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Mutagenic Effectiveness of Gamma Rays and EMS by Inducing Chlorophyll and Biochemical Mutations in Indian Mustard (*Brassica juncea*)

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ABSTRACT

PM 21 variety of Indian mustard i.e. *Brassica juncea* was irradiated with different doses of gamma rays along with combination of different concentrations of Ethyl Methane Sulfonate (EMS). To study the effectiveness of the mutagenic agents, different types of chlorophyll mutations i.e. albina, chlorina, xantha, virdis and albovirdis were isolated from the progenies. The experiment with 1500 Gy+0.75% EMS treatment had highest mutation frequency, while 1000 Gy+0.25% EMS treatment had the lowest mutation frequency. Overall, 1500 Gy of gamma rays dose had the highest mutagenic effect on plants generating maximum spectra of chlorophyll mutations. The antioxidant activity also improved in the viable mutants. Results from chlorophyll mutations and biochemical analysis suggested that gamma rays in combination with EMS treatment can be successfully used to generate variability in the germplasm of *B. juncea*.

Key words: Chlorophyll mutations, Indian mustard, gamma rays, EMS

INTRODUCTION

Indian mustard [*Brassica juncea* (L.) Czern and Coss] is one of the most important oilseed crops in India. Pusa Mustard 21 (PM 21) is a low erucic acid (2%) variety of Indian mustard. The available literature suggests that India has limited genetic variability in the primary gene pool. Mutation breeding is an effective method to introduce genetic variations in the germplasm in a short period of time. Among the different available mutagenic agents, irradiation has proven to be the most successful due to various reasons. It encourages the expression of recessive genes and produces new genetic variations (Choudhary and Jambhulkar, 2016). Out of the total mutant varieties of various crops developed using mutagenic agents, 89% of the varieties were developed using gamma rays, X rays, thermal and fast neutrons, whereas gamma rays alone account for about 60% of the mutant varieties (Beyaz and Yildiz, 2017). Another reason for which gamma rays are so reliable is because they do not present any threat to the environment and mankind. Gamma rays are considered to be the most penetrating of all the electromagnetic radiations. The energy level of gamma rays is from 10 to several hundred kilo electron volts (Saika, 2020). The present study investigated the mutagenic potential of different doses of gamma rays along with combination of EMS so as to open a window for developing newer varieties which could potentially improve production and quality of mustard.

MATERIALS AND METHODS

The present study was conducted on low erucic acid genotype of Indian mustard (*Brassica juncea*) i.e. PM 21 (Pusa Mustard 21). The seeds were ensured that they were mature, moisture free, disease free and pests free. The seeds were treated with different doses of gamma rays at BARC, Trombay, Mumbai,

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400085, India. The experiment consisted 1000, 1200 and 1500 Gy dose of radiation. Each irradiated variety was further treated with 0.25, 0.50 and 0.75% EMS concentrations. PM 21 seeds were also treated with only EMS in different concentrations of 0.25, 0.50 and 0.75%.

For determining the effect of irradiation on germination percentage, the petri dishes were covered with moist filter paper and 100 seeds of each experiment were spread individually with proper spacing on the filter paper. The petri dishes were kept at room temperature (25°C ± 2) for seven days. The filter paper was soaked with distilled water at an interval of 8 h to maintain constant moisture for the seeds. All sprouted seeds were considered as germinated. The seed germination percentage was calculated as:

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated in petri dish}}{\text{Total number of seeds}} \times 100$$

Field trial was conducted at ICAR-DRMR, Bharatpur, Rajasthan. The plants were sown in randomized block design with three replications. All recommended practices were followed to raise the progenies. The experiments were thoroughly screened regularly for standard chlorophyll mutations (albina, chlorina, xantha, viridis, alboviridis) throughout the crop season.

Mutation frequency was calculated as percentage of mutated M_2 progenies for chlorophyll mutations in each treatment.

$$\text{Mutation frequency} = \frac{\text{Number of mutated seedlings}}{\text{Number of seedlings analyzed for a particular dose}} \times 100$$

The mutagenic effectiveness was calculated with the following standard formula.

$$\text{Mutagenic effectiveness} = \frac{Mf}{Gy} \times 100$$

Where,

M_f = Mutation frequency, G_y = Radiation dose in Gray

The mutagenic efficiency was calculated in terms of injury and lethality.

$$\text{Injury} = \frac{M_f}{I} \times 100$$

$$\text{Lethality} = \frac{M_f}{L} \times 100$$

Where, I = % reduction in seedling height, M_f = Mutation frequency and L= % reduction in survival

LD_{50} is the dose for which half of the seeds treated with that dose of gamma rays do not survive or rather dies. Through LD_{50}, the correct dose to be given to the plants can be estimated. It also helps in deciding the effectiveness of a mutagen. With the help of germination percentage experiment, %lethality was calculated. A plot was formed between various doses of gamma rays administered and % lethality. LD_{50} was calculated using Probit analysis (Julia *et al.*, 2018).

According to the classification mentioned by Julia *et al.* (2018), following different kinds of chlorophyll mutations were observed and recorded.

In Albina mutations (Fig. 1), the seedling leaves were completely white. These were lethal mutations and the seedling survived only for 10-12 days after the emergence. In Xantha type of mutations (Fig. 2), the leaves of the seedlings were bright yellow in colour and the seedlings survived for 25-30 days. In Chlorina mutations (Fig. 3), the seedlings were yellowish green (pale green) in colour. The seedlings survived for reasonably longer period. Viridis mutations were viable mutations (Fig. 4). The seedling leaves were light green in colour which became normal green colour at later stages. In Albovirdis, the leaves of the seedlings were characterized by green base with white apex leaves.

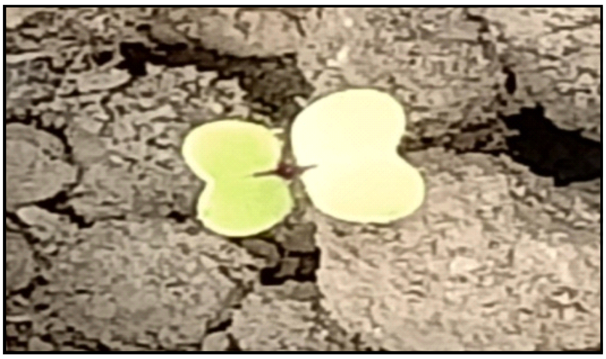

Fig. 1. Chlorophyll mutation albina.

Fig. 2. Chlorophyll mutation xantha.

Fig. 3. Chlorophyll mutation chlorina.

Fig. 4. Chlorophyll mutation virdis.

Not only physical chlorophyll mutations were observed in EMS and gamma rays treated seeds, but the seeds were also tested for biochemical changes to confirm the mutations.

The mutated seeds were defatted by using n-hexane as solvent. 500 mg seeds were crushed with the help of pestle and mortar. The seed meal was left dipped in hexane for overnight. Next day, the defatted seed meal was dried on a filter paper. 0.1 g defatted seed meal was homogenized in a 2 ml vial with 80% methanol. After keeping the homogenate overnight at room temperature, it was centrifuged at 3000 rpm for 4 min to obtain a clear solution. The pellet was discarded, and the supernatant was collected. The final volume of the supernatant was made up to 2 ml with 80% methanol.

Total antioxidant activity was estimated in defatted and methanolic extract of the samples using Sharma and Rai (2019) and Verma *et al.* (2019). To 100 µl of methanolic extract, 2.5 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate in the ratio of 1:1:1 was added). The reaction mixture was incubated in boiling water bath for 90 min. After cooling, the absorbance was measured at 695 nm. Calibration curve was prepared by measuring the absorbance of a series of standard solutions of ascorbic acid (0-50 µg/ml).

RESULTS AND DISCUSSION

The germination percentage decreased as the dose of gamma rays increased gradually from 1000 Gy (94%) to 1500 Gy (88%) indicating that lethal genetic changes might have taken place in the germplasm with higher doses of irradiation (Table 1). In the experiments, where both physical and chemical mutagenesis were performed, the germination percentage declined with the increasing concentration of EMS. Moreover, for similar treatment with EMS concentration, the germination frequency declined with increasing dose of gamma rays. The results are in agreement with Badukale *et al.* (2017). The LD_{50} for seed germination was calculated to be 4688 Gy which exceeded the present doses of gamma rays administered. The results are in agreement with the data as in none of

Table 1. Frequency of viable and non-viable chlorophyll mutants in Indian mustard

Dose	No. of M_2 seedlings	No. of mutants	Mutation frequency
0 Gy			
1000 Gy	120	1	0.83
1000 Gy + 0.25% EMS	40	0	0.00
1000 Gy + 0.50% EMS	60	1	1.66
1000 Gy + 0.75% EMS	80	2	2.50
1200 Gy	120	1	0.83
1200 Gy + 0.25% EMS	100	1	1.00
1200 Gy + 0.50% EMS	360	4	1.11
1200 Gy + 0.75% EMS	140	2	1.42
1500 Gy	100	2	2.00
1500 Gy + 0.25% EMS	120	5	4.16
1500 Gy + 0.50% EMS	100	5	5.00
1500 Gy + 0.75% EMS	40	5	12.5
0.25% EMS	700	5	0.71
0.50% EMS	280	4	1.40
0.75% EMS	100	4	4.00

the doses administered (1000, 1200 and 1500 Gy), 50% of the population died.

% injury for 1000, 1200 and 1500 Gy was found to be 3.37, 3.39 and 4.13%, respectively. Per cent lethality was found to be maximum for 1500 Gy dose i.e. 4.17%. The results are in agreement with the data as with increasing dose of gamma rays, % lethality should increase. A similar study on mutagenic efficiency with a different mutagen (sodium azide) was also performed by Gowtham *et al.* (2018) in Indian mustard.

For estimating the genetic effects of a mutagen, the frequency of chlorophyll and viable mutants in M_2 generation was mainly used as a dependable measure of genetic effect of mutagens. Mutation frequency was used as the indicator of mutagenic effect (Ambavane *et al.*, 2015; Garg *et al.*, 2022). In the individual experiments studied, the highest mutation frequency was recorded from 1500 Gy+0.75% EMS treatment (12.5) and the lowest was recorded from 1000 Gy+0.25% EMS treatment (0.00). The higher doses of gamma rays might have increased the damage in the genetic materials which could not be repaired during the process of plant growth, leading to the death of cells resulting in lethality (Ravichandran and Jayakumar, 2015). In the present study, it was observed that the most effective dose of gamma ray in producing chlorophyll mutation was 1500 Gy as the number of mutants observed was more as compared to other two doses of gamma rays.

In 1000 Gy gamma ray treatment and 1000 Gy + 0.50% EMS treatments only virdis mutations were observed (Table 2). In 1000 Gy + 0.75% treatment, 50% chlorina and 50% virdis mutations appeared. In 1200 Gy treatment, only chlorina mutations were spotted. In 1200 + 0.25% EMS treatment, only virdis mutations were observed. In 1200+0.50% EMS treatment, chlorina and xantha each contributed to 25% of the mutations, while virdis accounted for 50% of the mutations. In 1200 Gy+0.75% EMS treatment, 50% of the mutations were albino, while rest 50% were virdis. In 1500 Gy+0.25% EMS treatment, 20% mutations were albino, 60% were chlorine, while rest 20% were virdis. For 1500+0.50% EMS treatment, only chlorina mutations were observed. However, for 1500 + 0.75% EMS treatment only virdis mutations were observed. For 0.25% EMS, 40% chlorina and 60% virdis mutations were noticed. For 0.50% EMS treatment, 25% albino and 75% chlorina mutations occurred. For 0.75% EMS treatment, 25% each of albina and virdis mutations and 50% chlorina were observed. 1500 Gy had highest mutagenic effectiveness (0.133) as shown in Table 3.

Table 3. Mutagenic effectiveness of different doses of gamma rays

Dose	Frequency	Effectiveness
1000 Gy	0.83	0.083
1200 Gy	0.83	0.097
1500 Gy	2.00	0.133

Apart from the chlorophyll mutations, phenotypic changes were also observed in the mutated progenies like bold seed mutants (1200 Gy + 0.25% EMS), mutants with appressed siliqua (1200 Gy + 0.50% EMS) and

Table 2. Spectrum of chlorophyll mutations in Indian mustard

Dose	Frequency of chlorophyll mutations				
	Albina	Chlorina	Xantha	Virdis	Albovirdis
0 Gy	-	-	-	-	-
1000 Gy	-	-	-	100.00	-
1000 Gy+0.25% EMS	-	-	-	-	-
1000 Gy+0.50% EMS	-	-	-	100.00	-
1000 Gy+0.75% EMS	-	50.00	-	50.00	-
1200 Gy	-	100.00	-	-	-
1200 Gy+0.25% EMS	-	-	-	100.00	-
1200 Gy+0.50% EMS	-	25.00	25.00	50.00	-
1200 Gy+0.75% EMS	50.00	-	-	50.00	-
1500 Gy	50.00	-	-	50.00	-
1500 Gy+0.25% EMS	20.00	60.00	-	20.00	-
1500 Gy+0.50% EMS	-	100.00	-	-	-
1500 Gy+0.75% EMS	-	-	-	100.00	-
0.25% EMS	-	40.00	-	60.00	-
0.50% EMS	25.00	75.00	-	-	-
0.75% EMS	25.00	50.00	-	25.00	-

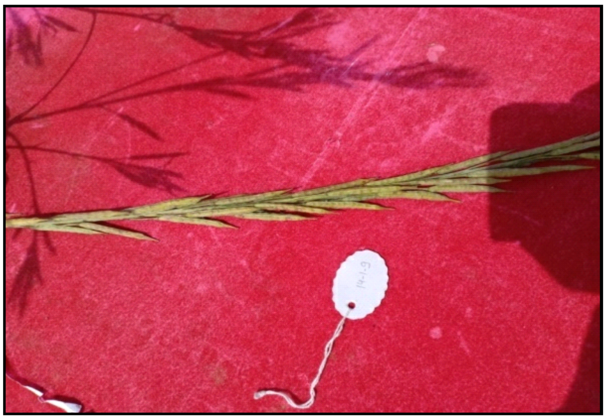

Fig. 5. Appressed siliqua mutant.

Fig. 6. Bold seed mutant.

Fig. 7. Dwarf mutant.

short-heighted plants/ dwarfs (0.25% EMS). These mutants were viable and survived in all of the progenies (Figs. 5, 6 and 7).

The antioxidant in Indian mustard is of utmost importance due to their free radical scavenging activity. The antioxidant activity in control samples was found to be in the range of 16 mg/g ascorbic acid equivalents. Whereas in the seeds treated with different combinations of gamma rays and EMS, the total antioxidant activity improved and increased up to 5-10% from the control in some of the samples. The improvement in antioxidant activity in mustard is beneficial for human health and reduces the risk of several health disorders related to oxidative stress. This opens a new window for the potential uses of mustard seed oil in food, pharmaceutical and cosmetic industries.

CONCLUSION

With the limited genetic variability in the germplasm of Indian mustard, sources must be searched extensively for inducing the mutations so that desirable traits can be incorporated and new varieties can be cultivated. Mutation breeding is one of the effective means to accomplish the goal. The different combinations of gamma rays and EMS as mutagenic mutants produced both non-viable chlorophyll mutants confirming the occurrence of mutations in the seed samples and useful viable mutants which showed morphological mutations like bold seeds and long silique. These morphological mutations give an extra edge as shorter varieties with bold seeds and long silique preferred by the farmers. The continuous improvement in these genotypes with mutations could ultimately lead to development of higher yielding varieties. The total antioxidant activity improved in some of the samples which is beneficial for human health. Thus, the present study concludes that gamma rays in combination with EMS can serve as a powerful mutagenic agent for generating the variability in the germplasm of *B. juncea*.

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