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# MUTATION FREQUENCY, EFFICIENCY AND EFFECTIVENESS OF GAMMA RAYS AND ETHYL METHANE SULPHONATE IN OKRA

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## Abstract

Where there is insufficient variation for a specific trait, or the available variation cannot be used due to hybridization barriers, mutagenesis may be used to create the variation. It is important to know the mutation frequency, efficiency and effectiveness of a particular mutagen dose. An experiment was conducted to determine mutagenic effectiveness and efficiency of gamma rays and Ethyl Methane Sulphonate (EMS) with the okra *Abelmoschus esculentus* L. Moench, cvs. $BCO^{-1}$ and Japanese Jhar Bhendi, at Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West-Bengal, India. Total mutation frequency was highest in the gamma irradiation treatment than EMS. Gamma radiation induced a higher frequency of chlorophyll mutants than did EMS. A higher mutagenic effectiveness, at lower concentration/dose, of the mutagen occurred. The 300 and 400 Gy doses of gamma irradiation and the 0.1% EMS produced high mutagenic efficiency. Gamma radiation was the most effective and efficient in inducing mutations in okra cultivars than was EMS.

***Key words***: *Abelmoschus esculentus*, chlorophyll mutants, effective dose, EMS, gamma radiation

## Introduction

Okra (*Abelmoschus esculentus* L. Moench) is grown under varying climatic conditions in almost all parts of India throughout the year. Okra has low seed germination during summer, and is attacked by pests and disease which can reduce yield. It is necessary to develop genotypes which are resistant to attack. The degree of variation in okra genotypes is limited and ways need to be found to increase variation.

Mutation is a sudden heritable change in an organism's genetic constitution. Applied mutagenesis is used to improve agronomic, pest and disease resistance, and post-harvest, traits where there is in-sufficient variation in available germplasm. Physical and chemical mutagens cause genes to mutate at rates over the spontaneous baseline, in this manner delivering novel attributes and widening genetic diversity of plants (Lagoda, 2007). Among physical mutagens, ionizing gamma rays are the most commonly used mutagens (Ashadevi *et al.,* 2017). They are electromagnetic radiations similar to X-rays in physical nature and action on the organism (Jadhav *et al.,* 2012) and among the chemical mutagens, Ethyl Methane Sulphonate ($CH_3SO_2OC_2H_5$) is used for inducing variability (Jayakumar and Selvaraj, 2003; Ashadevi *et al.,* 2017).

Mutations can be deleterious in nature. While conducting a mutation experiment, it is important to know mutation frequency, mutagenic efficiency and effectiveness of a particular mutagen dose. Mutation frequency is the proportion of mutated plants in the total population after exposure to a mutagenic agent. It is important to study mutagenic efficiency and effectiveness of a particular mutagen to recover a high frequency of useful mutations (Kumar and Mani, 1997). Mutagenic effectiveness is the number of mutations induced per unit dose of a mutagen in unit time; mutagenic efficiency is the extent of genetic damage caused by a mutagen dose when compared to the total biological damage in the first mutated ($M_1$) generation (Konzak *et al.,* 1964; Khan and Wani, 2006). Biological damage of a mutagenic agent is measured by lethality, injury and sterility. Lethality, the

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proportion of non-germinated seed, is an indication of the sensitivity of a genotype to a mutagenic dose (Gaul, 1958; Boranayaka, 2010). Some seed germinate, but show a variety of side effects which restrict growth and development into a complete plant, this is termed injury. Pollen of some mutants can be non-viable, with pollen sacs sometimes found to be empty resulting in sterility. An experiment was undertaken to study mutation frequency, efficiency and effectiveness of gamma rays and EMS in okra.

## Materials and Methods

Dried seed of okra, cvs. BCO-1 and Japanese Jhar Bhendi, were irradiated with doses of 100, 200, 300, 400 or 500 Gy $\gamma$ rays of $Co^{60}$ followed by treatment with 0.1, 0.2, 0.3, 0.4 or 0.5% EMS solution at the Department of Atomic Energy, Jadavpur Technical Campus, Kolkata, W.B., India, and in the laboratory of the Department of Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, India, respectively. Ethyl Methane Sulphonate, molecular weight of 14.16 g and specific gravity of 1.20, was purchased from Sigma Chemical Company (St. Louis, MO). Seed were soaked in distilled water for 6 hrs to activate the quiescent embryo before EMS treatment and to render cell membranes more permeable to a chemical mutagen causing quicker, direct, action to alter mutation frequency. Pre-soaked seed were placed in freshly prepared mutagenic solutions (using phosphate buffer of pH 7.0) with EMS solutions for 8 hrs at 25±2°C with intermittent shaking to provide uniform treatment to seed which were then thoroughly washed under running tap water for 1 hr to remove traces of chemical and then dried. For each treatment, 100 treated seed for each gamma ray level and EMS solution, with their controls, of each variety were sown in prepared beds at a spacing of 60 × 45 cm during spring-summer (February to July) season in the field in a completely randomized block design with 3 replications as the $M_1$ generation. Recommended cultural practices were followed to maintain good crop stand (Dhankhar and Mishra, 2001).

Seed germination, number of abnormal seedlings, chlorophyll mutants, seedling height and pollen sterility were recorded in the $M_1$ generation. The percent of $M_1$ plants emerging were averaged over 3 replications and reduction in germination was termed lethality (L). Average reduction in seedling height at 25 days after sowing over controls was termed injury (I). Pollen viability was determined by staining in 1% acetocarmine solution with the reduction in fertility compared to control plants and termed sterility (S).

Seed from all plants of each cultivar in the $M_1$ generation were bulked to produce the $M_2$ generation using the plant to row method in a completely randomized block design with 3 replications. In each replication 200 seed were sown. The $M_2$ population for each treatment and for each variety was screened to identify possible macro-mutants. Chlorophyll mutation frequency was determined as a percent of mutated $M_2$ progenies for chlorophyll deficiency (both viable and non-viable). Total mutation frequency (Mf) was determined as percent mutated $M_2$ progenies for chlorophyll deficient and other viable macro-mutants. Mutagenic effectiveness and mutagenic efficiency were measured as per the formulae of Konzak *et al.,* (1965).

## Results and Discussion

### Chlorophyll mutation frequency

Chlorophyll mutations give a standout amongst the most tried and true records for the assessment of genetic effects of mutagen treatments in various crop species. It is the most commonly occurring mutagenic effect in the plant kingdom. The frequency of chlorophyll mutants in $M_2$ generation is basically utilized as a trustworthy measure of genetic effects caused by mutagens (Nilan and Konzak, 1961).

Significant differences were observed between varieties (V), among doses (D) and for their interaction (V × D) in both mutagens Table 1. Among the various gamma irradiation doses Table 2, the mean chlorophyll mutation frequency was the highest (5.57%) at 300 Gy followed by 400 Gy (4.40%). Among EMS treatments Table 3, the highest chlorophyll mutation frequency was observed at 0.5% concentration (1.19) followed by 0.4% (1.18%). Comparison of Table 2 and 3 suggests that gamma irradiation had more chlorophyll mutation frequency than EMS treatment. Findings of this investigation corroborate earlier findings of Mahala *et al.* (2010) in the varieties of cluster bean RGC 936 and HGS 365. Contrastingly, Bhosle and Kothekar (2010) observed high chlorophyll mutation frequency in EMS treatments than Gamma rays in cluster bean.

The number of chlorophyll mutants obtained is also depended on the nature of genotype and mutagen. It varied conspicuously among the cultivars also. The higher chlorophyll mutation frequency found in Japanese Jhar Bhendi (4.55%) than BCO-1 (2.14%) in gamma irradiation Table 2 while BCO-1 (1.03%) had slightly high chlorophyll mutation frequency than Japanese Jhar Bhendi (0.96%) in case of EMS treatments Table 3. In BCO-1, the frequency of chlorophyll mutants was increased with the increase in dose of Gamma rays up to 300 Gy and sudden decrease was observed at 400 Gy followed by slight rise at 500 Gy while in Japanese Jhar Bhendi the increase in chlorophyll mutant frequency was tend

**Table 1:** ANOVA response for total mutation frequency, lethality, injury and sterility in okra.

| Source of variation | Gamma Irradiation | | | | | EMS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Chlorophyll mutation frequency | Total Mutation Frequency | Lethality | Injury | sterility | Chlorophyll mutation frequency | Total Mutation Frequency | Lethality | Injury | sterility |
| Varieties (V) | 43.76* | 27.23* | 12.52* | 1,383.6* | 1,203.8* | 0.29* | 0.03 | 84.0* | 45.07* | 599.07* |
| Dose (D) | 17.63* | 19.52* | 63.31* | 377.18* | 58.67 | 0.31* | 0.50* | 172.3* | 281.11* | 90.59* |
| Interaction VXD | 6.55* | 9.26* | 10.06 | 12.07 | 26.05 | 0.57* | 0.45* | 0.20 | 16.87 | 1.85 |
| Error | 1.67 | 1.97 | 30.44 | 37.7 | 72.71 | 0.03 | 0.033 | 9.51 | 8.56 | 32.03 |

*significant at 5% level

**Table 2:** Total mutation frequency due to gamma irradiation in $M_2$ generation of okra.

| Gamma irradiation (Gy) | Number $M_2$ plants examined | | Total mutants | | | | Chlorophyll mutation frequency (%) | | | Total mutation frequency (Mf)% | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Non-viable chlorophyll mutants | | Viable macro-mutants | | | | | | | |
| | BCO-1 | JJB | BCO-1 | JJB | BCO-1 | JJB | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean |
| 100 | 162 | 150 | 2 | 3 | 2 | 0 | 1.23 | 2.00 | 1.62 | 2.47 | 2.00 | 2.24 |
| 200 | 157 | 145 | 1 | 4 | 0 | 0 | 0.64 | 2.77 | 1.71 | 0.64 | 2.77 | 1.71 |
| 300 | 153 | 137 | 7 | 9 | 2 | 0 | 4.59 | 6.55 | 5.57 | 5.90 | 6.55 | 6.23 |
| 400 | 144 | 135 | 2 | 10 | 0 | 0 | 1.39 | 7.41 | 4.40 | 1.39 | 7.41 | 4.40 |
| 500 | 141 | 124 | 4 | 5 | 0 | 0 | 2.83 | 4.03 | 3.43 | 2.83 | 4.03 | 3.43 |
| Mean | | | | | | | 2.14 | 4.55 | | 2.65 | 4.55 | |
| | | | | | | | V | D | VXD | V | D | VXD |
| S.E (m) | | | | | | | 0.334 | 0.528 | 0.747 | 0.362 | 0.572 | 0.81 |
| C.D. | | | | | | | 1 | 1.581 | 2.237 | 1.084 | 1.714 | 2.424 |

**Table 3:** Total mutation frequency due to EMS in $M_2$ generation of okra.

| EMS (%) | Number $M_2$ plants examined | | Total mutants | | | | Chlorophyll mutation frequency (%) | | | Total mutation frequency (Mf)% | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Non-viable chlorophyll mutants | | Viable macro-mutants | | | | | | | |
| | BCO-1 | JJB | BCO-1 | JJB | BCO-1 | JJB | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean |
| 0.1 % | 149 | 156 | 2 | 1 | 0 | 1 | 1.35 | 0.64 | 0.99 | 1.35 | 1.28 | 1.32 |
| 0.2 % | 146 | 153 | 1 | 1 | 0 | 0 | 0.68 | 0.66 | 0.67 | 0.68 | 0.66 | 0.67 |
| 0.3 % | 137 | 146 | 1 | 1 | 0 | 0 | 0.73 | 0.68 | 0.71 | 0.73 | 0.68 | 0.71 |
| 0.4 % | 133 | 141 | 1 | 2 | 0 | 0 | 0.75 | 1.42 | 1.09 | 0.75 | 1.42 | 1.09 |
| 0.5 % | 124 | 136 | 2 | 1 | 0 | 0 | 1.61 | 0.74 | 1.18 | 1.61 | 0.74 | 1.18 |
| Mean | | | | | | | 1.03 | 0.96 | | 1.03 | 0.83 | |
| | | | | | | | V | D | VXD | V | D | VXD |
| S.E (m) | | | | | | | 0.05 | 0.07 | 0.10 | 0.05 | 0.07 | 0.11 |
| C.D. | | | | | | | 0.14 | 0.22 | 0.30 | N.S. | 0.222 | 0.314 |

increase with the rise in the dose of gamma rays up to 400Gy followed by a sudden decline at 500 Gy Fig. 1. The EMS at 0.2 and 0.3% almost induced similar frequencies of chlorophyll mutants in both the cultivars and interestingly, at the rest of the doses both the cultivars were oppositely responded in terms of chlorophyll mutant frequency Fig. 2. Kumar and Mishra (2006) observed the increase in chlorophyll mutation frequency with the increase in the doses of gamma rays.

Chlorophyll development in plants is by all accounts controlled by multiple genes situated close to the centromere and proximal segments of the chromosomes (Swaminathan, 1965). By and large, the nucleus genes control the biosynthesis of chlorophyll in plants through the long chain of biochemical reactions governed by multiple loci (Diana Svetleva, 2004). Restricted chromosome breakage by EMS treatment in these multiple gene regions may be attributed for getting chlorophyll lacking mutants (Gustafsson, 1965). As per Chopra (2005), the high frequency of chlorophyll transformations acquired with a mutagen, is because of particular activity of chemical and physical mutagens on genes governing chlorophyll production or the impacts on guanine in the G-C rich chloroplast genome.

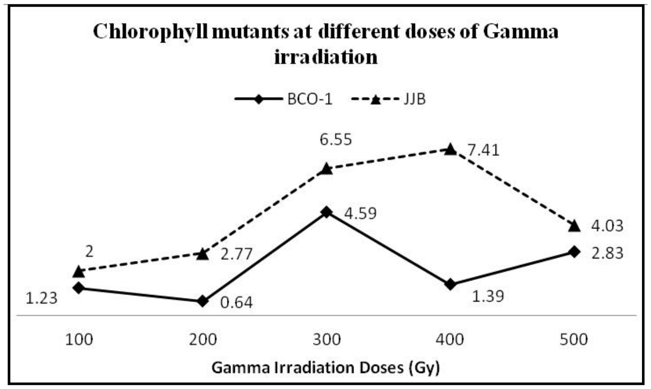


**Fig. 1:** Chlorophyll mutants at different doses of Gamma irradiation.

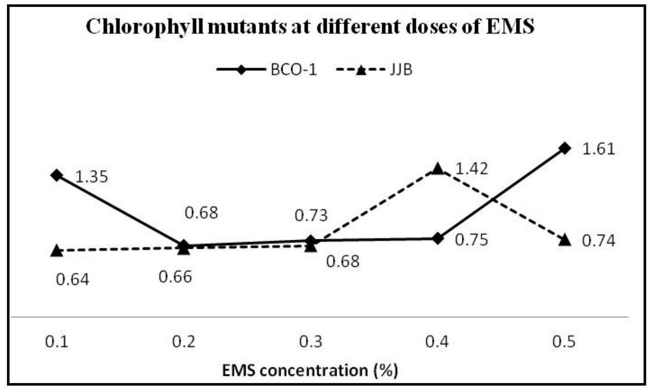


**Fig. 2:** Chlorophyll mutants at different doses of EMS.

## Total mutation frequency

Significant differences were observed for total mutation frequency at varieties, gamma irradiation doses and their interaction level. But, in case of EMS, there was no significant difference between the varieties for total mutation frequency. For lethality and injury, significant differences were there between the varieties and among the gamma irradiation doses but not for their interaction suggesting that there was no interaction effect for these traits. In case of sterility, significant differences were observed between the varieties only. In EMS treatments, significant differences were observed for lethality, injury and sterility between the varieties and doses, but, not for their interaction Table 1.

Mutation frequency was calculated by using chlorophyll mutants and other viable mutants. Previously several researchers like Konzak *et al.,* (1965), Datta and Biswas (1985) and Kumar and Mishra (2006) also used chlorophyll mutants for determining the mutation frequency. Perusal of Table 2 and 3 suggested that the gamma irradiation had high total mutation frequency than EMS. In BCO-1 gamma radiation at 500 Gy (2.83%) had moderate total mutation frequency Table 2 with high percentage of lethality, injury and sterility as 21.51%, 2051% and 39.35% respectively Table 4. In case of Japanese Jhar Bhendi 400 Gy (7.41%) radiation had more total mutation frequency Table 2 with high per cent of lethality and injury as 20.69% and 20.3% respectively Table 4. The highest total mutation frequency in BCO-1

**Table 4**. Lethality, Injury and Sterility due to gamma irradiation in $M_2$ generation of okra.

| Gamma | Lethality | | | Injury | | | Sterility | | |
|---|---|---|---|---|---|---|---|---|---|
| (Gy) | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean |
| 100 | 12.1 | 13.8 | 12.9 | 2.58 | 15.20 | 8.89 | 26.7 | 17.4 | 22.1 |
| 200 | 14.0 | 15.1 | 14.5 | 3.85 | 19.00 | 11.43 | 28.3 | 19.0 | 23.7 |
| 300 | 15.1 | 19.8 | 17.5 | 7.69 | 20.30 | 13.99 | 30.0 | 19.5 | 24.7 |
| 400 | 19.3 | 20.7 | 20.0 | 10.26 | 20.30 | 15.28 | 34.9 | 19.5 | 27.2 |
| 500 | 21.5 | 19.0 | 20.2 | 20.51 | 38.00 | 29.26 | 39.4 | 20.7 | 30.0 |
| Mean | 16.4 | 17.7 | | 8.98 | 22.56 | | 31.9 | 19.2 | |
| | V | D | V X D | V | D | V X D | V | D | V X D |
| S.E (m) | 1.42 | 2.25 | 3.19 | 1.59 | 2.51 | 3.55 | 2.20 | 3.48 | 4.92 |
| C.D. | N/A | N/A | N/A | 4.75 | 7.51 | N/A | 6.59 | N/A | N/A |

**Table 5:** Lethality, Injury and Sterility due to EMS in $M_2$ generation of okra.

| EMS | Lethality | | | Injury | | | Sterility | | |
|---|---|---|---|---|---|---|---|---|---|
| (%) | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean |
| 0.1 | 11.32 | 7.76 | 9.54 | 2.56 | 5.10 | 3.83 | 29.45 | 21.99 | 25.72 |
| 0.2 | 12.07 | 8.19 | 10.13 | 12.82 | 6.30 | 9.56 | 32.46 | 22.70 | 27.58 |
| 0.3 | 18.49 | 15.52 | 17.00 | 16.67 | 15.20 | 15.94 | 34.25 | 24.36 | 29.31 |
| 0.4 | 19.99 | 16.81 | 18.40 | 20.51 | 16.50 | 18.50 | 37.04 | 28.99 | 33.02 |
| 0.5 | 23.39 | 20.25 | 21.82 | 21.79 | 19.00 | 20.39 | 39.94 | 30.41 | 35.17 |
| Mean | 17.05 | 13.71 | | 14.87 | 12.42 | | 34.63 | 25.69 | |
| | V | D | V X D | V | D | V X D | V | D | V X D |
| S.E (m) | 0.80 | 1.26 | 1.78 | 0.76 | 1.19 | 1.69 | 1.46 | 2.31 | 3.27 |
| C.D. | 2.38 | 3.77 | N/A | 2.26 | 3.58 | N/A | 4.38 | N/A | N/A |

**Table 6:** ANOVA response for mutagen efficiency and mutatgenic effectiveness in okra.

| Source of variation | Gamma Irradiation | | | | EMS | | | |
|---|---|---|---|---|---|---|---|---|
| | Mutagen Efficiency based on Lethality | Mutagen Efficiency based on Injury | Mutagen Efficiency based on Sterility | Mutagenic Effectiveness | Mutagen Efficiency based on Lethality | Mutagen Efficiency based on Injury | Mutagen Efficiency based on Sterility | Mutagenic Effectiveness |
| Varieties (V) | 0.046* | 0.363* | 0.170* | 196.6* | 0.002* | 0.011* | 0.0001* | 0.007* |
| Dose (D) | 0.051* | 0.254* | 0.038* | 307.8* | 0.010* | 0.128* | 0.001* | 3.171* |
| Interaction VXD | 0.031* | 0.261* | 0.020* | 90.5* | 0.002* | 0.027* | 0.0005* | 0.056 |
| Error | 0.007 | 0.019 | 0.004 | 26.2 | 0.0001 | 0.002 | 0.0001 | 0.053 |

*significant at 5% level

(1.61%) and was recorded at 0.5% EMS whereas Japanese Jhar Bhendi (0.74%) had the highest total mutation frequency at 0.4% EMS Table 3.

Some lower doses of mutagenic treatments showed considerably high mutation frequency and less lethality, injury and sterility, whereas in some high dose treatments showed more mutation frequency, lethality, injury and sterility. Generally, mutagenic treatments that induce high mutation rate with lesser damage and ill effects are highly solicited because in mutation breeding programme only changes that are desirably accepted when they are free from undesirable genetic alteration. So, in that way such mutagenic treatments are desirable. In both the cultivars EMS at 0.1% Table 3 had high total mutation frequency (1.35%, 1.28%) and low lethality (11.32%, 7.76%), injury (2.56, 5.1%) and sterility (29.45%, 21.99%) as per Table 5. In BCO-1, gamma irradiation at a dosage of 100 Gy had moderate total mutation frequency (2.47%) with low injury (2.58%) Table 4 while 300 Gy dosage had high total mutation frequency (5.90) coupled with moderate levels of lethality (15.09%), injury (7.69%) and sterility (30.04%) Table 4. In case of Japanese Jhar Bhendi, the lower doses of gamma radiation had lower mutation frequency and also lower values of lethality, injury and sterility Table 4.

## Mutagenic effectiveness

Significant differences were observed for mutagenic effectiveness between varieties and doses in gamma irradiation as well as EMS. But, for their interaction (V x D), siginificant difference was observed only in gamma irradiation indicating that EMS doses did not interact with the varieties for mutagenic effectiveness Table 6.

Mutagenic effectiveness is defined as a measure of the frequency of mutation induced by a unit of mutagen (Konzak *et al.,* 1965). The degree of effectiveness of mutagen and the response of the variety was variable. Mutagenic effectiveness was high for BCO-1 (24.7) at 100 Gy irradiation and for Japanese Jhar Bhendi (21.8) at 300 Gy radiation suggesting higher mutagenic effectiveness at lower to moderate concentration/dose of the mutagen Table 7. Mutagenic effectiveness was high at 0.1% concentration in both the varieties Table 8. The mutagenic effectiveness of the EMS doses was very low when compared with the gamma irradiation in both the cultivars Table 7 and 8. Similar findings were recorded earlier in different crops (Kashid, 2004 in okra; Kumar *et al.,* 2007 in blackgram).

Among the two cultivars, BCO-1 emerged as the most responsive one in producing viable macro mutants Table 2. Lower to moderate doses of gamma irradiation (100 Gy and 300 Gy) proved to be the most effective followed by EMS treatment at 0.1 % concentration for inducing a broad spectrum of viable mutation in Okra. Similar observations of a general decrease in effectiveness with increasing doses of gamma rays irradiation were reported in cluster bean (Bhosle and Kothekar, 2010), in finger millet (Maduli and Misra, 2007; Ambavane *et al.,* 2015) and in Sesame (Rajaramadoss *et al.,* 2014).

## Mutagenic efficiency

Mutagenic efficiency is defined as the production of desirable changes which are free from associations with undesirable genetic alterations. This is generally measured by the proportion of the mutation frequency in relation to damages associated with mutagenic treatments as lethality, injury and sterility, etc. (Konzak *et al.,* 1965, Gaul *et al.,* 1972). Hence, mutagenic efficiency gives an idea of the proportion of mutation in relation to deleterious effects of the mutagen. Thus, mutagenic efficiency is most appropriate than mutagenic effectiveness in demining the efficiency of a mutagen. The higher efficiency of a mutagen indicates relatively less biological damage in relation to mutation-induced (Jain and Khandelwal, 2009).

Significant differences were observed for mutagenic efficiency based on injury, lethality and sterility at varieties, doses and interaction level in BCO-1 and Japanese Jhar Bhendi Table 6. In the cultivar BCO-1 Table 7 gamma irradiation at 300 Gy had high mutagenic efficiency for lethality (0.39) and sterility (0.20) and moderately high

**Table 7:** Mutagenic efficiency and effectiveness of gamma irradiation in okra.

| Gamma (Gy) | Mutagen Efficiency based on Lethality | | | Mutagen Efficiency based on Injury | | | Mutagen Efficiency based on Sterility | | | Mutagenic Effectiveness | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean |
| 100 | 0.20 | 0.15 | 0.18 | 0.96 | 0.13 | 0.55 | 0.09 | 0.11 | 0.10 | 24.7 | 20.0 | 22.3 |
| 200 | 0.05 | 0.18 | 0.12 | 0.17 | 0.15 | 0.16 | 0.02 | 0.15 | 0.09 | 3.20 | 13.8 | 8.50 |
| 300 | 0.39 | 0.33 | 0.36 | 0.77 | 0.32 | 0.55 | 0.20 | 0.34 | 0.27 | 19.7 | 21.8 | 20.7 |
| 400 | 0.07 | 0.36 | 0.22 | 0.14 | 0.37 | 0.26 | 0.04 | 0.38 | 0.21 | 3.50 | 18.5 | 11.0 |
| 500 | 0.13 | 0.21 | 0.17 | 0.14 | 0.11 | 0.13 | 0.07 | 0.19 | 0.13 | 5.70 | 8.1 | 6.90 |
| Mean | 0.17 | 0.25 | | 0.44 | 0.22 | | 0.08 | 0.24 | | 11.3 | 16.5 | |
| | V | D | VXD | V | D | VXD | V | D | VXD | V | D | VXD |
| S.E (m) | 0.02 | 0.03 | 0.05 | 0.04 | 0.06 | 0.08 | 0.02 | 0.03 | 0.04 | 1.32 | 2.09 | 2.95 |
| C.D. | 0.06 | 0.10 | 0.14 | 0.11 | 0.17 | 0.24 | 0.05 | 0.08 | 0.11 | 3.95 | 6.25 | 8.84 |

**Table 8:** Mutagenic efficiency and effectiveness of EMS in okra.

| Gamma (Gy) | Mutagen Efficiency based on Lethality | | | Mutagen Efficiency based on Injury | | | Mutagen Efficiency based on Sterility | | | Mutagenic Effectiveness | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean | BCO-1 | JJB | Mean |
| 100 | 0.12 | 0.17 | 0.15 | 0.52 | 0.25 | 0.39 | 0.05 | 0.06 | 0.06 | 2.09 | 1.99 | 2.04 |
| 200 | 0.06 | 0.08 | 0.07 | 0.05 | 0.10 | 0.08 | 0.02 | 0.03 | 0.03 | 0.53 | 0.51 | 0.52 |
| 300 | 0.04 | 0.04 | 0.04 | 0.04 | 0.05 | 0.05 | 0.02 | 0.03 | 0.03 | 0.38 | 0.35 | 0.37 |
| 400 | 0.04 | 0.08 | 0.06 | 0.04 | 0.09 | 0.07 | 0.02 | 0.05 | 0.04 | 0.29 | 0.55 | 0.42 |
| 500 | 0.07 | 0.04 | 0.06 | 0.07 | 0.04 | 0.06 | 0.04 | 0.02 | 0.03 | 0.50 | 0.23 | 0.37 |
| Mean | 0.07 | 0.08 | | 0.14 | 0.11 | | 0.03 | 0.04 | | 0.76 | 0.73 | |
| | V | D | VXD | V | D | VXD | V | D | VXD | V | D | VXD |
| S.E (m) | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 0.002 | 0.004 | 0.005 | 0.06 | 0.09 | 0.13 |
| C.D. | N/A | 0.03 | 0.04 | 0.03 | 0.05 | 0.07 | 0.007 | 0.012 | 0.016 | N/A | 0.28 | N/A |

for injury (0.77). The highest mutagenic efficiency for injury (0.96) was reported at 100 Gy. In Japanese Jhar Bhendi Table 7 the gamma radiation at 400 Gy was having the highest mutagenic efficiency for lethality (0.36) injury (0.37) and sterility (0.38). Though the lower doses of gamma radiation viz., 100 and 200 Gy had lower values of lethality, injury and sterility in Japanese Jhar Bhendi, the total mutation frequencies for these doses were less resulting in the lower mutagenic efficiencies. The mutagenic efficiency values of EMS are complementary to the mutation frequencies. More the dosage lesser the efficiency was observed. In both the cultivars, BCO-1 and Japanese Jhar Bhendi, low concentration of EMS (0.1%) had the higher mutagenic efficiency values for lethality (0.12, 0.17), injury (0.52, 0.25) and sterility (0.05, 0.06) as per Table 8. This higher mutagenic efficiency at lower concentrations was because of the lower levels of biological damage (Kashid, 2004; Jain and Khandelwal, 2009; Bhosle and Kothekar, 2010). Comparison of Table 7 and 8 depicted that mutagenic efficiency based on lethality, injury and sterility were higher for gamma irradiation than EMS. This finding was in conformity with the earlier reports in pigeon pea (Chary and Bhalla, 1988), Rice (Pillai *et al.,* 1993) and cluster bean (Dube *et al.,* 2011).

It can be concluded from the present experiment that gamma radiation is more efficient than EMS in inducing the mutations in okra. Total mutation frequency and mutagenic effectiveness at lethality, injury and sterility level were high for gamma radiation than EMS in Okra. Mean chlorophyll mutants were high in gamma radiation than EMS. The lower doses of EMS had the more effectiveness than higher concentrations owing to less damage at lower concentrations. But, in gamma rays the moderate doses (300 Gy in BCO-1 and 400 Gy in Japanese Jhar Bhendi) had the higher mutagenic efficiencies due to the lower total mutation frequencies at lower doses.

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