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Effect of combined doses of gamma ray and sodium azide (mutagenic agents) on the morphological traits of some varieties of okra (*Abelmoschus esculentus*)

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***Abelmoschus esculentus* (okra) is a popular health food because of its high fiber, vitamin C and folate content. It is also rich in antioxidants, a good source of calcium and potassium. Three varieties of okra: Jokoso, NHAe47-4 and Beru (a local variety) were irradiated with 20, 40, 60, 80 and 100 kr doses of gamma rays then soaked in 2.0 mM and 3.0 Mm of Sodium azide for 2h with the aim of determining the effects of the combined mutagens on their morphology. There were significant differences between the control and the treated seeds used in their germination, seedling survival, seeding length, survival to maturity and height at maturity. The effective concentrations and doses of combined mutagens for Beru and NHAe47-4 varieties were: 2.0 mM + 20 kr, 2.0 mM + 40 kr, and 2.0 mM + 60 kr and 3.0 mM + 20 kr, 3.0 mM + 40 kr, and 3.0 mM + 60 kr) while the effective concentrations and doses for Jokoso variety were: 2.0 mM + 20 kr, 2.0 mM 40 kr 2.0 mM 60 kr and 3.0 mM + 20 kr only. Jokoso variety was worse affected than any other. Sodium azide and gamma rays can be used for inducing mutation that will produce genetic variability in Okra.**

Key words: *Abelmoschus esculentus,* germination, genetic variability.

INTRODUCTION

Okra (*Abelmoschus esculentus)* is a perennial, often cultivated annually in temperate climates, and it grows to around 2 m tall. It is a flowering plant in the mallow family. It is related to such species as cotton, cocoa, and hibiscus. The leaves are 10 to 20 cm long, broad, palmately lobed with 5 to 7 lobes. The flowers are 4 to 8 cm in diameter, containing five white or yellow petals, with a red or purple spot at the base of each. The fruit is capsule-like and the length can be up to 18 cm long with pentagonal cross-section, containing numerous seeds. The crop is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits or pods containing round, white seeds. It is among the most heat- and drought-tolerant vegetable species in the world and it tolerates soils with heavy clay and intermittent moisture, but frost can damage the pods (Wikipedia, 2016).

Okra is a popular health food due to its high fiber,

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vitamin C, and folate content. It is also known for being high in antioxidants. The crop is rich in lutein which is an antioxidant found in several areas of the body including the skin and eyes. It is also a good source of calcium and potassium (Duvauchelle, 2012). Okra, known in many English-speaking countries as ladies' fingers, ochro or gumbo is valued for its edible green seed pods (https://en.wikipedia.org/wiki/OkraWikipedia, 2016).

A study carried out in 2009 revealed that okra oil suitable for use as a biofuel (Farooq, et al., 2010). It fibers were traditionally used to make rope for fish lines and game traps. It is suitable for paper and cardboard manufacture. Roasted okra seeds are used in some places as a substitute for coffee. The grounded pulp of *A. Caillei* stems is used as a stabilizer when making Pita beer in Ghana. Okra is a good source of calcium and oil. Oil from it seeds contains about 20% amino acid thus, it compares favorably with the oil found in poultry, eggs, and Soya beans. In genetics, a mutagen is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus, increases the frequency of mutations above the natural background level (https://www.ndsu.edu/pubweb/~mcclean/plsc431/mutation/mutation3.htm 21/4/2016). Mutation breeding has been done on okra by Norfadzrin et al. (2007) Manju and Gopimony (2009), Phadvibulya et al. (2009), Hegazi and Hamideldin (2010) and Muralidharan and Rajendran (2013) using different doses of gamma rays. Induced mutations have played a pivotal role in enhancing world food security, as new food crop varieties with various induced mutations have brought about a significant increase in crop production at locations people could directly access (Kharkwal and Shu, 2010). Induced mutations are significant as novel mutations are being isolated for enhanced nutrition quality of crop plants, for ex. micronutrients, protein, amino acids, fatty acids and vitamins (Navnath and Mukund, 2014).

Mutation, whether induced or natural, has played an important role in increasing variability leading to production of varieties of crop. Plant breeders induce mutation in crop varieties in order to produce high yielding and superior genotypes. In the process of breeding crop plants, the progress achievable tends to be limited by the variability present in nature, so that further progress in breeding becomes difficult. When mutation or change occurs in chromosomes, pure breeding lines occasionally give rise to cultivars having a different allele of a particular gene. Ionizing radiation produces a range of effects on DNA both through free radical effects and direct action: breaks in one or both strands can lead to rearrangements, deletions, chromosome loss, death if unrepaired; this is from stimulation of recombination, damage to/loss of bases (mutations) and crosslinking of DNA to itself or proteins. Induced mutations are significant as novel mutations are being isolated for enhanced nutrition quality of crop plants: micronutrients, protein, amino acids, fatty acids and vitamins (Navnath and Mukund, 2014). Another source of nutrition provision is from the neglected and underutilized crops. These and the major crop to enhance nutrition provision for the ever-growing human population (Jain and Suprasanna, 2011). Among physical mutagens, gamma radiation has been widely used for mutation induction for both seed and vegetative propagated crops. Recently, ion energy technology- heavy ion beam (HIB) and low energy ion beam (LIB) - is being used for mutation induction in wide ranging crops. HIB is predominantly used for inducing mutations in plants (Jain, 2010). This investigation seeks to induce profitable mutation and select the concentrations and doses of the mutagens that will enhance production of okra. Indeed, such a study is needful to unveil any desirable features for quantitative traits, agronomic, Phytochemical and pharmaceutical benefits.

MATERIALS AND METHODS

Plant source and mutagen treatment

Three varieties of okra NHAe47-4, Jokoso and a local variety called Beru were used for this research. Two improved varieties (NHAe47-4 and Jokoso) of Okra *A. esculatus* seeds were collected from Institute of Agricultural Research (Plant Science Department) Ahmadu Bello University, Zaria (11^0 04'N and $7^0$42'E) and a local variety was purchased in Sabo Main Market Zaria The Sodium azide used for this research was collected from Biochemistry Department A.B.U. Zaria. Soaking of the seeds was done in the Biological Sciences laboratory, Ahmadu Bello University, Zaria.

Standard agronomic practices (Good Agricultural Practices for Okra, 2008, "Thai Agricultural Standard (TAS)") during the experimental period were adopted in accordance with the enforcement of the Agricultural Standards Act B.E. 2551 (2008). National Bureau of Agricultural Commodity and Food Standards Ministry of Agriculture and Cooperatives.

Treatment of seeds with the mutagens

The okra seeds were air-dried and each variety divided into five sets of five hundred seeds and taken to the Centre for Energy Research and Development, Obafemi Awolowo University Ile-ife (7^0 28'N and 4^0 32'E) for radiation with Gamma cell 220 Cobalt 60 (Co^{60}).The doses of the Gama ray were 20 kr, 40 kr, 60 kr, 80 kr and 100 kr. The same sets of seeds were also soaked in 2.0 and 3.0 milimolar solution of Sodium azide respectively for one hour. The seeds were then washed with tap water to remove excess chemicals and exudates.

Planting of the seeds

Planting of both treated and the control seeds were done in Botanical Garden, Ahmadu Bello University Zaria, using the open garden to study the combined effect of the two mutagens. Each plot consisted of five rows and each row was four meters long and the intra row spacing of 50 cm and inter row spacing of 75 cm apart in three replications. Twenty seeds were planted per row. The layout follows 3×5 Randomized Complete Block Design (RCBD). The seeds were observed daily until maximum germination was achieved. Field studies were undertaken to determine the effects of

Table 1. Morphological characteristics of treated and untreated seeds of Beru variety (mean length in centimeter).

Mutagen	% Germination	Seedling height	Stem height at maturity	Leaf length	Fruit length
Control	97[a]	9.5[a]	28.00[a]	10.50[a]	5.60[a]
2.0mM + Gamma Rays 20Kr	50[b]	10.9[a]	11.37[c]	5.30[b]	5.50[a]
2.0mM + Gamma Rays 40 Kr	30[b]	5.3[c]	10.00[c]	5.10[b]	5.50[a]
2.0mM + Gamma Rays 60 Kr	40[b]	6.6[b]	12.25[c]	6.00[b]	-
2.0mM + Gamma Rays 100 Kr	10[b]	3.2[d]	-	-	-
3.0 mM + Gamma Rays 20Kr	10[b]	9.3[a]	12.17[c]	5.10[b]	4.50[a]
3.0 mM + Gamma Rays 40 Kr	20[b]	8.5[b]	-	-	-
3.0 mM + Gamma Rays 60 Kr	40[b]	6.6[b]	14.00[c]	5.30[b]	4.50[a]
3.0 mM + Gamma Rays 100 Kr	20[b]	5.1[c]	-	-	-

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

the combined mutagens on lethality and morphological injury. The growth of the 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: germination percentage, survival, and average plant height, seedling height, and root length, survival at maturity, time of flowering, fruit length and height at maturity.

Germination rate and percentage: Due to induced injury and delayed germination/ emergence of the treated seeds, the estimate was recorded at the time when the control population was considered 50 to 90%, that is, germination after a week of planting. The percentages of different treatment doses were then compared.

Seedling height: Seedling heights were measured in centimeter, using ruler from the ground level to the tips of the highest leaves.

Seedling survival: The estimates of seedling survival were taken to ascertain the relative emergence of seedling survival of the treatment population. The plants were considered dead if no photosynthetic tissue was observable. The seedling survival was observed after four weeks of the seedling emergence.

Survival to maturity: Estimates of the percentages of plants in each treatment at the time of maturity provided information on the severity of the injury induced by the mutagens. Treatment doses were then classified into low, medium, and high or lethal dose.

Delayed development: The recorded growths of the mutants were estimated in relation to the time of flowering of the plants as well as the variability in plant development within and between treatments. The time of emergence of flower buds was also recorded.

Heights at maturity: Measurements were done in centimeter. Whole plants, from the ground level to the highest point of the plants were measured. 50% of the population of each treatment (including the control) was selected at random and the mean of each taken. The percentage reductions in heights were also compared.

Statistical analysis

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

RESULT AND DISCUSSION

Mean performance of the morphological characters of okra

Tables 1 to 3 show the mean performance of the morphological characteristics of okra (*Abelmuscus esculantus.)*

Percentage germination

There were reductions in the germination and survival percentages with increasing concentrations of combined mutagens. Reductions in germination and survival percentages due to the effect of mutagens on various crop plants have earlier been documented by Mensah, (1977); Mensah and Akomeah (1997) and Mensah et al. (2005).

Beru, Jokoso and NHAe47-4 varieties were treated with 20 krad and 2 mM had 50% germination, and 100 krad and 2 mM had 10% germination. The seed sets were treated with 20 krad and 3 mM had 10% germination and100 krad and 3 mM had 20% germination. (Tables 1 and 3)

Seedling survival

The Beru variety treated with 2.0 mM+100 kr, 3.0 mM+40 kr, and 3.0mM +100 kr was not able to survive beyond three weeks after germination. Jokoso variety treated with 2.0 mM +100 kr, 3.0 mM + (40 kr, 60 kr, 100 kr) also did not survive beyond the seedling stage. NHAe47-4 variety treated with 2.0 mM +100 kr, 3.0 mM +100 kr did not survive beyond the seedling stage (Tables 1 and 3).

Seedling height

Seeds treated with combined mutagens that is, 2.0 mM +

Table 2. Morphological Characteristics of Treated and Untreated Seeds of Jokoso Variety (Mean in Centimeter).

Mutagen	% Germination	Seedling height	Stem height at maturity	Leaf length	Fruit length
Control	96[a]	6.70[a]	21.00[a]	10.00[a]	7.60[a]
2.0mM + Gamma Rays 20Kr	50[a]	8.5[a]	10.67[c]	5.90[b]	4.75[c]
2.0mM + Gamma Rays 40 Kr	20[b]	8.3[a]	8.00[c]	4.40[c]	4.00[c]
2.0mM + Gamma Rays 60 Kr	40[b]	6.0[a]	7.50[d]	7.30[b]	5.50[b]
2.0mM + Gamma Rays 100 Kr	10[b]	4.1[b]	-	-	-
3.0 mM + Gamma Rays 20Kr	50[a]	8.0[a]	14.25[b]	5.50[b]	4.00[c]
3.0 mM + Gamma Rays 40 Kr	30[b]	8.0[a]	-	-	-
3.0 mM + Gamma Rays 60 Kr	40[b]	6.3[a]	-	-	-
3.0 mM + Gamma Rays 100 Kr	10[b]	4.7[b]	-	-	-

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

Table 3. Morphological Characteristics of Treated and Untreated Seeds of NHAe47-4 (Mean in Centimeter).

Mutagen	% Germination	Seedling height	Stem height at maturity	Leaf length	Fruit length
Control	95[a]	9.10[b]	24.00[a]	9.80[a]	5.70[a]
2.0 mM + Gamma Rays 20Kr	50[a]	9.00[b]	11.67[d]	5.70[b]	4.00[b]
2.0 mM + Gamma Rays 40 Kr	40[b]	5.00[e]	5.75[f]	3.60[c]	3.00[b]
2.0 mM + Gamma Rays 60 Kr	20[b]	6.60[d]	8.00[e]	4.00[c]	2.50[c]
2.0 mM + Gamma Rays 100 Kr	10[b]	3.60[f]	-	-	-
3.0 mM + Gamma Rays 20Kr	30[b]	7.80[c]	12.17[d]	6.90[b]	4.70[a]
3.0 mM + Gamma Rays 40 Kr	40[b]	9.40[b]	9.17[e]	5.80[b]	2.75[c]
3.0 mM + Gamma Rays 60 Kr	20[b]	9.50[b]	6.75[f]	6.90[b]	5.00[a]
3.0 mM + Gamma Rays 100 Kr	10[b]	4.50[e]	6.00[f]	4.33[c]	1.00[d]

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

gamma rays of different concentrations have mean seedling heights as follows: 20 kr +2.0 Mm (10.50 cm); 40 kr +2.0 mM (4.97 cm); 60 kr +2.0 mM (6.50 cm), 100 kr +2.0 mM (3.25 cm), while those treated with 3.0 mM +the various doses of gamma rays were: 20 kr +3.0 mM (9.17 cm), 40 kr +3.0 mM (7.90 cm); 60 kr +3.0 mM (6.63 cm) and 100 kr +3.0 mM (5.13 cm) (Tables 1 and 3)

Stem height

Okra plants treated with combined mutagens showed decrease in stem height with increase in dose of the combined mutagens.

Leaf length

The seeds treated with the combined mutagens showed decrease in leaf length with increase in concentration of sodium azide and decrease in dose of Gamma rays.

Fruit length

The combination of both mutagens showed decrease in length with increase in the concentrations of Sodium azide solution and the doses of Gamma rays. Generally, the height of the seedlings, stem of the mature plants, length of the leaves and fruits length were inversely proportional to the doses of the two mutagens. (Tables 1 and 3)

Survival to maturity

Okra seeds treated with the two mutagens, (20 krad +2 mM, 40 krad + 2 mM, 60 krad + 2 mM and 20 krad and 3 mM) survived to maturity flowered but had stunted growth. Jokoso however, showed different characteristics. All those treated with 40 krad + 3 mM, 60 krad + 3 mM, died but those treated with 3 mM + 20 kr that survived (Tables 1 and 3). Beru variety treated with 20 krad + 3 mM survived till the flowering stages but those treated with 100 krad and 3 mM did not survive till the flowering stage. The NHAe47-4 variety treated with 20 krad + 2 mM, 40 krad + 2 mM, 60 krad + 2 mM survived till flowering stage but those treated with 100 krad + 2 mM died before reaching flowering stages. Those treated with 20 krad +3 mM, 40 krad + 3 mM, 60 krad + 3 mM survived till their flowering stages just as those treated with 100 krad +3 mM. It is worthy of note that all the sets of seeds of NHAe47-4 treated with all the various doses and concentrations of the combined mutagens survived to maturity budded but had stunted growth.

Development of fruits

When treated with combined mutagens, Beru variety delayed in flowering for up to three weeks even when the control plants was budded and flowered. The plants all attempted to bud but the majority of buds were aborted. Some tried to fruit but only one fruit or the maximum of four fruits developed. Jokoso variety delayed in flowering for more than three weeks but the control plants flowered and fruited. The plants had plenty buds but only few flowered and fruited. Some have just one fruit while those plants treated 20 kr +2 mM, and 20 kr+3 mM, had about five fruits. NHAe47-4 variety also had delay in producing flower buds for about three weeks while the control produced flower buds. In fact, these plants had numerous flower buds but only few buds were flowered and fruited. Those treated with 20 kr+2 mM and 20 kr+ 3 mM had up to six fruits but the rest had one or two fruits. Most of the buds died and fell off. This may be due to the combined effect of the two mutagens on the plants. This resulted in destroying the carpel. When some of the flower buds were opened, the carpels were seen like scattered red dots in the buds. The carpels probably were destroyed and no site for fertilization hence, on fruit formation. This may explain why there were a lot of bud formations but those buds did not develop into fruits.

DISCUSSION

It has been established that both radiation and chemical mutagens (Sodium azide and Gamma ray) play a role in enlarging genetic variable of quantitative character. This creates a scope for selection (Adamu et al., 2004). Mutations, or heritable alterations in the genetic material, may be gross, this may be at the level of the chromosome, or point alterations. This technically refers to mutations not visible as cytological abnormalities and/or those which map to a single "point" in experimental crosses. Point mutation can involve just a single nucleotide pair in DNA. Mutagens may be of physical, chemical or biological origin. They may act directly on the DNA, causing direct damage to the DNA, and most often result in replication error. Some however may act on the replication mechanism and chromosomal partition. Many mutagens are not mutagenic by themselves, but can form mutagenic metabolites through cellular processes; for example, through the activity of the cytochrome P450 system and other oxygenases such as cyclooxygenase (Kim and Guengerich, 2005). Such mutagens are called promutagens (http//www-personal.ksu.Edu/-bethmont/mutdes.htm\ mutagens, 2016).

In this research, Okra (*Abelmuscus esculantus* L.) showed differences in their response to gamma rays and sodium azide solutions even though phenotypic changes were observed. The techniques used could not demonstrate any specific chromosomal aberration. This however, does not mean that mutation did not take place since obvious morphological changes were recorded. Very interesting results have been obtained for many polygenic characters such as plant height, leaf length, fruit length and maturity and it was envisaged that combined mutagens will induce more variability in crops. In general, treatment with the mutagens (gamma rays and sodium azide) reduced germination, seedling survival, seedling height and plant height. This is possibly associated with dose. These observations were in agreement with earlier findings (Jagajanantham et al., 2013; Ashish et al., 2011 and Adamu et al., 2004).

There is high sensitivity with respect to germination and seedling injury. The seeds treated with highest dose of gamma ray and combined mutagens showed the highest reduction in percentage germination. Similar observation was made by Jadhav et al. (2012), who reported reduced germination percentage in all the M1 generation of okra treated with gamma ray and EMS. Ashish et al (2011) also observed reductions in the germination and survival percentages with increasing concentrations of two mutagens. Also, treatment with combined mutagens showed reduction in seedling height which may be due to a high frequency in induced deletions or translocations. Jagajanantham et al. (2013) reported the impact of chemical mutagens in germination percentage, plant height and number of leaves decreased with increasing concentration of DES. The plants treated with combined mutagens produced varieties with taller seedling height than the control. This showed that the combined mutagens can help to produce beneficial and desirable qualities. Jagajanantham et al. (2013) observed that the duration of the first flower increased with increasing concentration of DES and EMS. Those treated with 2 mM + 60 kr and 2 mM +100 kr showed reductions in seedling heights, which may be due to the inability of the Sodium azide to suppress the effect of the high doses of gamma radiation on the crops. Treatment with 3 mM + 20 kr also showed taller varieties than the control in Beru and Jokoso varieties but shorter seedling height in NHAe47-4 variety. With increase in dose of gamma rays on the three varieties of okra used in this research, the suppressing effect of sodium azide reduced with increases in dose of gamma rays. These rays were able to suppress the effect of sodium azide on the plants but in NHAe47-4 variety, the mean seedling height increased with combined treatment of 3 mM + 60 kr, then dropped remarkably when treated with 3 mM + 100 kr.

There were late flowering and maturity of the plants with increase in the dose of the combined mutagens. This may be due to the changes in genetic and physiological composition resulting from treatment with the two mutagens. This result is at variance with the works of Adamu et al. (2004) but in agreement with Jagajanantham et al. (2013). The result here may be due to the dose of the radiation and the effect of the chemical mutagens which suppressed early development of the crops.

The seeds treated with the highest dose of combined mutagens showed the highest reductions in the stem height, leaf length and fruit length. This is possibly associated with the doses and concentrations of the two mutagens. This is in agreement with the findings of Ashish et al. (2011) and Jagajanantham et al*.* (2013).

Conclusion

The result of this research showed that the effective concentrations of combined mutagens in Beru and NHAe47-4 varieties are 2.0 mM + 20 kr, 2.0 mM + 40kr, and 2.0 mM 60 kr and 3.0 mM + 20 kr, 3.0 mM + 40 kr, and 3.0 mM + 60 kr while in Jokoso variety, the effective doses are 2.0 mM + 20 kr, 2.0 mM + 40 kr, and 2.0 mM + 60 kr and 3.0 mM + 20 kr. Analysis of variance of the morphological characters indicated that there is a significant difference between the control plants and the plants treated with the two mutagens in all the three varieties of okra used. Genetics and cytogenetics analysis of induced mutation could contribute much to a deeper understanding of the mutation events in higher plants especially with the knowledge of the genetic contents of the chromosomes of crops. This finding offers very valuable material for physiological investigations of a single gene effects and the investigation of the process deserves more attention.

Conflict of Interests

The authors have not declared any conflict of interests.

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