	3	0	1	2
Ovate leaf	-	-	-	1	-	-	-	-	1	-	2	1	0	1
Purple leaf	-	1	1	-	-	-	-	-	-	1	3	2	0	1
Bold seeded	-	1	-	-	2	-	-	-	-	-	3	1	2	0
Small seeded	-	-	1	1	-	-	-	1	-	-	3	2	0	1
Orange seeded	-	-	-	1	-	-	-	-	-	5	6	1	0	5
Long siliqua	-	-	1	-	-	-	2	-	1	-	4	1	2	1
Short siliqua	-	1	3	2	5	2		2	4	2	21	6	7	8
Sterile siliqua	-	1	1	1	-	-	-	2	-	2	7	3	0	4
Partially sterile siliqua	-	-	-	-	1	-	-	-	1	2	4	0	1	3
Appressed siliqua	-	2	1	4	1	2	8	4	9	2	33	7	11	15
Purple siliqua	-	-	1	1	-	-	2	1	2	4	11	2	2	7
Total	-	16	18	24	23	12	21	19	31	35	199	58	56	85
Morphological mutation freq.(%)	-	2.78	3.20	4.09	3.81	2.03	3.63	3.98	6.05	6.94	3.99	3.36	3.16	5.69

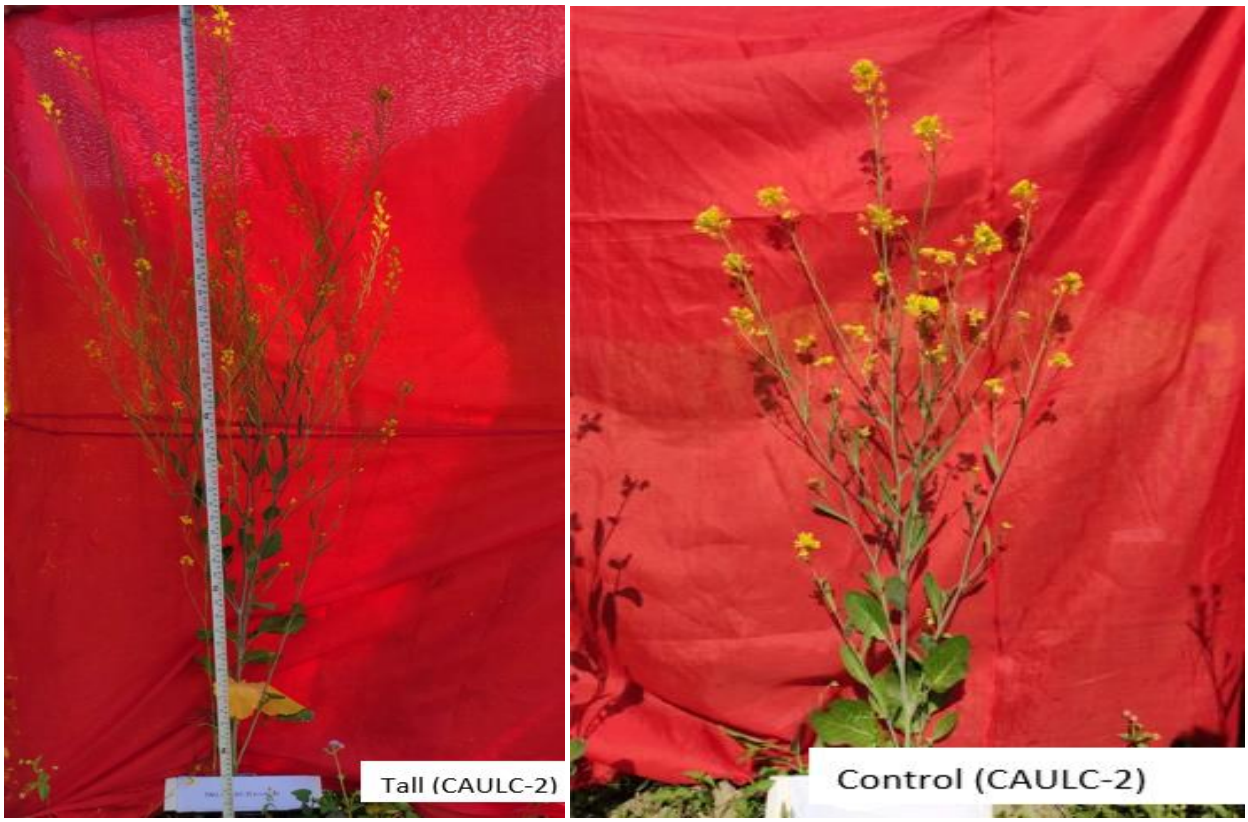
Table 2: Spectrum and frequency of morphological mutation in M_2 generation of NRCHB-101

Type of mutation		Mutagenic treatment										Mutagen		
	Control	1000 Gy	1100 Gy	1200 Gy	0.3% EMS	0.5% EMS	0.7% EMS	1000Gy+ 0.5%EMS	1100Gy+ 0.5%EMS	1200Gy+ 0.5%EMS	Total	Gy	EMS	Gy+EMS
No. of M_2 plants studied	455	524	551	478	530	563	546	519	493	525	4729	1553	1639	1537
Tall	-	1	-	-	2	2	2	1	-	2	10	1	6	3
Dwarf	-	-	1	-	-	-	-	-	-	1	2	1	0	1
Profuse basal branches	-	-	1	2	2	1	1	-	3	-	10	3	4	3
High no. of branch	-	-	-	1	-	1	-	-	1	1	4	1	1	2
Early flowering	-	6	9	8	6	8	13	7	10	11	78	23	27	28
Late flowering	-	-	1	-	-	1	1	-	-	1	4	1	2	1
Early maturing	-	4	5	7	5	8	11	4	7	10	61	16	24	21
Narrow leaf	-	-	-	-	-	1	-	-	-	1	2	0	1	1
Broad leaf	-	-	1	-	-	1	1	-	-	-	3	1	2	0
Rounded leaf	-	-	-	1	1	-	-	-	-	-	2	1	1	0
Ovate leaf	-	-	-	-	-	1	-	-	1	-	2	0	1	1
Purple leaf	-	-	1		-	1	2	-	-	-	4	1	3	0
Bold seeded	-	2	1	2	-	1	-	2	2	2	12	5	1	6
Small seeded	-	-	-	-	1	-	-	1	-	1	3	0	1	2
Orange seeded	-	-	-	-	-	-	-	-	-	-	0	0	0	0

Long siliqua	-	-	1	1	-	2	1	-	2	3	10	2	3	5
Short siliqua	-	-	-		-	-	2	-	1	-	3	0	2	1
Sterile siliqua	-	1	-	1	-	-	-	-	-	-	2	2	0	0
Partially sterile siliqua	-	2	-	1	-	-	-	1	-	-	4	3	0	1
Appressed siliqua	-	4		1	3	2	2	6	5	1	24	5	7	12
Purple siliqua	-	1	3	1	3	1	2	3	2	3	19	5	6	8
Total	-	21	24	26	23	31	38	25	34	37	259	71	92	96
Morphological mutation freq. (%)	-	4.01	4.35	5.44	4.34	5.51	6.96	4.82	6.89	7.05	5.48	4.57	5.61	6.24

1a: Mutant with profuse basal branches **1b:** Mutant with high number of branches

Fig 1: Mutations affecting plant growth habit



2a: Tall mutant

2b: Dwarf mutants

Fig 2: Mutations affecting plant height

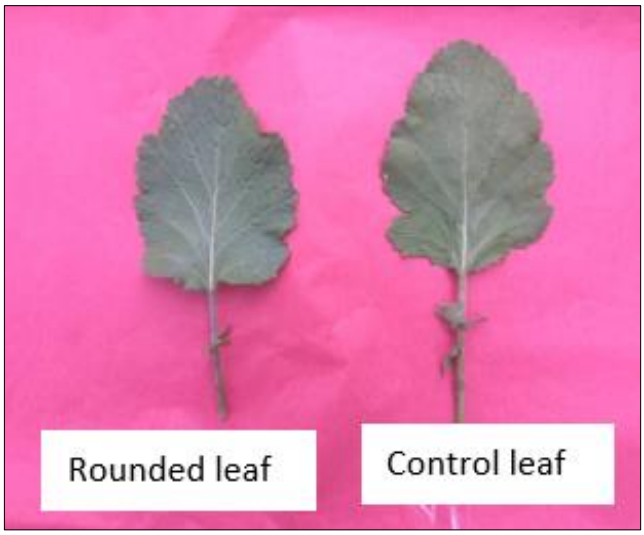
3a: Early flowering mutants

3b: Late flowering mutant **3c:** Early maturing mutant

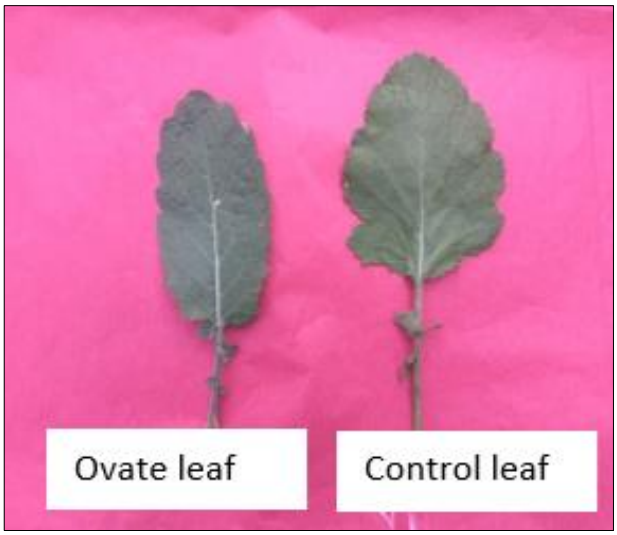
Fig 3: Mutations affecting maturity

4a: Narrow leaf mutant

4b: Broad leaf mutant



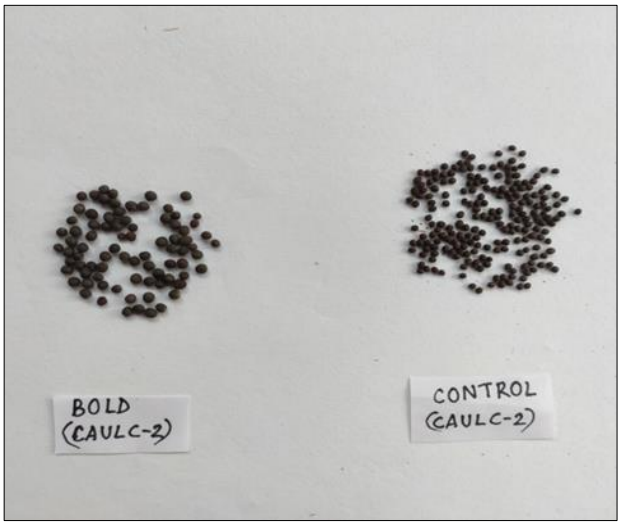
4c: Rounded leaf mutant



4d: Ovate leaf mutant

4e: Purple leaf mutants

Fig 4: Mutations affecting foliage



5a: Bold seeded mutant

5b: Small seeded mutant

5c: Orange seeded mutant

Fig 5: Mutations affecting seed size and colour

6a: Long siliqua mutant

6b: Short siliqua mutant

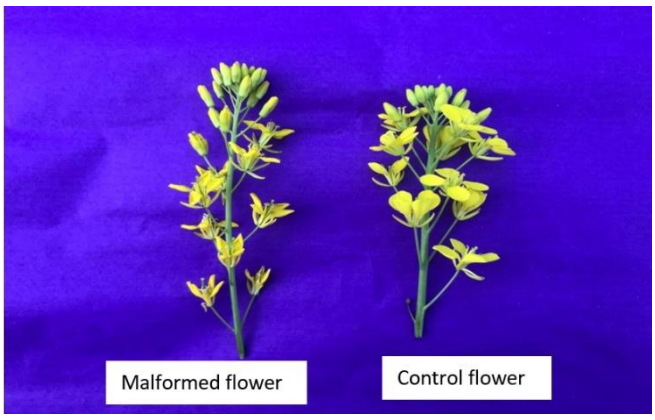
6c: Sterile siliqua mutant

6d: Purple siliqua mutant

6e: Appressed siliqua mutant

Fig 6. Mutations affecting siliqua characteristics

7a: White flower mutant



7b: Malformed flower mutant

Fig 7: Mutations affecting flower morphology

Conclusion

The present investigation revealed that isolation of plants with altered flower color, plant growth habit, plant height, maturity duration, foliage, seed size, seed colour and siliqua characteristics is possible in Gamma rays and EMS treated plant population. It provides greater chances for the selection of desired characters. The morphological mutants studied can be utilized for identification and characterization of Indian mustard genotypes.

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