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Stimulating Genetic Variability on Growth And Yield of Okra (*Abelmoschus esculentus)* Using Sodium Azide

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ABSTRACT
As interest in mutant breeding grows, so does the amount of study being done on employing mutagens to induce mutations for genetic variety. As a result, breeders' only means of producing variety is through mutation breeding. An experiment was conducted to induce genetic variability using sodium azide on varieties of okra (Ablemoschus esculentus). Okra seeds were treated with different sodium azide concentrations (0mM, 2mM, 4mM, and 6mM). The research was carried out at the Department of Agronomy Federal University Dutsin-ma. The objectives of the research were to evaluate the effect of sodium azide (NaN3) on the growth and yield of okra, identify the mutagen that induces beneficial mutation with minimum lethality, select the variety of okra that produce the best mutants in terms of yield and agronomic traits. The pre-soaking of okra seed in distilled water, treatment with mutagen, seed germination test, and germination percentage were done in the laboratory. The treated and the control seeds were planted in nylon bags on the field. Each treatment was in three replications. The field layout was Completely Randomized design (CRD). The result revealed that plots treated with 2mM, 4mM, and 6mM of sodium azide recorded decreased growth, yield, and yield components with an increase in concentrations of the mutagens in okra varieties studied in comparison with untreated plots. However, treatment with 2mM and 4mM performed better than those treated with 6mM. Therefore, treatment with a low concentration of Sodium Azide is recommended for the okra varieties used in this research.

Keywords:
Sodium azide,
Mutagens,
Variability,
Okra,
Millimolar,
Concentration.

INTRODUCTION

Okra, (*Abelmoschus esculentus*), is a popular crop cultivated across the world's tropical and subtropical climates. Mature pods can be utilized as a source of mucilage and as animal feed in addition to being consumed as a vegetable. Mature seeds are powdered and used as a coffee replacement in addition to being used to produce oil. Confectioneries also utilize a variety of plant components as sizing or thickening agents. Numerous illnesses have been treated with the plant's medical application. There are reports of okra plants having anticancer, antibacterial, and hypoglycemic properties (Baghery and Kazemitabar, 2015), antimicrobial (De Carvalho *et al*., 2011), antiulcer (Atodariya *et al*., 2013), antihyperlipidemic (Sabitha *et al*., 2011), and hypoglycemic activities (Tomoda *et al*., 1989, Sabitha *et al*., 2011). Okra has a rapid growth cycle, easy cultivation, pest resistance, and high nutritional value. It is sensitive to diseases such as yellow vein mosaic virus, Cercospora leaf spot, fusarium wilt, *etc*. (Baghery and Kazemitabar, 2015).

Any crop to be used in a breeding programme that aims to produce high-yielding cultivars with other desired traits must have some degree of variability. Induced mutations have the potential to provide valuable diversity in quantitatively inherited traits under certain conditions (Baghery and Kazemitabar, 2014). Considerable progress has been made in inducing mutations of okra [*Abelmoschus esulentus* (L.) Moench] for improvement of their certain characters, but detailed studies are lacking (Ashish *et al.*,2011). Anwar *et al.*, (2009) have proposed the use of okra (*A. esculentus*) seed oil for fuel/biodiesel production. Mutation promotes the genetic diversity for the desired qualities in a variety of agricultural plants (Guangfeng, 2017). Sodium azide is a potent mutagen that has been shown to restrict the growth of plant components on okra effectively. Mutation enhances the genetic diversity of the desired qualities in a variety of agricultural plants (Guangfeng, 2017). Sodium azide is a potent mutagen that has been shown to restrict the growth of plant components on okra effectively. To generate mutants with desirable traits that can be bred with other cultivars or as improved varieties for farmers, and consumers, and to produce okra seed that can be identified or labeled as being mutagenic or having a mutagenic provenance. The study aims to evaluate the effect of sodium azide (NaN3) in inducing genetic variability in some varieties of okra.

MATERIALS AND METHODS

The experiment was conducted in a completely randomized design with three replications at the take-up site of Federal University Dutsin-Ma, Katsina State in the year 2023. Dutsin-Ma lies between latitude 12º 27' 22''N and longitude 7º 30' 83'' E. The farm is situated within latitude 12^0 17' 40'' N, and longitude $7^0$27' 19''E. Federal University Dutsin-Ma Take off Campus.

Source of Plant Materials

Dry hybrid seeds of two improved okra (Gombo and Heirloom) varieties were obtained from the central market in Kano State. The treatments of seeds with chemical mutagens were done in the chemistry laboratory of Federal University Dutsin-MA, Katsina State. Seeds were presoaked in distilled water for 6 hours and then to induce mutation they were transferred to 2mM, 4mM, and 6mM concentrations of Sodium azide solution for 6 hrs. After 6-hour periods of treatment, the treated seeds were decanted and thoroughly washed using distilled water to remove the residual effects of the mutagens. Untreated seeds were presoaked in water for 6 hours and used as the controls. The Beakers used were appropriately and carefully labeled and arranged according to their respective plot number, and name of okra variety, and the various concentrations were labeled on the beakers. That is One beaker per treatment. There were twenty-four (24) plots per replication.

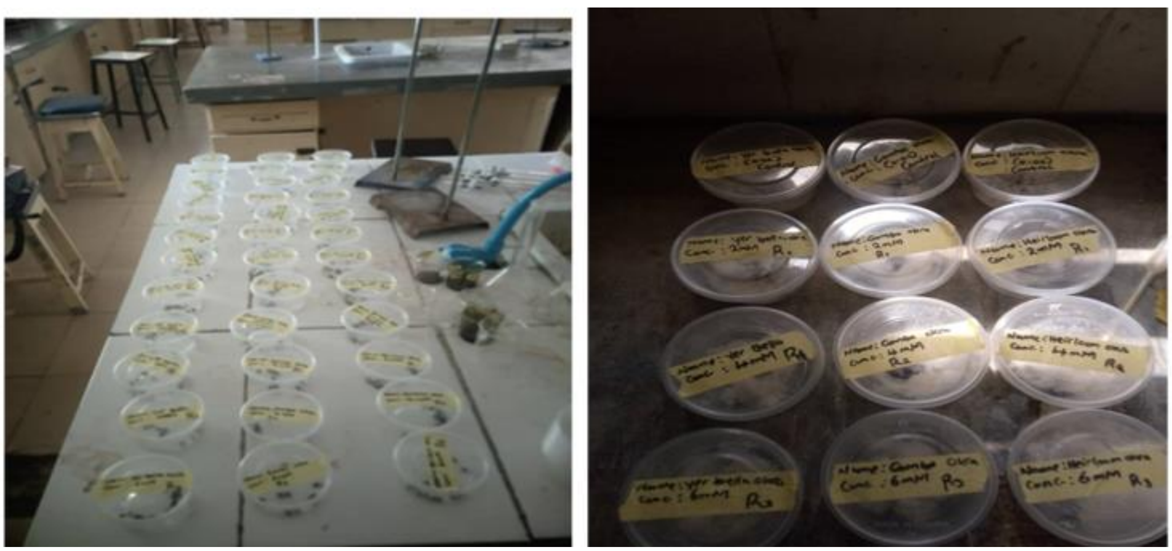

Plate 1: Soaking of the okra seeds in mutagens

Agronomic and Morphological Traits Measured include

Laboratory germination test, Percentage germination (field), Plant Height (cm), Number of pods per plant, Pod length (cm), Pod circumference (mm), Seed number per pod, Pod weight (g), 100 seed weight (g) and Seed yield per plant. Data collected were analysed using two-way Analysis of variance (ANOVA) using SAS (SAS, 2002). Duncan's multiple range test (DMRT) was used to separate the means.

RESULTS AND DISCUSSION

The mutational effects of Sodium azide were seen on okra concerning its germination percentage, pod length, pod number, pod circumference, number of seeds, seed weight, and seed yield per plant respectively and this agreed with the findings of Adamu and Aliyu, 2007 where he observed the mutational effects on tomato. The data gathered in the study were analysed and summarised in Tables 1 - 4

Effect of Sodium Azide on Percentage Germination, Seedling height, plant height, Length of Pod, and Number of Gombo Okra Variety

Table 1 shows that the treatment of seeds with various concentrations of Sodium Azide significantly affected the percentage germination of Gombo okra seeds. Treatment with 6mM sodium azide recorded the lowest percent germination compared with other treatments with 2 and 4mM and the control. Also, a significant effect was observed in treating seeds of Gombo okra with varying concentrations of sodium azide on seedling height at 2 weeks after sowing (WAS) and plant height at 7 WAS. At 2 WAS, the control plots significantly produced the tallest seedlings as compared to seedlings which received varying levels of sodium azide. Thus, the longest seedlings were recorded from the control plots, while the shortest plant height was observed from plants treated with 6 mM concentration of sodium azide. Similar results were recorded by Maryam (2017); and Maryam and Hussaini (2023).

Numerous crops, including cluster beans (Velu *et al*., 2007), cowpea (Gnanamurthy *et al*. 2013), sesame (Anbarasan *et al*. 2013), rice (Talebi *et al*., 2012), soybean (Satpute & Fultambkar 2012), pearl millet (Ambli & Mullainathan 2014), and pigeon pea (Ariraman *et al*. 2014) have all shown similar results regarding the impact of mutagens. In a similar vein, Warghat *et al*. (2011) found that, in contrast to the control, sodium azide and gamma ray mutagens reduced the musk okra (*Abelmoschus moschatus*) germination and survival percentage. According to Jadhav *et al*. (2012), okra seeds treated with EMS and gamma ray mutagens had less germination. Additionally, they noticed a rise in the death rate. Jagajanantham *et al*. (2013) observed that okra seedlings' germination and survival were reduced with the administration of EMS and DES mutagens. During the trial, there was no significant difference in pod number and pod length on the sodium azide-treated plants.

Table 1: Influence of sodium azide on seedling height, plant height, pod number, and pod length of Gombo Okra Variety

	Percentage germination	Seedling Height (cm)	Plant Height (cm)	Pod Number	Pod Length
Treatment	**2 DAS**	**2 WAS**	**7 WAS**	**7 WAS**	**10 WAS**
Control	9.50[a]	11.75[a]	54.65[a]	7.25[a]	9.37[a]
2mM	10.75[a]	9.2[b]	43.65[bc]	5.25[a]	5.57[a]
4mM	10.00[a]	9.40[b]	47.57[b]	4[a]	6.92[a]
6mM	7.25[b]	8.45[b]	41.05[c]	4.75[a]	6.12[a]
SE+	0.478	0.284	1.869	1.118	1.164
Significance	*	*	*	NS	NS

Means with the same letter are not significantly different at a 5% level of probability

Effect of sodium azide rates on yield components of Gombo okra variety

No significant difference was observed in pod circumference, seed number, 100 seed weight, and seed yield of okra with the sodium azide-treated plants during the trial (Table 2). However, the highest pod weight of 25.60g was obtained from the plants on control plots. This was followed by those that were treated with 4mM sodium azide. The remaining treatment showed pod weights which were not significantly different. There was a general decrease with the increased doses of Sodium azide for all the yield components. However, they were not significantly different statistically except for pod weight.

Table 2: Influence of sodium azide on yield components of Gombo okra variety

Treatment	**Pod Circumference**	**Pod weight**	**seed numbers**	**100 seed weight**	**Seed Yield**
Conc. Rate	9WAS	9WAS	18WAS	18WAS	18WAS
Control	24.15[a]	25.60[a]	297.75[a]	4.37[a]	4.8[a]
2mM	20.6[a]	14.65[b]	258.75[a]	3.87[a]	3.67[a]
4mM	19.02[a]	21.67[ab]	226.5[a]	4.3[a]	3.97[a]
6mM	17.52[a]	13.35[b]	217[a]	4.05[a]	3.22[a]
SE+	3.854	3.13	55.056	0.635	0.81
Significance	NS	*	NS	NS	NS

Means with the same letter are not significantly different at a 5% level of probability

Effect of sodium azide rates on percentage germination, plant height, and number of pods of Heirloom okra variety

There was no significant difference between the control plants and the treated plants for percentage germination, seedling and plant height, pod length, and pod number of Heirloom okra during the trial (Table 3). Okra plants on the control plots and those on plots treated with 2mM Sodium azide significantly and consistently produced the highest seedling, plant height, pod number, and pod length as compared to other treatments. There was a decreased performance with an increased concentration of Sodium azide (4Mm and 6Mm). The effect of the sodium azide may have resulted in reduced plant height leading to significant differences ($P<0.05$) observed in various treatments. However, statistically, there was a significant in the performance of Heirloom okra for seedling height, plant height, and pod length. At the same time, there was no significant difference between the control plants and the treated plants in their germination percentage and pod number. Xin Yuan (2021) reported chemical mutagens to have inhibitory effects on the transcriptome of soya bean leading to an increase or decrease in most of the crop growth and yield attributes.

Table 3: Influence of Sodium Azide on Percentage Germination, Seedling height, Plant height, Pod number, and Pod length of Heirloom okra variety

Treatment	Percent germination	Plant height (cm)		Pod number	Pod length (cm)
Conc. Rate	2DAS	2 WAS	7 WAS	9WAS	10WAS
Control	17^{a}	12.97^{a}	48.15^{a}	6.25^{a}	8.02^{a}
2mM	17.75^{a}	13.05^{a}	47.02^{a}	6.25^{a}	7.87^{a}
4mM	15.75^{a}	11.42^{b}	40.10^{b}	6.25^{a}	4.30^{b}
6mM	17^{a}	12.47^{ab}	42.25^{b}	2.25^{a}	2.82^{b}
SE$\pm$	0.931	0.421	1.353	1.296	0.97
Significance	NS	*	*	NS	*

Means with the same letter are not significantly different at a 5% level of probability

Effect of sodium azide rates on yield and yield components of Heirloom okra variety

There was a significant effect of sodium azide on pod diameter, pod weight, and 100 seed weight of heirloom okra. Okra plants on control plots produced significantly wider pod circumferences than those treated. Also, okra plants on the control plots and those on plots treated with 2mM sodium azide significantly produced heavier pod weight as compared to other treatments. Similarly, heavier 100 seed weight was recorded from the control plots and those of plants treated with 2mM sodium azide, while the least 100 seed weight was obtained from plants treated with 4 and 6mM sodium azide (Table 4).

Table 4: Influence of sodium azide on yield components of Heirloom okra variety

Treatment	Pod diameter	Pod weight	seed numbers	100 seed weight	seed yield
Conc. Rate	9WAS	9WAS	18WAS	18WAS	18WAS
Control	29.82^{a}	26.20^{a}	311^{a}	4.32^{a}	3.5^{a}
2mM	26.17^{ab}	28.17^{a}	301.75^{a}	4.27^{a}	3.25^{a}
4mM	20.12^{ab}	17.47^{ab}	202^{a}	3.05^{b}	2.7^{a}
6mM	13.60^{b}	9.97^{ab}	173^{a}	2.70^{b}	2.07^{a}
SE+	4.041	3.874	45.725	0.361	0.512
Significance	*	*	NS	*	NS

Means with the same letter are not significantly different at a 5% level of probability

The mosaic virus was seen in the okra plants. The Okra mosaic virus (OMV) and cucumber mosaic virus (CMV) is a plant virus that infects okra plants with devastating results. Mosaic is a member of the potyvirus genus and is transmitted by aphids. This virus causes yellowing and distortion of leaves, resulting in reduced yield quality of okra. All the treatments were affected with the mosaic virus, though, the control (0.00) produced better yield even though they were affected with the mosaic virus to the same degree. Those treated with sodium azide concentrations of 2mM, 4mM, and 6mM had delays in fruiting and produced few fruits.

Plate 2: Okra plant before the infection with mosaic virus

Plate 3: Okra plants after infection with mosaic virus

Plate 4: Severity of okra mosaic virus on okra plant

CONCLUSION

The study focused on the use of the chemical mutagen sodium azide to induce genetic variability in two varieties of okra (*Abelmoschus esculentus*) and its impact on the growth and yield of the crop. Treatment with sodium azide resulted in a significant decrease in germination percentage, seedling height, and plant height, with the 6 mM concentration having the most pronounced negative effects. The control group (untreated seeds) had the highest germination rate and produced the tallest seedlings and plants. There was a significant impact on yield components, with pod diameter, pod weight, and 100-seed weight being affected by sodium azide treatment. There was no significant difference in the number of pods produced between control and treated plants. Control plants produced significantly wider pod circumferences, heavier pod weights, and higher 100-seed weights than treated plants. The study noted the presence of mosaic virus in the okra plants, affecting all treatments to a similar extent. It caused yellowing and distortion of leaves, potentially impacting yield quality. The study observed that the severity of the negative effects increased with higher concentrations of sodium azide (6 mM had the most adverse impact). Treatment with 2mM and 4mM concentrations were comparatively better in terms of growth and yield compared to 6mM. The study provides insights into the potential use of sodium azide as a mutagen to induce genetic variability in okra. The results suggest that while mutagenesis can lead to genetic variation, there is a trade-off between the extent of variation induced and the negative impact on growth and yield. It's worth noting that mutation breeding is a valuable tool in crop improvement, but the selection of mutagen type and concentration is a critical aspect of the process. Mutagenesis can introduce both beneficial and detrimental traits, and breeding programs must carefully evaluate the trade-offs to ensure that the desired characteristics are enhanced while minimizing undesirable effects. Additionally, monitoring and managing disease susceptibility in the mutated plants is important for overall crop success. Careful consideration of the mutagen concentration is necessary to strike a balance between creating genetic diversity and maintaining crop productivity.

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