

# Effect of Gamma Ray and Sodium Azide on the Germination, Survival and Morphology of Varieties of Okro (*Abelmoschus esculantus* L. moench)

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## Abstract

Okro, *Abelmoschus esculantus* L. Moench is an important medicinal herb of the family Malvaceae. The oil is used in various pharmaceutical industries due to its high medicinal properties. Three varieties of okro seeds: Jokoso, NHAe47-4 and a local variety (Beru) were exposed to varying concentrations of sodium azide (NaN<sub>3</sub>) in the range of 1.0mM to 4.0mM and gamma rays 60Co doses in the range 20-100 kR respectively. Morphological traits of the genotypes were measured according to the coding criteria specified by the Standard International Crop Descriptor for okro. Variations in the percentage germination, survival, seedling height, stem height; leaf length and the fruit length were recorded. There were significant differences between the control and the treated seeds in all the characters considered. Effect of the mutagenic treatments resulted in decreasing percentage germination, survival, and average stem height, leaf length and fruit length with increase in concentration and doses of the mutagens. The effective concentration of sodium azide solution for okro seeds are: 1.0mM, 2.0mM, 3.0mM, and 4.0mM. While the effective doses of Gamma rays for okro seeds are 20kR, 40 kR, and 60 kR for the varieties used in this research. All the three varieties responded similarly to the concentrations of Sodium azide. Sodium azide and gamma rays can be used for inducing mutation that will produce genetic variability in Okro.

**Key Words:** Concentrations; Agronomic Characters; Genotypes; Mutagenic Treatments;

## 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 mutation has been established as an important tool for improvement of certain traits in the existing germplasm. Mutations are of two type viz., natural and artificial or induced mutation. Frequency of natural mutation is very low and hence, artificial mutation with application of mutagens is followed to get

better genetic variability. Various types of chemicals capable of inducing mutation in plants had been found out. They are Ethyl Methane Sulphonate (EMS), Methyl Methane Sulphonate (MMS), diethylsulphate (DES), Ethylene Imine (EI), Hydroxyl Amine (HA), n-nitroso-n-ethyl urea (NEU), Nitrous Acid (NA), sulphur mustard, 5-bromouracil, Sodium Azide (SA) etc. [5].

Considerable progress has been made on induce mutations of okro [*Abelmoschus esculantus* (L.) Moench] for improvement of their certain characters, but detailed studies are lacking. Traits like germination, pollen sterility, plant length, fruit length, survival to maturity and mortality are much affected by mutagenic treatment. Therefore, an attempt has been made to study the effect of mutagenic treatment on agronomic characters of okro. 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 [1].

The mutagenic effects of sodium azide have been documented in previous reports. [6] 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 [3] reported that gamma ray mutagenesis can be expected to yield severe phenotypic high proportion of useful mutations with normal yielding properties. In plant cells; the nucleus is considered the principal site of damage by ionizing radiation [1].

## Materials and Methods

### Plant Source and Mutagen Treatment

Two improved varieties ( Table ) of Okro, *Abelmoschus esculantus* L. Moench seeds were collected from Institute of Agricultural Research (Plant science department) and a local variety was purchased in Sabo main market Zaria and taken to Centre for Energy Research and Development, Obafemi Awolowo University Ile-ife (7° 28'N and 4° 32'E) for radiation with Gamma cell 220 Cobalt 60 (Co<sup>60</sup>) part of the seeds were treated with Sodium azide

in the laboratory in Biological Sciences department, Ahmadu Bello University (A.B.U.), Zaria.(11° 04'N and 7°42'E) The Sodium azide used for this research was collected from Biochemistry department A.B.U. zaria.

S/No	Genotypes	Description	Source
1	Beru	Local variety	Local market
2	Jokoso	Improved variety	Institute of Agricultural Research, Zaria
3	NHAe47-4	Improved variety	Institute of Agricultural Research, Zaria

### Planting of the Seeds

The experiment was categorized into two treatment groups. I.e. sodium azide and gamma rays. In first category, seeds were presoaked in buffer solution for two hours then soaked in sodium azide solutions of 1.0mM, 2.0mM, 3.0mM and 4.0mM for one hour after which the treated seeds were washed in tap water to remove excess chemicals and exudates from the seeds and in second category; 20,40,60,80 and 100 kR doses of gamma rays using Co60 as a source in gamma chamber. The seeds were observed daily until maximum germination was achieved. Planting of sets of seeds treated Sodium Azide, those treated with Gamma ray and the control seeds were done in Botanical garden, Ahmadu Bello University Zaria, using the open garden to study the effect of the two mutagens on each set of okro seeds. Each plot consisted of five rows and each row was four meters long with the intra row spacing of 50cm and inter row spacing of 75cm apart in three replications. Eight hills of two seeds each were planted per row. The layout follows 3×5 Randomized Complete Block Design (RCBD).

### Data Collection

The growth of plant was estimated in relation to the time of flowering, maturity and variability in plant development within and between treatments. The following data were collected: percentage germination, seedling height, and leaf length, survival at maturity, fruit length and height at maturity.

### Statistical Analysis

The data collected were subjected to the following statistical methods for analyzing the data: Mean (average) percentages, analysis of variance (ANOVA) and t-test was used to determine if there was any significant difference between the two mutagens.

## Results and Discussion

The T-Test Between The Two Mutagens Shows There Is Significant Difference In The Leave Length And Fruit Length For Nhae47-4. There Was No Significant Difference Between The Effects Of The Two Mutagens For The Other Parameters Of The Nhae47-4. Also, There Was No Significant Difference Between The Effects Of The Two Mutagens For The All Parameters Of Beru And Jokoso (Table 1). This Shows That The Two Mutagens Have Similar Effect On The Okro Varieties. This Is In Line With the Work Of [1].

**Table 1:** Result of t-test between Gamma ray and Sodium azide for all the varieties

Variety length	Seedling Height	Stem Height	Leave length	Fruit
Beru	0.376808	1.066381	1.023216	0.309004
Jokoso	1.408269	0.369527	0.093874	-0.14275
NHAe47-4	-0.03261	2.652084	2.389943*	4.834658*

\* Significant at the 5% level of probability

The present investigation revealed that the germination and survival percentages of the three varieties of okro seeds decrease 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 [9, 10].

It was estimated that for all the three varieties, germination % ranges from 80% to 95% observed at 1Mm to 4mM of sodium azide and 20% to 75% at 20 to 100kR of gamma rays. (Tables 2-4). The impact and tolerance of the biological material to the mutagens were marked in all the three varieties in terms of germination and seedling survival. This observation remained in parity with [11, 1, 5]. In this investigation, germination percentage, seedling height, average plant height, leaf length and fruit length decreases with increasing dose/concentration (Table 2- 4) and the viable mutants observed could be used as a dependable measure of genetic effect of mutagen. The maximum viable mutants were observed in chemical mutagen (sodium azide) while, minimum in physical mutagen (Gamma rays) shown in (Table 2-4). Similar results were also reported by [7, 4] in okro.

The seedling height, average plant height, leaf length and fruit length in different treatments showed significant reductions in the higher doses of the mutagens. The results revealed that the most efficient concentration for inducing mutations in the three varieties using sodium azide is 1, 2 and 3 Mm and 20, 40 and 60 kR for gamma rays. [8] Reported that the effectively of gamma rays induced mutations in okro cv. selection 2-2 with gamma irradiation ranges between 10 to 60 kR. Also [1] reported that Mutagen (0.80 kR. and 0.20 %) significantly produced more branches, more buds and number of fruits/plants than the control plants. [2] Reported similar results in *Trigonellafoenumfraeum*.

**Table 2:** Effect of Sodium Azide (SA) and Gamma Rays (GR) on agronomic characters of Beru Variety (Mean Length in centimeter)

Mutagen	Percentage (%) Germination	Seedling Height	Stem Height at Maturity	Leaf Length	Fruit Length
Control	97a	9.5a	28.00a	10.50a	5.60a
Gamma Ray 20 Kr	65a	8.5b	25.17a	8.92a	4.80a
Gamma Ray 40 Kr	75a	8.6b	21.13b	6.25b	4.90a
Gamma Ray 60 Kr	75a	7.2b	14.67c	6.50b	4.36a
Gamma Ray 100 Kr	50b	4.8c	5.67d	1.92c	-
SodiumAzide 1.0	96a	7.4b	-	-	-
SodiumAzide 2.0	95a	9.8	12.75c	5.80b	6.00a
Sodium Azide 3.0	95a	2.4d	13.25c	5.40b	3.83b
Sodium Azide 4.0	90a	6.5c	10.50c	5.90b	3.50b

Means followed by the same letter in the column are not significantly different (P>0.05)

**Table 3:** Effect of Sodium Azide (SA) and Gamma Rays (GR) on some agronomic characters of Jokoso Variety (Mean in Centimeter)

Mutagen	Percentage (%) Germination	Seedling Height	Stem Height at Maturity	Leaf Length	Fruit Length
Control	96a	6.70a	21.00a	10.00a	7.60a
Gamma Ray 20 Kr	40b	6.8a	14.80b	8.42a	6.0b
Gamma Ray 40 Kr	55a	6.4a	11.91b	6.25b	3.25d
Gamma Ray 60 Kr	40b	6.4a	9.67c	4.67c	-
Gamma Ray 100 Kr	20b	2.7b	Died	-	-
SodiumAzide 1.0	85a	5.2b	12.17b	5.40b	5.50b
SodiumAzide 2.0	85a	3.8b	9.17c	6.40b	4.30c
Sodium Azide 3.0	80a	5.2b	14.00b	9.50a	5.13b
Sodium Azide 4.0	80a	2.8b	11.00c	5.60b	4.50c

Means followed by the same letter in the column are not significantly different (P>0.05)

**Table 4:** Effect of Sodium Azide (SA) and Gamma Rays (GR) on some agronomic characters of NHAe47-4 (Mean in Centimeter)

Mutagen	Percentage (%) Germination	Seedling Height	Stem Height at Maturity	Leaf Length	Fruit Length
Control	95a	9.10b	24.00a	9.80a	5.70a
Gamma Ray 20 Kr	40b	6.50d	21.75b	9.83a	5.70a
Gamma Ray 40 Kr	50a	5.40e	16.33c	6.83b	5.30a
Gamma Ray 60 Kr	65a	5.60d	11.83d	8.58a	4.75a
Gamma Ray 100 Kr	35b	3.70f	5.00f	-	-
SodiumAzide 1.0	95a	4.90e	9.50e	3.80c	3.50b
SodiumAzide 2.0	95a	11.00a	9.25e	4.70c	3.63b
Sodium Azide 3.0	80a	2.80f	8.50e	3.40c	3.00b
Sodium Azide 4.0	80a	2.00g	-	-	-

Means followed by the same letter in the column are not significantly different (P>0.05)

## Summary and Conclusion

The outcomes of this research revealed that the most efficient concentrations for inducing mutations in the three varieties using sodium azide are: 1, 2 and 3 Mm and 20, 40 and 60 kR for gamma rays.

None of the mutants performed better than the control except in Beru variety where 2Mm of sodium Azide produces longer seedling height and fruit length (9.8cm and 6.0cm respectively). Hence the doses and concentrations of the mutagens induce variable mutations on the Okro varieties used in this research.

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