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Assessment of chlorophyll mutation in M_2 generation of Indian mustard (*Brassica juncea* L. Czern and Coss)

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Abstract
The experiment was carried out during *rabi* 2018-19 and rabi 19-20 in the experimental field of College of Agriculture, Central Agricultural University, Imphal. Seeds of two genotypes of Indian mustard *viz*., CAULC-2 (local cultivar) and NRCHB-101 were exposed to three doses (1000, 1100 and 1200 Gy) of gamma rays, three concentrations of ethyl methanesulphonate (0.3, 0.5 and 0.7%) alone and in various combinations (1000Gy + 0.5%, 1100Gy + 0.5% and 1200Gy + 0.5%). Five different types of chlorophyll mutants *viz*., albina, chlorina, xantha, viridis and alboviridis were isolated from different treatments in M_2 generation. Combination treatment of 1100 Gy+ 0.5% EMS produced the highest frequency of chlorophyll mutation in both the genotypes. Alboviridis and viridis types of mutants were more frequent in CAULC-2 and NRCHB-101 respectively, whereas albina type was least frequent in both the genotypes. CAULC-2 was more sensitive to mutagenic treatment as compared to NRCHB-101.

Keywords: Gamma rays, ethyl methanesulphonate, chlorohyll mutation, mustard

1. Introduction
Mutation breeding has proved to be a very useful additional tool for creating variability in plant breeding. It is considered as an efficient supplier of genetic variability producing non-or slight gene erosion (Mick *et al*., 1990) [1]. 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 chlorophyll mutations in mutagenesis experiments. The mutagenic effect is being reflected in the form of segregation of chlorophyll mutants and it serves as a good indicator to forecast the spectrum of genetic variability that can arise from the mutated sectors (Sengupta and Datta, 2005) [2]. Leaf colour mutations are one kind of most frequently observed mutation in both spontaneous and induced mutant populations, and are often used as a measure to assess the effectiveness of various mutagens. Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromere and proximal segment of chromosome (Swaminathan, 1964) [3]. Majority of agricultural crop plants exhibit a high percentage of chlorophyll mutations. The spectrum of induced chlorophyll variations reveals the presence of viable mutations in mutagen treated population. The probability of producing desirable mutations and genetic variability by artificial means is theoretically higher in self-pollinated crops (Welsh, 1981) [4] like mustard. The frequency of chlorophyll 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 chlorophyll mutations in M_2 generation of two genotypes of Indian mustard *viz*. CAULC-2 and NRCHB-101. It also attempted to determine chlorophyll mutation rate including the families segregating for one or more types of chlorophyll mutations in M_2 generation.

2. 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 gammas 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. Seeds of M_1 plants were harvested separately and were grown as individual M_2 families in separate line during *rabi* 2019-20. The treated and control material were screened for the frequency of chlorophyll in M_2 generation. Chlorophyll mutations were scored and classified as per Gustafsson (1940) [5] and Gaul (1965) [6]. The spectrum and frequency of chlorophyll mutations for different treatments were computed.

$$Chlorophyll\ mutation\ frequency\ (\%) = \frac{Total\ no.of\ chlorophyll\ mutants}{\text{Total number of M2 seedlings observed}} \times 100$$

3. Results and Discussion

Spectrum and frequency of chlorophyll mutation in M_2 generation of CAULC-2 and NRCHB-101 is presented in Table 1. Chlorophyll mutation frequency in M_2 generation is the most reliable measure for induced genetic variation. Gustafson (1940) and Gaul (1965) [6] have classified chlorophyll mutations into albina, xantha, chlorina, virdis, maculata, albo-virdis, albo-xantha, striata etc. Though chlorophyll mutations are considered to be less important from crop improvement point of view, still they act as indicators of effectiveness of mutagenic treatments in inducing other mutations observed in M_2 generation.

In the present study, five types of chlorophyll mutants *viz*. albina, chlorina, xantha, viridis and alboviridis types were isolated at seedling stage in M_2 generation (Table 1). Similar type of chlorophyll mutants were earlier reported by Khan and Tyagi (2010) [7] in soybean and Gupta *et al*. (2012) [8] in Indian mustard. The occurrence of alboviridis and viridis type was most frequent in genotypes CAULC-2 and NRCHB-101 respectively, and albina was the least in both the genotypes. The highest frequency of viridis type was earlier reported by Yadav (1992) [9] and Gupta *et al*. (2012) [8] in *B. juncea*. The reason for the appearance of greater number of viridis may be attributed to involvement of polygenes in the chlorophyll formation (Nilan and Konzak, 1961) [10] and the genes are probably more responsive to mutagen. Differential occurrence of mutations could be due to differential mode of action of mutagen on different base sequences in various genes.

For the genotype CAULC-2, the lowest chlorophyll mutation frequency i.e. 0.05% was observed at 1000 Gy, 0.3% EMS and 1000Gy+0.5%EMS, and the highest chlorophyll mutation frequency i.e. 0.21% was recorded at combination treatment of 1100Gy+0.5%EMS. However, for the genotype NRCHB-101, the lowest chlorophyll mutation frequency (0.04%) was observed at 0.3% and 0.5% EMS treatment, and the highest (0.14%) was evident at 1100Gy+0.5%EMS. Therefore, combination treatment of 1100 Gy+ 0.5% EMS produced the highest frequency of chlorophyll mutation in both the genotypes. In general, the highest frequency of chlorophyll mutations was observed in combination treatment in both the genotypes, followed by EMS treatment in CAULC-2 and gamma rays in NRCHB-101. These findings were in accordance with the results obtained by Ramezsani and More (2014) in grasspea and Khan and Tyagi (2010) [7] in soybean.

It was observed that the mutagens used differed from each other in their ability to induce chlorophyll mutation in different genotypes. It may be due to differential amount of chromosome mutations induced in M_1 in different genotypes (Yadav, 1992) [9]. Marked varietal differences were present in terms of induction of chlorophyll mutations at different dose/concentration of gamma-rays and EMS. The variety CAULC-2 appeared to be more sensitive towards the mutagenic treatment as compared to NRCHB-101. Swaminathan (1964) [3] suggested that differences in mutation spectrum and rate in different genotypes might be due to differences in the location on genes in relation to the centromere. The varietal difference to mutagens as described by Krishnaswamy (1983) [12] was due to level of differentiation of the apical and lateral meristem. Those have already differentiated at the time of mutagenic treatment expected to respond differentially from those were yet not differentiated (Rines, 1985) [13]. Another interpretation was varietal difference might be due to difference in uptake or metabolism of the mutagen, cellular repair mechanism or mode of action of the mutagens.

Estimation of mutation frequency on the basis of M_2 plants gives the best estimate of actual mutation frequency. Even from breeder's point of view, the frequency of mutation expressed on M_2 population basis is more realistic and helpful. Moreover, the absence of chlorophyll mutants in the M_1 generation and their appearance in M_2 generation indicates the recessive nature of chlorophyll mutation. Chlorophyll development seems to be controlled by many genes located on several chromosomes, and mutation in this gene may induce chlorophyll mutations.

Table 1: Spectrum and frequency of chlorophyll mutation in M_2 generation of CAULC-2 and 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
CAULC-2														
No. of M_2 plants studied	2270	2165	2318	2406	2115	2372	1940	1872	1938	2006	19132	6889	6427	5816
Albina	-	-	-	1	-	-	-	-	-	-	1	1	-	-
Chlorina	-	1	1	-	-	1	-	-	1	-	4	2	1	1
Xantha	-	-	1	-	1	-	1	1	-	1	5	1	2	2
Viridis	-	-	1	-	-	1	-	-	2	1	5	1	1	3
Alboviridis	-	-	-	1	-	2	1	-	1	2	7	1	3	3
Total	-	1	3	2	1	4	2	1	4	4	22	6	7	9

Chlorophyll mutation frequency%	-	0.05	0.13	0.08	0.05	0.17	0.10	0.05	0.21	0.19	0.11	0.09	0.11	0.15
NRCHB-101														
No. of M_2 plants studied	2235	2074	2421	1884	2477	2314	1782	2015	2114	1973	19054	6379	6573	6102
Albina	-	-	-	-	-	-	-	-	1	-	1	-	-	1
Chlorina	-	1	2	1	-	-	-	-	-	-	4	4	-	-
Xantha	-	-	-	-	-	-	-	-	2	-	2	-	-	2
Viridis	-	-	-	-	1	1	1	-	-	2	5	-	3	2
Alboviridis	-	-	-	1	-	-	-	2	-	-	3	1	-	2
Total	-	1	2	2	1	1	1	2	3	2	15	5	3	7
Chlorophyll mutation frequency %	-	0.05	0.08	0.11	0.04	0.04	0.06	0.09	0.14	0.10	0.08	0.08	0.05	0.11

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