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**Research Article**

# EFFECT OF SODIUM AZIDE AND GAMMA RAYS TREATMENTS ON PERCENTAGE GERMINATION, SURVIVAL, MORPHOLOGICAL VARIATION AND CHLOROPHYLL MUTATION IN MUSK OKRA (*ABELMOSCHUS MOSCHATUS* L.)

**ASHISH R. WARGHAT[1]*, NANDKISHOR H. RAMPURE[2], PRASHANT WAGH[2]**

**[1]Defence Institute of High Altitude Research, Defence R & D Organization C/o 56 APO, Leh 194101, India, [2]Department of Botany, R.T.M. Nagpur University, Nagpur 440033, India. Email:ashishwarghat@hotmail.com**

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**ABSTRACT**

*Abelmoschus moschatus* medik. L. is an important medicinal herb of family Malvaceae. Ambrette oil is used in various pharmaceutical industries due to its high medicinal properties. Seeds of musk okra (*Abelmoschus moschatus*) were exposed to varying concentrations of sodium azide ($NaN_3$) in the range 0.05-0.20 % and gamma rays $^{60}$co doses in the range 20-80 kR. Variations in the percent germination, survival, seedling height ($LD_{50}$), chlorophyll mutants were recorded during this experiment. The $LD_{50}$ values based on survival percentage were fixed at 0.10 % for sodium azide and 80 kR. gamma rays respectively. Effect of the mutagenic treatments were resulted in decreasing percentage germination, survival, average plant height, number of buds and number of branches but increasing total leaf area, number of flower and number of fruits/plants. Sodium azide and gamma rays treatments were produced leaf shape variation and chlorophyll mutants. In the present study, gamma rays was provide to be more effective and efficient mutagen for producing robust/high yields and can be required to obtain useful mutants in *A.moschatus.*

**Keywords**: Sodium azide, Gamma rays, $LD_{50}$, Chlorophyll mutant

## INTRODUCTION

Mutation is a sudden heritable change in an organism. Which may be structural or functional? But generally structural changes occur. It is produced by changes in base sequence of gene and it can be spontaneously or artificially both in seed and vegetative propagated crops. But seed is the most commonly used material for mutational studies because it can tolerate physical conditions. Induced mutations have recently become the subject of biotechnology and molecular investigation leading to description of the structure and function of related genes. Induced mutation in plant is an effective tool for crop improvement. The mutagenic effects of sodium azide have been documented in previous reports. Kleinhofs *et al.*[1] reported that sodium azide is a very potent mutagen in barley and induced chlorophyll deficiency as well as a wide range of morphological and physiological mutants and Froicer[2] reported that gamma ray mutagenesis can be expected to yield severe phenotypic high proportion useful mutations with normal yielding properties. In plant cells; the nucleus is considered the principal site of damage by ionizing radiation. The process of leading to radiation damage may be summarized as: the initial physical, which lasts only a minute fraction of a second; the physico-chemical stage lasting about $10^{-16}$s; the chemical stage lasting a few seconds and the biological stage in which the time scale from tens of minutes to tens of years depending on the particular symptoms. Sparrow *et al.*[3] reported that there was a direct relationship between the radiosentivity of an herbaceous plant and average volume occupied by a chromosome in the cell nucleus. Larger the chromosome volume, more sensitive is the plant and hence, smaller the radiation dose required to kill the plant or to cause a certain degree of damage. Improved quality and quantity of crops have been achieved through radiation-induced mutation. However, the harmful effects of deleterious mutation include death in the embryonic state, inability to reproduce, increased Susceptibility to disease and decrease in life expectancy of a few months.

The seed of this plant are known as Ambrette seed, which contain moisture 11.14 %, protein 2.3 %, starch 13.35 % and fatty oil 14.5 %. Seeds yield a volatile oil known as musk seed oil or Ambrette seed oil, which is in high demand at national, international and pharmaceutical industries level due to its anticancerous, aphrodisiac and antidepression properties documented by Verma *et al.*[4].To increase the production of this crop there is a need to have a better understanding of its genetic background. However, there is a lack of information about varieties of *A. moschatus*, which require variability because of their pollination status. The present study was therefore undertaken to fill the gap in knowledge of the genetic background of the crop and assess the effect of the two mutagens on the plant. The present investigation was undertaken to study the effect of chemical and physical mutagen in different concentration and dose treatments on germination, survival, $LD_{50}$ and chlorophyll mutation. Such a study is needful to unveil any desirable features for quantitative traits, agronomic, Phytochemical and pharmaceutical benefits.

## MATERIALS AND METHODS

### Germination

Dry and healthy seeds of musk okra were obtained from 'Shree Shell', Nagpur were used for this investigation. The experiment was categorized into two treatment groups. i.e. sodium azide and gamma rays. In first category, seeds were exposed to 0.05-0.20 % concentration of sodium azide for 18 hrs. After that washed under running tap water to remove excess chemicals and exudates from the seeds and in second category; 20,40,60,80 kR. doses of gamma rays using $^{60}$co as a source in gamma chamber. The seeds were observed daily until maximum germination was achieved.

### Pot experiments

Pot studies were undertaken to determine the effects of the two mutagens on survival, average plant height, number of flower, total leaf area and number of branches. Number of fruits/plant and number of bud were determined to evaluate the effect on yield. The effect of the two mutagens on lethality, morphological injury and chlorophyll mutations were also investigated.

## RESULT AND DISCUSSION

There were reductions in the germination and survival percentages with increasing concentrations of two mutagens. Reductions in germination and survival percentages due to the effect of mutagens on various crop plants have earlier been documented by Mensah[5], Mensah *et al.*[6]; Mensah *et al.*[7] However the effects were more pronounced in *A. moschatus.* $LD_{50}$ value was calculated on the basis of 50 percent reduction of germination seeds count. The present investigation exhibited that the germination percentage of *A. moschatus* decreased with the increase in the Dose or concentration of the mutagens were used to find out the $LD_{50}$ values for further studies. It was estimated that using 50 % reduction in seed germination observed at 0.10 %

sodium azide and 80 kR. of gamma rays. (Fig 1 & 2). The impact and tolerance of the biological material to a mutagen was marked in *A. moschatus* in terms of germination and survival. In the present investigation, germination, survival percentage, average plant height and total leaf area was decreased with increasing dose/concentration shown is (Table 1) and the viable mutants were observed is mainly used as a dependable measure of genetic effect in mutagen reported by Nilan *et al.*[8]and Gautam *et al.*[9] The maximum viable mutants were observed in physical mutagen (Gamma rays) while, minimum in chemical mutagen (sodium azide) shown in (Table 2).

**Albino**: In this type of chlorophyll mutation, leaves were white in color due to absence of all pigment. This was leaded to the death of the plants at 10-15 days after germination.

**Leaf shape:** It is also induced by mutagen, due to vary in shape of leaves. (Fig 3)

Keeping in view, it was observed that Gamma rays were more pronounced in inducing chlorophyll mutations than sodium azide. However, the number of buds and branches in different treatments indicated significant reductions in the higher doses of the mutagen only. The results revealed that the most efficient concentration for inducing mutations in *A. moschatus* using sodium azide is 0.20 % and 80 kR. gamma rays. Leaf abnormalities with mutagenic treatment were observed, albino and other included changes in shape of leaves and chlorophyll deficiencies. The abnormalities were mainly due to nature. Mutagen (0.80 kR. and 0.20 %) significantly produced more branches, more buds and number of fruits/plants than the control plants. Similar results were reported by Biswas *et al.*[10] in *Trigonella foenumfraeum*.

**Table 1: Effect of sodium azide (SA) and gamma rays (GR) on some agronomic characters of *A. moschatus***

| Parameters | | Concentrations | | | | |
|---|---|---|---|---|---|---|
| | | Control<br>Control | 0.05%<br>20 kR. | 0.10 %<br>40 kR. | 0.15%<br>60 kR. | 0.20 %<br>80 kR. |
| Germination (%) | SA | 100 | 30 | 35 | 40 | 40 |
| | GR | 100 | 50 | 30 | 50 | 70 |
| Survival (%) | SA | 100 | 70 | 84.6 | 55 | 40 |
| | GR | 100 | 55 | 40 | 75.2 | 45 |
| Average plant height (cm) | SA | 32.3 | 34.2 | 29.7 | 35.4 | 30.6 |
| | GR | 32.3 | 31.6 | 31.9 | 28.7 | 23.3 |
| No. of flower | SA | 5.1 | 5.3 | 4.8 | 5.8 | 6.2 |
| | GR | 5.1 | 6.3 | 5.4 | 5.5 | 10.9 |
| No. of bud | SA | 9.5 | 8.2 | 7.1 | 6.2 | 8.5 |
| | GR | 9.5 | 7.9 | 6.3 | 11.2 | 12.1 |
| No. of fruits/plant | SA | 5.1 | 5.3 | 4.8 | 5.8 | 6.2 |
| | GR | 5.1 | 6.3 | 5.4 | 5.5 | 10.9 |
| Total leaf area | SA | 23.8 | 36.5 | 22.9 | 39.2 | 48.6 |
| | GR | 23.8 | 43.6 | 28.6 | 43.7 | 58.2 |
| No. of branches | SA | 11.9 | 10.5 | 12.5 | 8.9 | 11.5 |
| | GR | 11.9 | 12.5 | 12 | 9.3 | 11.3 |

SA= Sodium azide, GR= Gamma rays

**Table 2: Chlorophyll mutation observation**

| Sr. No. | Concentration | No. of Mutation | Type of Mutation |
|---|---|---|---|
| 1 | Control | No | No |
| 2 | 0.05 % | 2 | 1 Leaf shape |
| 3 | 0.10 % | 2 | 1 Leaf shape +1 albino |
| 4 | 0.15 % | 2 | 1 Leaf shape |
| 5 | 0.20 % | 1 | 1 Leaf shape+1 albino |
| 6 | 20 kR | 4 | 3 leaf shape+ 1albino |
| 7 | 40 kR | 1 | 1 leaf shape |
| 8 | 60 kR | 4 | 3 leaf shape +1 albino |
| 9 | 80 kR | 11 | 9 leaf shape +2 albino |

**Percentage germination**

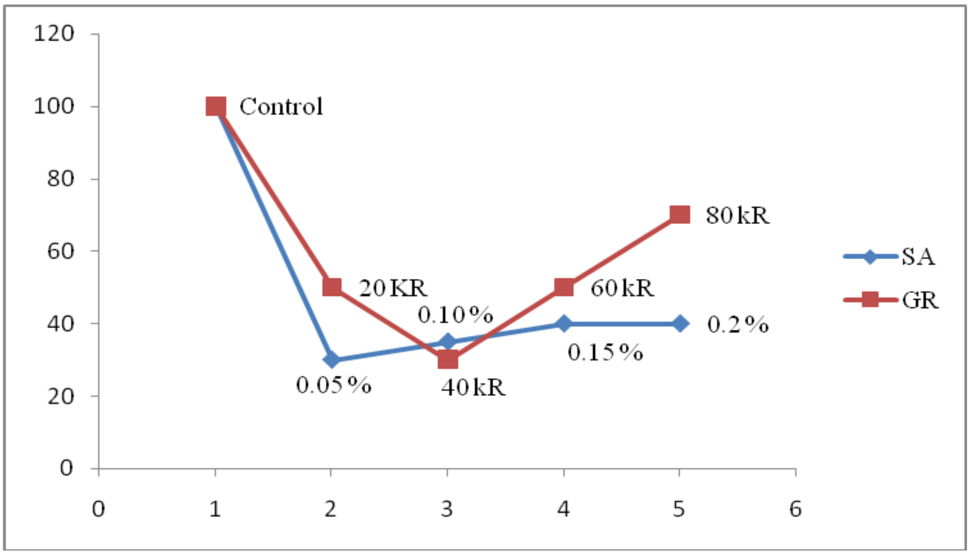



**Fig. 1: Effect of sodium azide and gamma rays on the germination percentage of *A. moschatus***

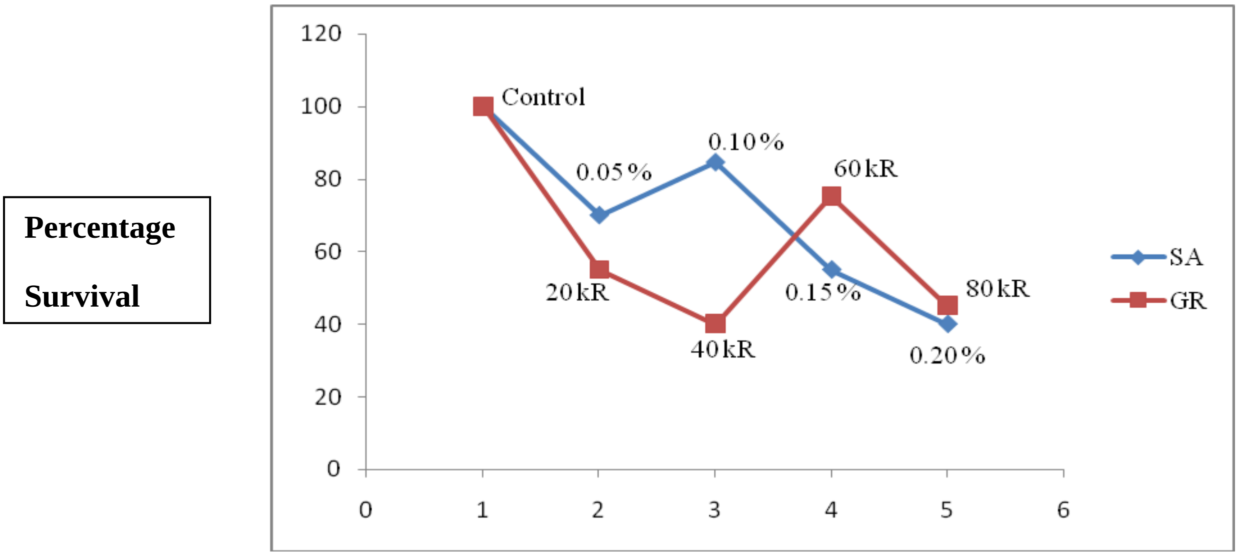
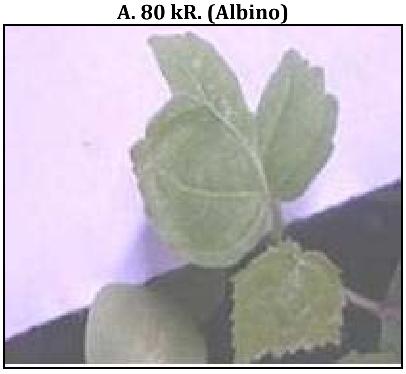


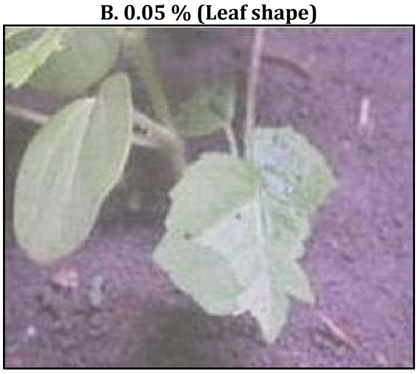

**Fig. 2: Effect of sodium azide and gamma rays on survival percentage of *A. moschatus***

**A. 80 kR. (Albino)**

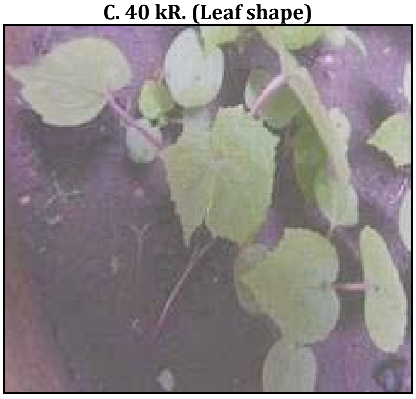
**B. 0.05 % (Leaf shape)**



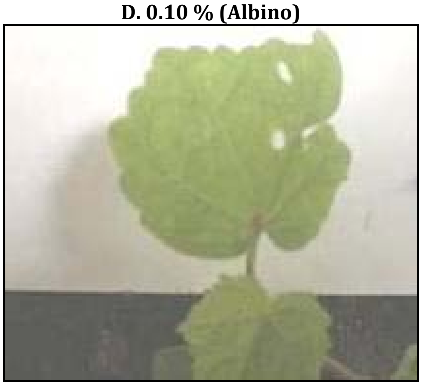
**C. 40 kR. (Leaf shape)**



**D. 0.10 % (Albino)**



**E. 20 kR. (Leaf shape)**



**F. 0.15 % (Leaf shape)**

**Fig. 3: Chlorophyll mutation**

**CONCLUSION**

However, plants with higher yield were observed under (0.20 % and 80 kR.) of sodium azide and gamma rays. The useful mutant isolated through the present study need to be tested further on a wider scale to establish any changes in chromosome or allele frequency and also to assess its performance in later generations.

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