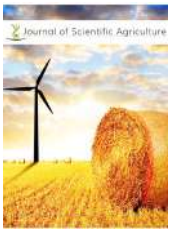
Journal of Scientific Agriculture 2025, 9: 146-157
doi: 10.25081/jsa.2025.v9.9499
https://updatepublishing.com/journal/index.php/jsa

Research Article



ISSN: 2184-0261

Genetic analysis of the effect of sodium azide (chemical mutagen) induction on three Cameroonian Okra (*Abelmoschus esculentus* (L.) Moench) varieties' growth characteristics, yield, and enzymatic activities

Raphaël Siméon Njock[1], Benoit Constant Likeng-Li-Ngue[1,3], Luther Fort Mbo Nkoulou[2,3], Jude Manga Ndjaga[1,3]*, Hermine Bille Ngalle[1], Joseph Martin Bell[1]

[1]Department of Plant Biology, Faculty of Science, University of Yaounde I, P.O Box 812, Yaounde, Cameroon, [2]Mbalmayo Agricultural research Centre (CRAMB), Institute of Agricultural Research for Development (IRAD) P.O Box 2123, Nkolbisson, Cameroon, [3]Research and Support Center for Agro-pastoral Producers in Cameroon (CRAPAC), BP 812 Yaoundé, Cameroon

ABSTRACT

Abelmoschus esculentus is a plant with nutritional and medical properties, therefore environmental circumstances are progressively affecting production. The aim of this study was to contribute to the development of genetic variability in three okra varieties exposed to sodium azide (SA). In this study, sodium azide (0 g/L, 2 g/L, 4 g/L, and 6 g/L for 6 hours) was administrated to three okra seeds (Locale 1, Yellen, and Hire) in the field. Descriptors like seed germination rate, plant height (PH), stem diameter (SD), number of leaves per plant (NLP), foliar area (FA), number of fruits per plant (NFP), and number of seeds per fruit (NSF) were used to analyze genetic variability, broad-sense heritability (H^2), and genetic advance (GA) in three okra varieties. The results showed that the Yellen variety had the highest percentage of seed germination (100% in M_1 and M_2 for control (T0), to 41.67% (M_1) and 44.44% (M_2) for T3) as well as POX (71.01% for M_1 and 128.84% for M_2) and CAT (198.03% for M_1 and 125.80% for M_2) activities. It also had the highest NFP in the T0, with values between 1.25±2.29 in M_1, and 14.42±2.11 in M_2 ($p<0.05$). In terms of productivity, the Hire variety outperformed with elevated FW1F (16.21 g for M_1, and 25.87 g for M_2) and DW1F (15.44g for M_1, and 23.10g for M_2) values. However, the explained variance of the induction of sodium azide was R^2=0.101 for growth parameters, R^2=0.377 for productivity parameters, and R^2=-0.218 for enzymatic activities. Following M_2 generations, characters FA and NFP displayed high genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad-sense heritability, and genetic advance as percent of mean (GAM) levels. Therefore, chemical mutagenesis offers a straightforward method for introducing mutations within plants through hybridization, which can increase favorable crop improvement programs.

Received: February 24, 2025
Revised: July 26, 2025
Accepted: July 28, 2025
Published: August 08, 2025

***Corresponding Author:**
Jude Manga Ndjaga
E-mail: judemng@gmail.com

KEYWORDS: *Abelmoschus esculentus*, Azoture azide, Agronomic parameters, Enzymes, Hybridization, Genetic advance, Heritability

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is a critically important plant in the Malvaceae family (Nizamani *et al.*, 2020). All of its parts (roots, stems, leaves, fruits, and seeds) are used for a variety of employs, including food, medicine, crafts, and industry (Marius *et al.*, 1997). The plant is recognized for its high carbohydrate, protein, oil, potassium, magnesium, iodine, vitamin A, and vitamin C levels (Das *et al.*, 2019). Okra is a powerful antioxidant due to its high lutein concentration (Maryam & Kasimu, 2016), has anti-ulcer and anti-carcinogenic qualities, and is an excellent regulator of hypoglycemia (Maryam, 2017). However, its oil is acceptable for use as a biofuel (Anwar *et al.*, 2010).

Despite its many advantages, a significant drop in production would be accompanied with a variety of biotic (Diallo *et al.*, 2022), and abiotic limitations that have a negative impact on it (Gnago *et al.*, 2010). In addition to the 239 million undernourished people in Sub-Saharan Africa, an additional 266 million people may face hunger by 2080 (Capot & Perus, 2013), whereas regular consumption of this plant, while not a cure-all, could make a significant contribution to resolving this issue while also improving people's overall health. The solution to the aforementioned problems obviously lies in the ongoing improvement of the genetic diversity within this plant via induced mutations. Mutation can be induced by physical and/or chemical mutagens to create genetic variety, resulting in new varieties with better characteristics (Arulbalachandran *et al.*, 2009). However, plant breeding by crossing or mutation breeding has been studied to acquire good agronomical characteristics such as enhanced product quality and productivity, reduced vegetative phase, and resistance to pests and diseases (Dewi *et al.*, 2016).

Traits emerging from induced mutagenesis may be heritable (Roychowdhury & Tah, 2013). Anthropogenic mutagenesis is separated into two axes: the use of radiation and the application of chemical substances. Physical mutagenesis employs radiation (gamma rays, X-rays, ions, etc.), whereas chemical mutagenesis mostly uses alkylating chemicals such as ethyl methanesulfonate, methyl methanesulfonate (Oladosu *et al.*, 2016), colchicine, and sodium azide (Misra *et al.*, 2017). The findings of Bhat *et al.* (2005) revealed that chemical mutagens were more effective in *Vicia faba* L. than physical mutagens. Thus, using these might be promising for producing genetic variants in new individuals with specific desirable agronomic qualities (Aviya & Mullainathan, 2018; Sable *et al.*, 2018). However, SA is a chemical mutagen that has been shown to be highly mutagenic in a variety of species, including plants, and can produce genetic diversity, affecting plant growth and productivity (Lunn & Sansone, 2012). Recently studies of Njock *et al.* (2024), showed that the application of mutagens to specific varieties of okra farmed in Cameroon resulted in a considerable increase of carbohydrates, proteins, and polyphenols.

However, SA is regarded as one of the most effective chemomutagens utilized to cause mutations in agricultural plants (Pour *et al.*, 2023). As a result, the action of this chemical agent can have a favorable or negative effect on life (organisms), particularly their quantitative characteristics (Mosisa *et al.*, 2013). NaN_3 has been used to select various plant species, including maize (Eze & Dambo, 2015), rice (Herwibawa & Kusmiyati, 2017), wheat (Srivastava *et al.*, 2011), and tomato (El Kaaby *et al.*, 2015). Furthermore, while considerable research has been conducted on the mutagenic efficiency of okra cultivars growing in Cameroon, their usage in the production of genetic variability remains inadequate from an agronomic and enzymatic aspect (polyphenol oxidase, peroxidase, catalase, and phenylalanine ammonialyase).

Quantitative genetic indices like phenotypic and genotypic coefficients of variation (PCV and GCV), heritability, and genetic gain are commonly employed to assess plant species genetic diversity (Sundari *et al.*, 2022; Angel *et al.*, 2024). Genetic variety remains an important requirement for any crop improvement activity, and the development of a trait is entirely determined by the amount of variability present in the base material. However, understanding a trait's heritability and genetic advance would provide a genetic improvement advantage (Johnson *et al.*, 1955; Patil *et al.*, 2015). Both factors can be used in breeding plans for simple selection. To this end, selection is more effective when traits have a high heritability and GAM, and which indicate the fraction of genetic variance that might be offered down to the next generation (Patil *et al.*, 2015). According to Angel *et al.* (2024), the additive genetic effect would play an important role in generating high heritability while also rendering selection an effective tool in afterwards separating generations. The current study aims to examine genetic diversity following sodium azide treatment in three okra varieties, in order to generate a population of high-yielders.

MATERIALS AND METHODS

Plant Material

The trials were conducted at FONDAF (Foyer Notre Dame de la Forêt) plots in Bipindi locality (N 2°58'8.97'' and E 9°56'9.08''), Ocean Department, South Cameroon, across two cropping seasons in 2022 (M_1: first generation and M_2: second generation). This area experiences a humid tropical equatorial environment with an average annual rainfall of 1,700 mm. The vegetation type is low and medium-altitude dense humid evergreen equatorial forest, consisting of old secondary forests and forest fallows (Anonymous, 2015). Three *Abelmoschus esculentus* seed varieties were used: Locale 1 (high productive) from the experimental fields of the Plant Genetics and Improvement Unit (PGIU) of the Yaounde I, and Hire (productive) and Yellen (productive) from the Cameroon company Agri-Espoir.

Treatment of Okra Seeds with Sodium Azide (NaN_3)

Sodium azide, a chemical mutagen was purchased in India from VWR Chemical and supplied to Cameroon via Biosciences and Technologies (BST). The okra seeds were treated with a chemical mutagen (NaN_3) using the method described by Esson *et al.* (2018). Okra seeds from all three varieties were pre-treated by soaking them in distilled water for six hours (for destroy harmful microflora on the grains). After pre-treatment, they were immersed in mutagen NaN_3 solutions at various concentrations (0 g/L for treatment T0, 2 g/L for treatment T1, 4 g/L for treatment T2 et 6 g/L for treatment T3, which was only done in M_1) (Table 1) for six hours before being deeply rinsed with distilled water to remove any mutagen residues. Finally, the seeds were wrung out and sowed in the field using an experimental set-up. Untreated seeds were used as control. The experimental design was a block model (3 blocks), completely randomized with three parameters (03 varieties × 4 concentrations (g/L) of NaN_3 × 01 period of mutagen exposure). Finally, following mutagen induction, seed germination and

Table 1: The different applications of sodium azide on two generations of okra

NaN_3 Treatment (g/L)	Local 1		Hire		Yellen	
	M_1	M_2	M_1	M_2	M_1	M_2
T0 (control)	0	0	0	0	0	0
T1	2	0	2	0	2	0
T2	4	0	4	0	4	0
T3	6	0	6	0	6	0

M_1=first mutant generation; M_2=second mutant generation; NaN_3=sodium azide

agronomic parameters were measured during the first generation of mutants' (M_1) growth season. Plants from the M_1 generation that are planted and treated with NaN_3 generate to plants from the M_2 generation, which will likewise be evaluated (growth traits, yields, and enzymatic activities) the following growth season (Table 1).

Agronomic Parameters Determination

Thanks to the procedures described by some authors, the growth parameters such as plant height (Maryam & Kasimu, 2016), stem collar diameter (NGuessan *et al.*, 2021), number of leaves (NGuessan *et al.*, 2021), and foliar surface (Hoyt & Bradfield, 1962) per plant were evaluated in the Local 1, Hire and Yellen varieties at M_1 and M_2. However, the percentage of seed germination (%) was calculated depending on the concentrations of NaN_3 used for treatment using the formula opposite: PSG (%) = (NEP/NSS) × 100. Where: PSG (%) = percentage of seed germination; NEP= number of emerged plants; NSS = number of seeds shown.

Likewise, at maturity, three okra varieties from each of the M_1 and M_2 generations were assessed for all desired productivity parameters. For various doses of NaN_3, alongside the T0 control, quantitative data were obtained, including number of fruits per plant (NFP), number of seeds per fruit (NSF), fresh weight of one fruit (FW1F), and dry weight of one fruit (DW1F).

Extraction of Total Soluble Proteins

The extraction procedure employed is slightly modified from that described by Lanaud *et al.* (1986). 400 g of fresh leaves (okra) were mashed in a mortar (with melting ice) with 0.125 g of polyvinylpyrrolidone (PVP) for each variety. The crushed material was combined with 5 mL of phosphate buffer (pH 7.2-7.3). After 10 min of incubation on ice, the mixture was centrifuged at 3500 rpm for 10 min at 4 °C (Centrifuge 5702 R) and the recovered supernatant formed the crude enzyme extract was collected. Each trial was performed thrice.

Enzymes Essays

Determination of Phenylalanine ammonialyase (PAL)

PAL assay was conducted according to the method described by Okey *et al.* (1997) with minor modifications. The reaction mixture contained with 1 mL enzyme extract, 0.5 mL of L-phenylalanine (12 mM) and 0.5 mL of borate buffer (pH 8.8). The PAL activity was measured at 290 nm and expressed in nmol min/mL fresh weight. Experiments were conducted in three replicates.

Determination of catalase (CAT)

CAT activity was determined using the method Cakmak and Horst (1991), which involves a drop in optical density at 240 nm owing to H_2O_2 consumption. The reduction in absorbance was monitored for 3 min. The reaction mixture consists of 100 µL of 0.1 M phosphate buffer (pH 7), 2 µL of 0.3% H_2O_2, and 30 µL of enzyme extract. The Cat activity was measured in triplicates and was expressed as ΔA 240/min/mg fresh weigh.

Determination of peroxidase (POX)

POX activity was assessed according to Rodriguez and Tames (1982). The reaction mixture contained 1 mL of 0.05 M phosphate-citrate buffer (pH 4.6), 1 mL of 40 mM guaiacol, 0.5 mL of 26 mM H_2O_2 and 100 µL enzyme extract. The changes in absorbance at 470 nm were recorded at 30 s interval for 5 min using a Jenway 6305 spectrophotometer. The enzyme activity was expressed as ΔA470/min/g fresh weigh. All the experiments were repeated thrice.

Determination of polyphenol oxidase (PPO)

PPO activity was determined as described by Kemmenn and Broumer (1964). The reaction mixture consisted of 2.5 mL of 0.1 M acetate buffer (pH 6.0) and 0.5 mL of 10 mM catechol. The activity was measured in triplicates and was expressed as change in absorbance (ΔA495/min/g fresh weigh) at 495 nm.

Estimating Genetic Parameters

Growth, productivity, and enzyme activity characteristics were calculated using phenotypic and genotypic coefficients of variation, broad-sense heritability, and genetic gain as a percentage of the mean.

Phenotypic and genotypic coefficients of variation

Johnson *et al.* (1955) proposed the following formulas for calculating phenotypic and genotypic coefficients of variation:

- Phenotypic coefficient variance (PCV): $PCV = \frac{\sqrt{\sigma^2 p}}{\overline{x}} \times 100$
- Genotypic coefficient variance (GCV): $GCV = \frac{\sqrt{\sigma^2 g}}{\overline{x}} \times 100$

Where: $\sigma^2 p$ = phenotypic variance, $\sigma^2 g$ = genotypic variance, and $\overline{x}$ = average value of character. However, PCV and GCV estimates collapse into three categories: low (> 10%), moderate (10-20%), and high (> 20%).

Broad-sense heritability (H^2)

H^2 was measured using the formula proposed by Imran and Kramer (1951):

$$H^2 = \frac{\sigma^2 g}{\sigma^2 p} = \frac{\sigma^2 p - \sigma^2 E}{\sigma^2 p} = \frac{\sigma^2 I - \sigma^2 i}{\sigma^2 I}$$

Where: $\sigma^2 I$ = phenotypic variance, $\sigma^2 i$ = environmental variance. Dabholkar (1992) classified broad-sense heritability values as high (>60%), moderate (30-60%), and low (>30%).

Genetic gain

Allard's (1960) method was used to compute the genetic gain from selection based on the broad-sense of heritability and the phenotypic standard deviation (SD). The formula is as follows:

$$G = K \times H^2 \times \sqrt{(\sigma^2 p)} \text{ and } GG = \frac{G}{X} \times 100$$

Where: GG = genetic gain from selection, K= constant based on the selection intensity (top 3%=1.75), $\sigma^2 p$= standard deviation of the phenotypic variance, H^2= broad-sense heritability. However, genetic gain from selection calculates collapse into three categories: high (>20%), moderate (10-20%), and low (>30%) (Johnson *et al.*,1955).

Data Analysis

The data obtained for the three parameters analyzed were treated to a one-way analysis of variance (ANOVA) using IBM SPSS software 25th version (SPSS, Inc., Chicago, IL, USA). The difference between the various means±standard deviation was compared using the Tukey's HSD test at 0.05 levels. Multiple linear regression (MLR, software R version 4.1.2 (R Development Core Team 2022)) and principal component analysis (PCA, SPAD 5.5) were used to investigate the effect of sodium azide exposure on the growth, productivity, and enzyme activity parameters of three okra varieties in M_1 and M_2.

RESULTS

Growth Traits

Effect of NaN_3 on seed germination

In generations M_1 and M_2, concentration T0 (control, without NaN_3) had a nearly 100% seed germination rate, outperforming the other three concentrations of the three varieties tested (T1, T2 and T3, $p<0.05$) (Figure 1). However, we discovered that increasing the concentration of NaN_3 in the seeds caused a decrease in germination rate, regardless of the variety ($p<0.05$) (Figure 1). Despite the reported drop, seed germination rates were higher in M_2 than M_1 (Figure 1). Although exhibiting a slightly greater germination rate than the other two varieties (Hire et Yellen), the Locale 1 variety sax a drop ranging from 13.89 to100 % for M_1 and 25 to 100 % for M_2, with T1 to T3 concentrations (Figure 1).

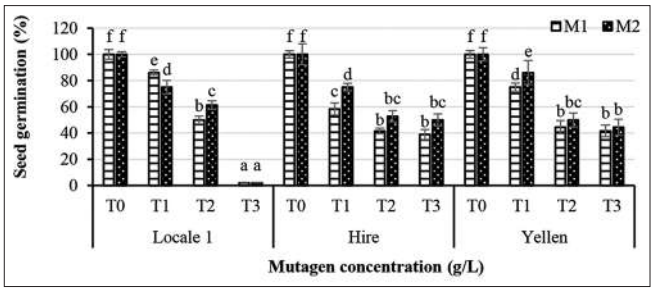


Figure 1: The effect of NaN_3 on germination percentage of seeds from three different okra varieties at M_1 and M_2. Values (mean±SD, n=3) with same letters are not significantly different at p<0.05

Effect of NaN_3 on plant height

The control (concentration T0) of the three varieties superior to the other concentrations (T1, T2, and T3) in M_1 and M_2 (Figure 2). Average plant height decreased in Locale 1 (46 to 47 % for M_1 and 18 à 34 % for M_2), Hire (15 to 29 % for M_1 and 23 to 28 % for M_2), and Yellen (27 to 41 % for M_1 and 24 to 40 % for M_2) as the concentration of NaN_3 increased (Figure 2). However, M_2 recorded higher average plant heights than M_1, with the exception of Yellen, where T3 concentration was somewhat higher in M_1 (25.77 cm) than M_2 (20.01 cm) (Figure 2).

Effect of NaN_3 on stem diameter

In the T3 concentration of the Locale 1 variety, the average collar diameter was just non-existent, in contrast to the Hire (6.71 cm for M_1 and 9.21 cm for M_2) et Yellen (8.17 cm for M_1 and 9.57 cm for M_2) varieties at this concentration (Figure 3). Howbeit, regardless of the concentration T0 (18.33 cm), T1 (12.75 cm) T2 (10.92 cm) and T3 (9.57 cm) the average collar diameters of the Yellen variety were larger in M_2 than those of the Locale 1 and Hire varieties (Figure 3).

Effect of NaN_3 on number of leaf per plant

Regardless of concentration or variety, M_2 had a larger average number of leaves per plant than M_1. Locale 1 and Yellen (M_2) varieties have less average number of leaves per plant (Figure 4). There was no significant change between concentrations T1 and T2 for variety in M_1 ($p<0.05$), but Hire showed a small increase in the number of leaves per plant from T1 to T2 (4.93%) and T2 to T3 (5.02%), whereas Locale 1 and Yellen did not showed it in M_2 (Figure 4).

Effect of NaN_3 on foliar area

The average foliar area of Hire and Yellen varieties exceeded that of the Locale 1 variety (M_1 and M_2) (Figure 5). The Yellen variety had the highest percentage decreases in average foliar area due to T1, T2 and T3 concentrations, ranging from 63 to 67% in M_1, and 54 to 66% in M_2 (Figure 5).

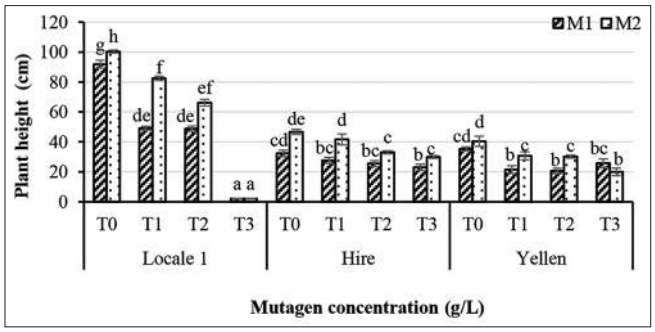


Figure 2: The effect of NaN_3 on average plant height from three different okra varieties at M_1 and M_2. Values (mean±SD, n=3) with same letters are not significantly different at p<0.05

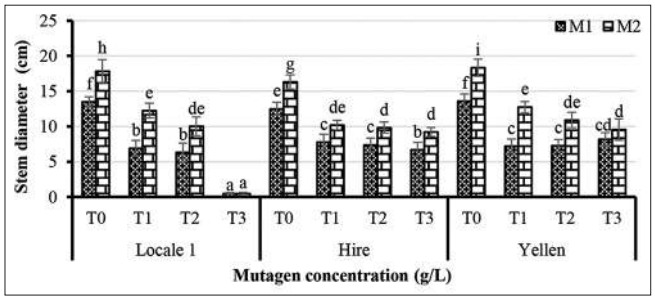


Figure 3: The effect of NaN_3 on average stem diameter from three different okra varieties at M_1 and M_2. Values (mean±SD, n=3) with same letters are not significantly different at p<0.05

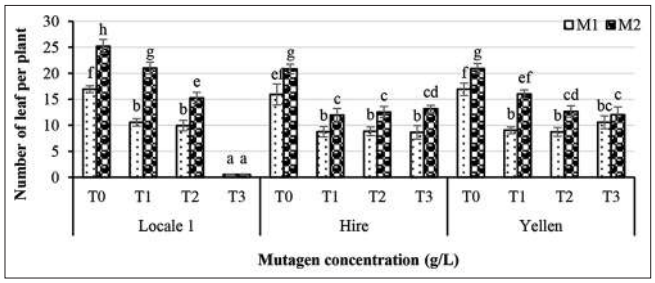


Figure 4: The Effect of NaN_3 on average number of leaves per plant from three different okra varieties at M_1 and M_2. Values (mean±SD, n=3) with same letters are not significantly different at p<0.05

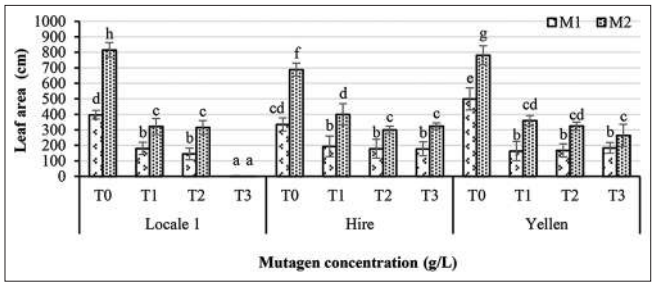


Figure 5: The Effect of NaN_3 on average foliar area per plant from three different okra varieties at M_1 and M_2. Values (mean±SD, n=3) with same letters are not significantly different at p<0.05

Productivity Traits

In the three okra varieties studied, and in the absence of NaN_3, the treatment yielded relatively high values for number of fruit per plant (from 8.08 to 10.25 for M_1, and 11.83 to 14.42 for M_2), fresh weight of one fruit (from 14.22 to 22.13 for M_1, and 16.74 to 25.87 for M_2), dry weight of one fruit (from 10.44 to 15.44 for M_1, and 19.36 to 23.10 for M_2) and number of seeds per fruit (from 90.33 to 93.30 for M_1, and 95.33 to 96.67 for M_2) in M_1 et M_2, as opposed to T1, T2, and T3 which were treated with NaN_3 (Table 2). As the concentration of NaN_3 increases, the values of all four fruit descriptors decrease, regardless of variety ($p<0.05$). However, we have observed a lack of value in the Locale 1 variety for the T3 treatment (Table 2).

Variations of enzymatic activities in the okra leaves

Figure 6 depicts the effects of different NaN_3 doses (0, 2, 4, and 6 g/L) on enzymatic activity (PAL, PPO, CAT, and POX) in the leaf tissues of three okra varieties (Locale 1, Hire, and Yellen) investigated. In plants treated with SA, all the enzymatic activities were slightly increased when compared to the control (non-treated plant: T_0). Whatever enzyme activity was investigated in our study, the NaN3-treated Yellen variety outperformed the other two varieties in M_1 and M_2 [POX (0.34 to 0.59 ΔA420/min/g protein for M_1 and 0.26 to 0.59 ΔA420/min/g proteins for M_2), CAT (0.20 to 0.60 ΔA 240/min/mg of protein for M_1, and 0.31 to 0.70 ΔA 240/min/mg of protein for M_2), PPO (0.091 to 0.22 ΔA 290/min/mg of protein for M_1, and 0.078 to 0.24 ΔA 290/min/mg of protein for M_2), and PAL (0.12 to 0.36 nmol min/mL of protein for M_1, and 0.15 to 0.31 nmol min/mL of protein for M_2)] (figure 6).

Relationship between Parameters Investigated

Multiple linear regression (MLR)

Table 3 indicates that the variance inflation factor (VIF) values for the variables contained in the model are less than 5 in yield and growth parameters, with the exception of NLP (8.24). Contrarily to VIF values higher than 5 for all of the enzymatic activities investigated (Table 3). However, NFP (0.409, $p<0.01$), FW1F (0.979, $p<0.01$) and CAT activity (0.356, $p<0.01$) displayed a positive and highly significant effect, but POX activity was highly significant and negative effect (Table 3).Therefore, the variation explained by induction sodium azide on three okra varieties in M_1 and M_2 was R^2=0.377 for yield, R^2=0.101 for growth, and R^2=-0.218 for enzymatic activities (Table 3).

Principal component analysis (PCA)

A PCA was generated to visually represent the affinities between the three varieties as well as the relationships between all of the parameters (growth, productivity, and enzymatic) evaluated (Figure 7). For M_1 (PC_1: 75.69% and PC_2: 24.31% of the total variability) and M_2 (PC_1: 82.08% and PC_2: 17.92% of the total variability), the first two principal components represented 100% of the total variability of all the characteristics investigated (Figure 7). In M_2, the plant height and number of leaves per plant of Locale 1 variety were dominating features in the PC_1, whereas the stem diameter, number of fruits per plant and peroxidase activity were the most prominent features in the PC_2 (Yellen variety) (Figure 7). In contrast, in M_1, apart from the dominance

Table 2: Productivity traits (means±SD) of okra varieties after NaN_3-treated

Varieties	Conc.	NFP		FW1F (g)		DW1F (g)		NSF	
		M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
Locale 1	T0	8.08±2.40^{c}	14.08±2.05^{d}	14.22±1.12^{cd}	23.70±2.19^{d}	10.44±2.52^{cd}	19.36±1.15^{c}	91.00±3.21^{a}	96.67±4.92^{a}
	T1	3.42±1.02^{a}	10.27±1.60^{c}	10.31±1.43^{b}	27.51±1.82^{f}	7.10±1.23^{b}	24.02±2.02^{d}	79.00±4.17^{b}	80.33±2.78^{b}
	T2	3.17±1.50^{a}	5.92±2.30^{a}	7.88±2.11^{a}	14.66±1.32^{a}	5.87±1.18^{a}	12.42±1.03^{a}	71.67±1.02^{c}	72.33±1.21^{c}
	T3	0	-	0	-	0	-	0	-
Hire	T0	8.25±2.99^{c}	11.83±2.85^{c}	16.21±1.33^{e}	25.87±1.65^{e}	15.44±2.01^{f}	23.10±3.12^{d}	93.30±2.14^{a}	95.33±3.02^{a}
	T1	4.50±1.11^{ab}	5.75±1.22^{a}	13.52±2.24^{c}	20.11±0.73^{c}	9.55±1.52^{c}	17.04±1.23^{b}	83.33±2.55^{b}	84.00±1.06^{b}
	T2	4.00±1.78^{a}	5.67±1.35^{a}	10.38±1.17^{b}	19.95±1.19^{c}	8.66±1.23^{bc}	17.00±0.51^{b}	84.33±2.09^{b}	82.00±0.90^{c}
	T3	3.83±1.63^{a}	6.25±2.01^{a}	10.45±1.09^{b}	20.82±1.32^{c}	8.77±0.95^{b}	17.02±1.81^{b}	83.67±2.33^{b}	84.67±1.08^{b}
Yellen	T0	10.25±2.90^{d}	14.42±2.11^{d}	16.74±1.88^{e}	22.13±2.08^{a}	13.00±1.12 ^{e}	19.52±1.16^{c}	90.33±5.12^{a}	96.33±6.98^{a}
	T1	3.92±1.33^{a}	7.83±2.12^{b}	12.13±2.06^{c}	24.43±1.08^{d}	9.65±1.02 ^{c}	20.86±2.42^{c}	79.67±2.78^{c}	80.67±2.02^{c}
	T2	3.67±1.51^{a}	7.00±2.71^{ab}	12.69±1.71^{c}	23.05±2.22^{d}	9.43±1.45 ^{c}	19.49±1.12^{c}	66.00±4.11^{d}	72.33±2.72^{d}
	T3	5.75±1.05^{b}	6.90±3.01^{ab}	10.18±1.05^{b}	16.87±2.07^{b}	8.22±2.45 ^{bc}	17.95±1.01^{b}	84.00±2.12^{b}	85.33±1.98^{b}

NFP=Average number of fruit per plant; FW1F=Average fresh weight of one fruit; DW1F=Average dry weight of one fruit; NSF=Average number of seeds per fruit

Con.=concentration in NaN_3. The means followed by the same letter in the same column are not significantly different in the Turkey HSD test at ($P<0.05$)

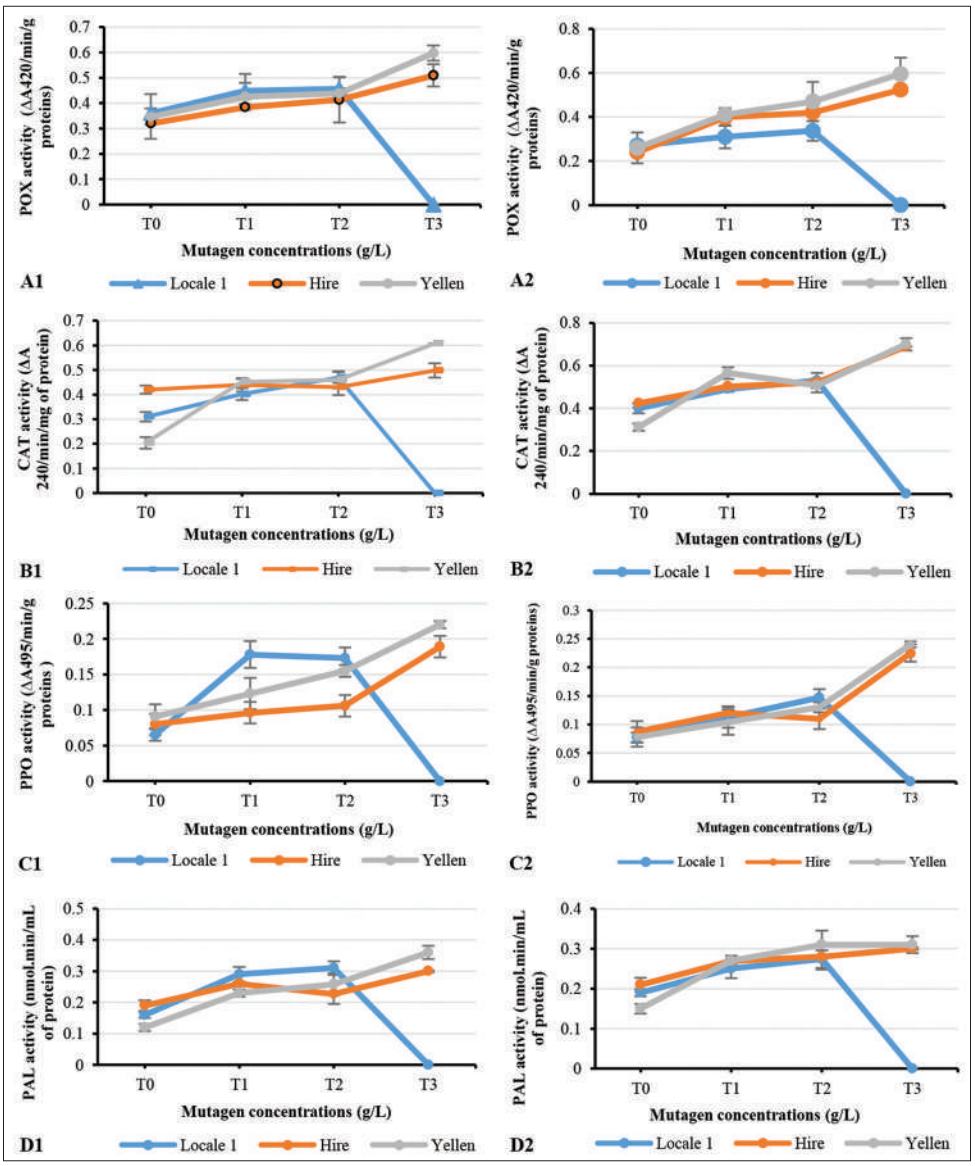


Figure 6: Effect of NaN_3 on four enzymatic activities in three okra varieties in M_1 and M_2. M_1: A1 (POX), B1 (CAT), C1 (PPO), D1 (PAL) and M_2: A2 (POX), B2 (CAT), C2 (PPO), D2 (PAL). *Significantly different at p<0.05*

Table 3: Multiple linear regression analysis of variables (growth and yield parameters, and enzymatic activities) studied in generations M_1 and M_2

Yield parameters				Growth parameters				Enzymatic activities			
Variables	Estimate	P(>|t|)	VIF	Variables	Estimate	P(>|t|)	VIF	Variables	Estimate	P(>|t|)	VIF
Intercept	3.354	<0.001***		Intercept	1.619	<0.001***		Intercept	5.266	<0.001***	
NFP	0.139	0.409**	3.49	NLP	0.276	0.134*	8.24	CAT	0.356	0.718**	5.750
FW1F	0.148	0.979**	3.49	FA	-0.004	0.167ns	4.38	POX	-21.32	w0.967**	5.800
DW1F	0.002	0.324*	2.65	PH	-0.026	0.200*	3.09	PAL	-6.477	0.863*	28.37
				SG	0.020	0.494ns	5.20	PPO	7.088	0.373*	27.27
				SD	0.361	0.411ns					
R^2	0.377			R^2	0.101			R^2	-0.218		

*=Significant; **=highly significant; ns=no significant; VIF=variance inflation factor; R^2=coefficient of determination; NFP=average number of fruit per plant; FW1F=average fresh weight of one fruit; DW1F=average dry weight of one fruit; NLP=average number of leaves per plant; FA=average foliar area; PH=average plant height; SG=average seed germination; SD=average stem diameter; CAT=catalase, POX=peroxidase; PAL=phenylalanine ammonialyase; PPO=polyphenol oxidase

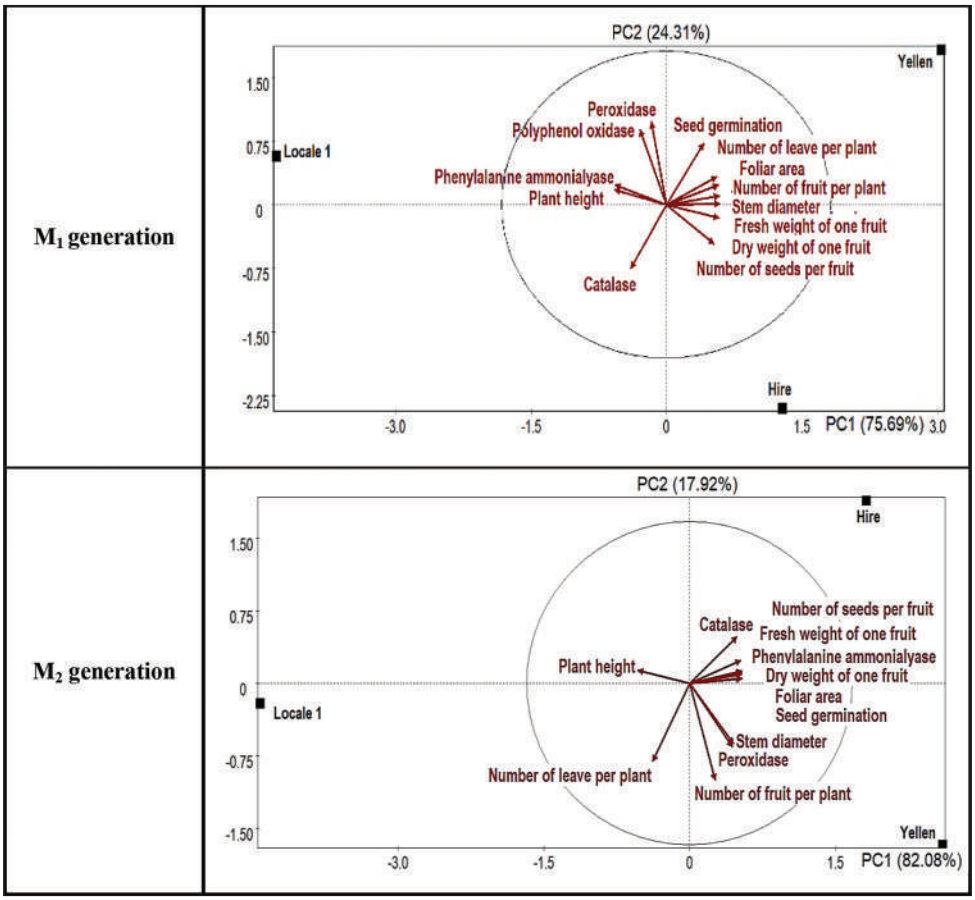


Figure 7: Principal component analysis based on growth traits, productivity and enzymatic activities from three okra varieties in M_1 and M_2

of plant height in PC_1, we observe significant enzymatic activities (POX, CAT, PPO, and PAL) in Locale 1, but the Yellen and Hire varieties were the most influenced by the other parameters (growth and productivity) that dominate PC_2 (Figure 7).

Estimates of variability parameters for different traits

After analyzing the genetic variances in M_2 generations of three okra varieties, we observed that the phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all descriptors studied after different mutagen treatments (Table 4). The PH (60.66), PPO (56.32), and NFP (44.08) traits of the three studied, respectively growth, enzyme system, and yield parameters, were the highest in PCV (Table 4). The FA trait had the highest value in GCV (44.77), with a PCV value of 51.12 (Table 4). Broad-sense heritability ranged from 12.01% (POX) to 77.40% (FA) (Table 4). The descriptors FA, SG, and NFP all had high broad-sense heritability estimates with 77.40%, 72.66% and 64.76% respectively. In contrast, moderate estimates of broad-sense heritability were obtained for PAL, DW1F, PH, NLP, and SD, with percentages ranging from 30% to 60% (Table 4). The

Table 4: Estimates of variability, broad-sense heritability, and genetic advance as a percent of mean for growth, yield, and enzymatic activities in okra generations M_2

Character	Mean	Range		Coefficient of variation		Heritability in broad-sense (H^2)	Genetic advance (GA)	Genetic advance as percentage of mean (GAM)
		Min.	Max.	GCV	PCV			
NLP	12.76	8.92	21.04	22.40	39.06	47.33	0.23	26.67
FA	312.96	245.57	657.16	44.77	51.12	77.40	0.38	80.77
PH	38.47	40.89	96.11	12.24	60.66	40.72	0.06	5.09
SG	63.78	41.22	100.00	30.60	38.27	72.66	0.36	50.42
SD	9.77	6.35	15.95	21.05	33.90	54.02	0.27	26.94
NFP	6.44	5.11	12.33	31.24	44.08	64.76	4.63	94.52
FW1F	15.78	8.67	22.12	16.13	28.19	22.03	3.06	26.36
DW1F	13.12	6.54	19.73	16.01	28.36	36.41	3.03	33.06
NSF	76.51	40.03	94.98	10.64	32.33	27.22	2.75	3.65
CAT	0.43	0.20	0.65	19.95	41.84	12.23	0.11	0.85
POX	0.37	0.09	0.59	6.94	50.85	12.01	0.00	1.95
PAL	0.23	0.06	0.33	11.28	42.44	33.07	0.01	6.18
PPO	0.12	0.09	0.22	8.13	56.32	18.10	0.05	2.02

Min.=minimum; Max.=maximum; GCV=genotypic coefficient variance; PCV=phenotypic coefficient variance; NFP=average number of fruit per plant; FW1F=average fresh weight of one fruit; DW1F=average dry weight of one fruit; NLP=average number of leaves per plant; FA=average foliar area; PH=average plant height; SG=average seed germination; SD=average stem diameter; NSF=average number of seed per fruit; CAT=catalase, POX=peroxidase; PAL=phenylalanine ammonialyase; PPO=polyphenol oxidase

genetic advance was given as a percentage of mean values that varied from 0.85 (CAT) to 94.52 (NFP) (Table 4). However, the FA and NFP traits revealed the highest levels of heritability (77.40% and 64.76%) and GAM (80.77% & 94.52%), respectively. We additionally noticed a high value of 4.63 in genetic gain (GA) for the NFP character (Table 4).

DISCUSSION

Three Cameroonian okra varieties were subjected to a chemical mutagen-induced mutation method in order to generate novel genetic diversity that could affect crop development and productivity. After induced mutagenesis, all of the resulting phenotypes are inherited. However, the M_2 generation of the three varieties examined performed well in all of the traits investigated (growth, yield, and enzymatic activity), as opposed to the M_1 generation (Locale1, Yellen, & Hire), which received various sodium azide treatments. It developed into necessary to focus efforts on a particular number of M_2 generation (Srivastava *et al.*, 2011). Our research demonstrated that the mutagen used during the intense cell division process of germination would have had an inhibitory action, slowly leading to partial, then total, inhibition of the seed germination rate depending on the dose of NaN_3. In fact, emergence and germination are linked biological processes. Similarly, a progressive decrease in the values of this parameter was observed when the effects of increasing doses of this mutagen were observed on four distinct varieties of *Lycopersicon esculentum* (Adeosun *et al.*, 2020). It was discovered in *Capsicum annuum* that large quantities of this mutagen gradually lowered physiological and biological processes to the point of preventing cell division (Omeke, 2021). This finding is consistent with the results obtained in the T3 treatment (0% seed germination rate in Locale 1). Furthermore, the plant died as a result of applying sodium azide at high quantities, which was above the suggested dose (Zeinullina *et al.*, 2023). According to research by Naaz *et al.* (2019), a change in the mitochondrial apparatus may result in an ATP deficit and an interruption in germination. NaN_3's activity would result in a decrease in emergence and a corresponding fall in germinate performance. Similar findings were reported by Viani *et al.* (2019), who highlighted that the O-acetylserine sulfhydrylase enzyme produced the molecule that conferred this mutagen its effectiveness. It is believed that this organic azide molecule, which enters the cell nucleus, interacts with genomic DNA to alter amino acids, changing the way that proteins that are probably made regularly function (Mosisa *et al.*, 2013; Srivastava *et al.*, 2019). This could account for the variation in the plant's reaction that was noticed.

The reduction in leaf number per plant and leaf area of all three varieties in this study was caused by a decrease in the mechanisms associated with leaf renewal, which are related to leaf enlargement, as a result of the NaN_3 treatments. Similar results were reported in the study of Yafigham *et al.* (2018), who indicated that increasing NaN_3 concentrations ranging from 0 to 1.60 mM will diminish leaf number production in *C. annuum*. Notwithstanding the current experiment's outcomes, earlier research on plant species including *L. esculentum*, *A. moschatus*, and *S. indicum* showed that sodium azide improved leaf performance (Mensah & Obadoni, 2007; Aminu *et al.*, 2017). Specifically, the reaction of all three varieties of *L. esculentum* subjected to this mutagen showed that sodium azide might promote the formation of leaf primordia, leading to a higher number of leaves in treated individuals in comparison to the control sample (Aminu *et al.*, 2017). It was determined that chromosomal damage, disrupted enzymatic activities, and an accumulation of protein and mineral metabolism were the causes of the reduction in leaf development values (leaf abnormalities) (Grover & Virk, 2017).

However, as the stem girth in particular gives the plant tremendous strength to survive in the field, plant height is also an important characteristic of okra. In the three varieties we utilized, we observed a decrease in plant height and girth diameter in our data. It is believed that this decrease results

from NaN_3's detrimental effects on the processes involved in leaf production and the growth of leaf blades. These findings suggest a reduction in photosynthetic activity, which in turn suggests a reduction in the total amount of energy and organic matter produced, both of which are necessary for the plant's growth in width (auxesis) and height (meresis). Our findings are consistent with those of (Mensah & Obadoni, 2007; Sheikh *et al.*, 2012), who noted that the mutagen's effect on *S. indicum* and *T. aestivum* would be due to sodium azide acting as a respiratory inhibitor and depriving the cells of the energy required for mitosis, which would cause a decrease in height. As per reference (Omeke, 2021), the induction of a reduction in stem diameter by sodium azide is ascribed to the obliteration of genetic material and the suppression of cell division, which subsequently hinders the expansion of stem thickness. When increasing NaN_3 concentrations of 0-1.60 mM, 0-2.5 mM, 0.5-2 mM, and 0-0.05% were applied to *C. annuum* (Yafizham *et al.*, 2018), *Oryza sativa* (Dewi *et al.*, 2016), *T. aestivum* (Türkoğlu *et al.*, 2023), and *Phaseolus vulgaris* (Mosisa *et al.*, 2013), it was observed in a practical illustration that the plant specimens shrank in comparison to the controls. There are several reasons that could support the quantitative decrease in the percentage of plant material produced when sodium azide is used. These include perturbations in the stability of growth regulators and promoters, such as brassinosteroids, which result in altered plant development; and physiological disruption of chlorophyll production in mutants (Mitchum *et al.*, 2006; Gnanamurthy *et al.*, 2013). Similarly, two elements contribute to the shorter (semi-dwarf) plants that are produced when this mutagen is applied. First of all, it modifies gibberellin production, which is responsible for inducing stem elongation. Second, the final link in the electron transfer chain that influences breathing is inhibited by azide ions *sativa* (Dewi *et al.*, 2016). On the other hand, an increase in plant height was noted for increasing amounts of 0-0.4% and 0-0.5% sodium azide applied, respectively, to *Diospyros lotus* (Mensah & Obadoni, 2007) and the Kvartet variation of *Panicum miliaceum* (Zeinullina *et al.*, 2023).

The complex effects of NaN_3 treatment on plants vary with concentration and involve a variety of interactions that can both accelerate and inhibit growth. The ability of some enzymes to react to biotic or abiotic stress is linked to their variety susceptibility and determines how they respond. In addition, sodium azide is a salt, which may induce abiotic stress (salt stress). As NaN_3 concentration increased, POX, CAT, PAL and PPO were found to increase in Locale 1, in contrast to the fall in these parameters' values that was noted somewhat in Hire and more prominently in Yellen. Differences in the mutagen's absorption or metabolism in plant cells could be the cause of the diversity in these reactions (Yafizham *et al.*, 2018). Jude *et al.* (2016) and Elian *et al.* (2021) showed that these enzymes are thought to be biochemical indicators of stress resistance, both biotic and abiotic. Sodium azide functions as a nitric oxide (NO) donor when H_2O_2 and catalase are present, as well as a catalase inhibitor. Here, we demonstrated how reactive nitrogen species produced by catalase-catalyzed oxidation of NaN_3 can aid in tyrosine nitration when H_2O_2 is present (Ogino *et al.*, 2001). Heme-containing enzymes such cytochrome oxidase, peroxidase, and catalase are inhibited by sodium azide. According to Khan *et al.* (2009) seedlings treated with NaN_3 yield mutant plants that have more capacity for antioxidation than normal plants. In particular, PPO activity is one of the plant's most potent defensive tools because of its capacity to create quinones (Elian *et al.*, 2017). They are useful markers for measuring the effects of the mutagen in this regard. With the intention of enabling the plants to subsequently have a continuous and sufficient production of fruit biomass as in M_2, the increase observed in Locale 1 would be the result of the activation of new genes, enhancing the character associated to the creation of this enzymatic resource. Furthermore, the genetic uniqueness of this variety would promote the development of a self-regulating system aimed at mitigating or even eliminating the inhibitory, detrimental effects of NaN_3. Thus, this situation would be to blame for the conflict observed between the morphological, agronomic and enzymatic responses recorded in Locale 1. Depending on the varietal specificity of the drug, the stress caused by its salty characteristic could result in disorders that cause an increase or decrease in the synthesis of these enzymes (POX, CAT, PAL, and PPO). Because peroxidases are physiologically ubiquitous and naturally play a significant role in plant protection, sodium azoture can easily increase the production of these enzymes (Diao *et al.*, 2019). Prior studies assessing the effects of increasing NaN_3 doses on peroxidase and catalase activities on *P. sativum*, *Vicia faba*, and *Helianthus annuus* have demonstrated that this mutagen is useful in gradually raising the values of these enzyme parameters (Elfeky *et al.*, 2014; Hamouda *et al.*, 2014). Moreover, *P. sativum* and *Vicia faba* showed a six-fold increase in peroxidase activity after being exposed to high concentrations of NaN_3 in comparison to their controls (Hamouda *et al.*, 2014). Furthermore, in *P. sativum* and *V. faba* (Saad-Allah *et al.*, 2014) and *T. aestivum* (Sheikh *et al.*, 2012), an increase in total protein (including enzymes) was seen in proportion to the increase in NaN_3 concentrations from 0 to 4 mM and then from 0 to 0.04%, respectively. As a carcinogenic salt, sodium azide induces metabolic problems, which are characterized by an excess of H_2O_2, which raises the activity of peroxidase (Elfeky *et al.*, 2014). According to (Zeinullina *et al.*, 2023), the genetic apparatus would be influenced by the increase in enzyme activity that results from the mutational effect of NaN_3. This could increase plant resistance to harmful environmental factors and pathogens, resulting in higher yields and improved quantitative characteristics (plant weight, fruit weight, number of fruits, etc.). It is well known that chromosomal aberrations, or modifications to the DNA molecule's structure (a disarray of the regular nucleotide arrangement), are the source of NaN_3-induced mutations. These aberrations result in genetic information changes that are translated into various expressions, like the three varieties examined here (Yafizham *et al.*, 2018). Sodium azide disrupts the enzymatic activity of the plant, according to (Liamngee *et al.*, 2017).

Genetic variability is essential for any crop improvement initiative. The analysis of variance of three okra varieties for growth parameters (NLP, FA, PH, SG, and SD), yield (NFP, FW1F, DW1F, and NSF), and enzyme activities (CAT, POX, PAL, and PPO) reveals genetic variability in M_2 generation

material. All of the characters resulting from treatment (sodium azide) under study displayed a wide range of values, indicating substantial possibility for exploiting the traits across the selection process. The analysis of variance for the 13 descriptors of the three okra varieties revealed that PCV (60.66 for plant height) was greater than GCV (44.77 for foliar area) for all features, showing that the environment influences the expression of the traits. These findings are consistent with those reported by Sundari *et al.* (2022), who discovered that the values of the agronomic characteristics tested on *Sesamum indicum* were more significant in PCV than GCV. This would suggest that the observed variation is due to both environment influences and genetics. According to Ramya *et al.* (2017)'s observations on restorer lines of *Penniqetum glaucum*, the GCV offers a valuable approach for examining the variability of different phenotypes since it depends the heritable genetic part of total variation. However, heritability in broad sense and predicted genetic advance as a percentage of the mean are essential genetic parameters of the trait characteristic under selection. This study found high estimates of broad-sense heritability (H^2>60%) for foliar area (H^2<77.40%), seed germination (H^2<72.66%), and number of fruits per plant (H^2<64.76%). These results are consistent with those of Singh *et al.* (2014) and Ramya *et al.* (2017). Dry weight of one fruit (H^2=36%) recorded moderate estimates of broad-sense heritability. Yahaya (2015) reported comparable findings about grain yield per plant. The results of Mohammadi *et al.* (2012) also found that the descriptors fruit per plant and yield of fruit per plant are the most important in okra growing. The most significant values of genetic advance were for number of fruit per plant (94.52%), foliar area (80.77%), seed germination (50.42%), and dry weight of one fruit (33.06%), highlighting that these descriptors are governed by additive gene effects and that selection will benefit the enhancement of these characters in okra. Previous studies by Panigrahi *et al.* (2014) and Seck *et al.* (2023) yielded similar results on *Vigna mungo* and *Oryza sativum*, respectively. Genetic gain might be deemed the yearly efficiency improvement obtained through selective breeding. However, our research presented that foliar area (H^2 =77.40% and GAM=80.77%) and number of fruit per plant (H^2=64.76% and GAM=94.52%) exhibited high estimates of broad-sense heritability and genetic advance as percent of mean, highlighting that these descriptors may be developed further using basic selection approaches. This would imply that the broad-sense heritability value linked to genetic advance as percent of mean was more trustworthy than heritability estimates alone for character selection (Vijay *et al.*, 2015; Ramya *et al.*, 2017). Muluken *et al.* (2016), for example, demonstrated that combining high heritability and high GAM would offer more information than each parameter isolated, revealing how genes cooperate and are transformed.

CONCLUSION

Three different okra cultivars were treated to sodium azide (NaN_3), and their genetic variability was examined in order to identify exploitable growth, productivity, and enzymatic characteristics. Through mutagenesis using varying concentrations of sodium azide on okra seeds, our varieties Locale 1 (plant height), Yellen (seed germination and number of fruits per plant), and Hire (weights of a fresh seed and a dry seed) demonstrated the best results, regardless of the M_1 and M_2 generations. On the other hand, a number of growth and yield characteristics showed a negative correlation with sodium azide administration, indicating that the plant was doing better when it came to enzymatic indicators of resistance (bioprotection), such as PPO, CAT, PAL, and POX. The production process of the facility can incorporate these enhanced features. Likewise, quantitative genetic parameters (variability, heritability, and genetic advance) might be helpful in the selection of certain trait. Through hybridization, these enhanced characteristics can be integrated with other, sensitive, and untested varieties. Therefore, other plant species with high food intake that have not yet been examined for this mutagen should also be exposed to this genetic enhancement technique.

AUTHORS' CONTRIBUTION

Raphael Siméon NJOCK: Writing original draft, methodology, writing – review and editing. Benoît Constant LIKENG-LI-NGUE: Conceptualization, writing review and editing, investigation. Luther Fort MBO NKOULOU: Methodology, writing review and editing. Jude MANGA NDJAGA: Methodology, writing review, editing, investigation, formal analysis, and visualization. Hermine Bille NGALLE and Joseph Martin BELL: Writing review and editing, Supervision.

REFERENCES

Adeosun, C. A., Elem, K. A., & Eze, C. D. (2020). Mutagenic effects of sodium azide on the survival and morphological characters of tomato varieties. *Nigerion Journal of Biotechnology, 37*(1), 55-62. https://doi.org/10.4314/njb.v37i1.6

Allard, R. W. (1960). *Principles of Plant Breeding*. New York, US: John Wiley and Sons.

Aminu, Y., Bala, B. U., Kabiru, H. I., & Musbahu, A. A. (2017). Induced growth and yield responses to seasonal variation by sodium azide in tomato (*Lycopersicon esculentum* Mill.). *Bayero Journal of Pure and Applied Sciences, 10*(1), 226-230. https://doi.org/10.4314/bajopas.v10i1.45S

Angel, D. P., Ahamed, M. L., Satish, Y., Patro, T. S. S. K., Prakash, K. K., & Srivastava, R. K. (2024). Genetic variability analysis for yield and important agronomic traits in association panel of pearl millet. [*Pennisetum glaucum* (L.) R. Br.]. *International Journal of Advanced Biochemistry Research, 8*(9), 843-848. https://doi.org/10.33545/26174693.2024.v8.i9Sj.2230

Anonymous. (2015). *Plan communale de développement de Bipindi*. Yaoundé, Cameroon: OAL, ATIPAD & PNDP.

Anwar, F., Rashid, U., Ashraf, M., & Nadeem, M. (2010). Okra (*Hibiscus esculentus*) seed oil for biodiesel production. *Applied Energy, 87*(3), 779-785. https://doi.org/10.1016/j.apenergy.2009.09.020

Arulbalachandran, D., Mullainathan, L., & Velu, S. (2009). Screening of mutants in black gram (*Vigna mungo* (L.) Hepper) with effect of DES and COH in M generation. *Journal of Phytology, 21*, 213-218.

Aviya, K., & Mullainathan, L. (2018). Studies on effect of induced mutagenesis on Finger millet (*Eleusine coracana* (L.) gaertn.) VAR-CO 13 in M1 generation. *Horticultural Biotechnology Research, 4*, 23-25. https://doi.org.10.25081/hbr.2018.v4.3485

Bhat, T. A., Khan, A. H., & Parveen, S. (2005). Comparative analysis of meiotic abnormalities induced by gamma rays, EMS and MMS in *Vicia faba* L. *The Journal of Indian Botanical Society, 84*, 45-48.

Cakmak, I., & Horst, W. J. (1991). Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, peroxidase activities in root tips of soybean (*Glycine max* L.). *Physiologia Plantarum, 83*(3), 463-468. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x

Capot, S., & Perus, D. (2013). *Les Enjeux De La Propriété Industrielle*

Appliques Aux Semences Végétales. Cahiers Du Lab. RII: Documents De Travail, Laboratoire de Recherche sur l'Industrie et l'Innovation. Université de la Côte d'Opale.

Dabholkar, A. R. (1992). *Elements of Bio Metrical Genetics* (pp. 57-116) New Delhi, India: Concept Publication company.

Das, S., Nandi, G., & Ghosh, L. K. (2019). Okra and its various applications in drug delivery, food Technology, health care and pharmacological aspects-a review. *International Journal of Pharmaceutical Sc*ience*, 11*(6), 2139-2147.

Dewi, K., Agustina R. Z., & Nurmalika, F. (2016). Effects of blue light and paclobutrazol on seed germination, vegetative growth and yield of black rice (*Oryza sativa* L. "Cempo Ireng"). *Journal of Biotropian, 23*(2), 85-96. https://doi.org/10.11598/btb.2016.23.2.478

Dewi, K., Meidiana, G., Sudjino, S., & Suharyanto, S. (2016). Effects of Sodium Azide (NaN_3) and cytokininon vegetative growth and yield of black rice plant *(Oryza sativa* L. Cempo Ireng'). *AIP Conference Proceedings, 1755*(1), 130005. https://doi.org/10.1063/1.4958549

Diallo, A., Goudiaby, A. O. K., Diedhiou, M. A. A., Sambou, A., Niang Y. D., & Fall, B. F. (2022). Characteristics and weed dynamics of okra (*Abelmoschus esculentus* L) and tomato (*Solanum lycopersicum* L) under the effect of different org anic fertilizers. *International Journal of Biological and Chemical Sci*ences*, 16*(6), 2804-2820. https://doi.org/10.4314/ijbcs.v16i6.26

Diao, M., Dembele, R. H., Konate, K., & Dicko, M. H. (2019). Étude comparative des peroxydases de dix (10) plantes supérieures couramment rencontrées au Burkina Faso. *Internationa*l *Journal of Biological and Chemical Sciences, 13*(6), 2533-2545. https://doi.org/10.4314/ijbcs.v13i6.9

El Kaaby, E. A. J., Al-Ajeel, S. A., Al-Anny, J. A., Al-Aubaidy, A. A., Abdulrazaq, A., & Ammar, K. (2015). Effect of the chemical mutagens sodium azide on plant regeneration of two tomato cultivars under salinity stress condition *in vitro*. *Journal of Life Sciences, 9*, 27-31.

Elfeky, S., Abo-Hamad, S., & Saad-Allah, K. M. (2014). Physiological impact of sodium azideon *Helianthus annus* seedlings. *International Journal of Agronomy and Agricultural Research, 4*(5), 102-109.

Elian, D. H., Fotso, Djeuani, A. C., Djamndo, D. M., & Omokolo, N. D. (2021). Evaluation des activités polyphénoloxydases, peroxydases et l'accumulation des composés phénoliques dans la résistance du manioc stimulé au Benzo (1, 2, 3) thiadiazol-7-carbothionic acid-s-méthyl ester vis-à-vis de *Colletotrichum gloeosporioides* Penz. *International Journal of Biological and Chemical Sciences, 15*(3), 950-965. https://doi.org/10.4314/ijbcs.v15i3.9

Elian, H. D. B. (2017). *Micropropagation du manioc (Manihot esculenta Crantz) et évaluation des marqueurs biochimiques de résistance à Colletotrichum gloeosporioides Penz* Doctoral dissertation. Université de Yaoundé 1.

Esson, A. E., Adebola, M. I., & Yisa A. G. (2018). Frequency of mutation, lethality and efficiency of ethyl methane sulphonate and sodium azide on foxtail millet (*Setaria itálica* [L.] P. Beauv.). *Journal of Scientific Agriculture, 2*, 9-13. https://doi.org/10.25081/jsa.2018.v2.845

Eze, J. J., & Dambo, A. (2015). Mutagenic effects of sodium azide on the quality of maize seeds. *Journal of Advanced Laboratory Research in Biology, 6*(3), 76-82.

Gnago, J. A., Danho, M., Agneroh, T. A., Fofana, I. K., & Kohou, A. G. (2010). Efficacité des extraits de neem (*Azadirachta indica*) et de papayer (*Carica papaya*) dans la lutte contre les ravageurs du gombo (*Abelmoschus esculentus*) et du chou (*Brassica oleracea*) en Côte d'Ivoire. *International Journal of Biological and Chemical Sciences, 4*(4), 953-966. https://doi.org/10.4314/ijbcs.v4i4.63035

Gnanamurthy, S., Dhanavel, D., & Bharathi, T. (2013). Effect of chemical mutagenesis on biochemical activity and variability, heritability and genetic advances in *Zea mays* (L.). *International Journal of Current Science, 3*(4), 57-61.

Grover, I. S., & Virk, G. S. (1984). Induced chlorophyll mutants in mungbean (*Vigna radiate*). *Acta Botanica Indica, 12*(2), 138-147.

Hamouda, M., Saad-Allah, K., & Kasim, W. A. (2014). Molecular and physiological responses of *Pisum sativum* and *Vicia faba* to sodium azide. *International Journal of Agronomy and Agricultural Research, 4*(6), 46-61.

Herwibawa, B., & Kusmiyati, F. (2017). Mutagenic effects of sodium azide on the germination in rice (*Oryza sativa* L. cv. Inpago Unsoed 1). *Journal Agroteknologi, 7*(2), 9-14. https://doi.org/10.24014/ja.v7i2.2759

Hoyt P., & Bradfield, R. (1962). Effect of varying leaf area by partial defoliation and plant density on dry matter production in corn. *Agronomy Journal, 54*(6), 523-525.

Imran, M., & Kramer, H. (1951). Segregation for yield, height and maturity following a soya bean cross. *Agronomy Journal, 43*(12), 605-609. https://doi.org/10.2134/agronj1951.00021962004300120005x

Johnson, H. W., Robinson, H. F., & Comstock, R. E. (1955) Estimates of Genetic and Environmental Variability in Soybeans. *Agronomy Journal, 47*(7), 314-318. https://doi.org/10.2134/agronj1955.00021962004700070009x

Jude, M. N., Pierre, E. O., Louise, O. M., Christian, D. K. J., & François, D. P. (2016). Heritability of the tolerance to *Phytophthora megakarya* Bras. and Grif. of *Theobroma cacao* L. in terms of their necrosis length, phenolic contents and activity of enzymes. *International Journal of Biosciences, 8*(5), 249-261.

Kammenn, V., & Broumer, D. (1964). Increase of polyphenoloxidase activity by a local virus infection in uninoculated of leaves. *Virology, 22*(1), 9-14. https://doi.org/10.1016/0042-6822(64)90042-X

Khan, S., Al-Qurainy, F., & Anwar, F. (2009). Sodium azide: a chemical mutagen for enhancement of agronomic traits of crop plants. *Environment We International Journal of Science and Technology, 4*, 1-21.

Lanaud, C. (1986). Utilisation des marqueurs enzymatiques pour l'étude génétique du cacaoyer. *Café Cacao Thé, 30*, 259-270.

Liamngee, S. M., Ogah, J. J., Amagu, K. T., Kwon-Ndung, E. H., Iorkor, D., & Tervershima, J. E. (2017). Mutagenic action of sodium azide on germination and emergence in landraces of *Phaseolus vulgaris* L. on the Jos Plateau Agro-Ecological Zone. *IOSR Journal of Agriculture and Veterinary Science, 10*(2), 64-70. https://doi.org/10.9790/2380-1002016470

Lunn, G., & Sansone, E. B. (2012). *Destruction of hazardous chemicals in the laboratory*. New York, US: Wiley.

Marius, C., Gerard, V., & Antoine, G. (1997). Le gombo, *Abelmoschus esculentus* (L.) Moench, une source possible de phospholipides. *Agronomie et Biotechnologies, Oléagineux, corps gras, lipides, 5*, 389-392.

Maryam, A. H. (2017). Effect of Gamma Ray and Sodium Azide on the Germination, Survival and Morphology of Varieties of Okro (*Abelmuscus esculantus* L. moench). *International Journal of Horticulture & Agriculture, 2*(1), 1-4. https://doi.org/10.15226/2572-3154/2/1/00107

Maryam, A. H., & Kasimu, A. A. (2016). Effect of combined doses of gamma ray and sodium azide (mutagenic agents) on the morphological traits of some varieties of okra (*Abelmoschus esculentus)*. *African journal of Agricultural Research, 11*(32), 2968-2973. https://doi.org/10.5897/AJAR2016.11085

Mensah, J. K., & Obadoni, B. (2007). Effects of sodium azide on yield parameters of groundnut (*Arachis hypogaea* L.). *African Journal of Biotechnology, 6*(6), 668-671.

Misra, S., Dubey, S., & Bist, R. (2017). Sodium azide induced mutagenesis in wheat plant. *World Journal of Pharmacy and Pharmaceutical Sciences, 6*(10), 294-304.

Mitchum, M. G., Yamaguchi, S., Hanada, A., Kuwahara, A., Yoshioka, Y., Kato, T., Tabata, K., Kamiya, Y., & Sun, T. P. (2006). Distinct and over lapping roles of two gibberellin 3-oxidases in *Arabidopsis* development. *The Plant Journal, 45*(5), 804-818. https://doi.org/10.1111/j.1365-313X.2005.02642.x

Mohammadi, M., Ramezanpour, S., Navabpour, S., Soltanloo, H., Kalateharabi, M., & Kia, S. (2012). Genetic analysis and heritabilities of resistance to *Mycosphaerella graminicola* in wheat. *Crop Breeding Journal, 2*(1), 35-42. https://doi.org/10.22092/cbj.2012.100414

Mosisa, G., Muthuswamy, M., & Petros, Y. (2013). Sodium Azide: a chemical mutagen for enhancement of agronomic traits in *Phaseolus vulgaris* (Linn). *Journal of Theoretical and Experimental Biology, 10*(1-2), 21-25.

Muluken, D., Wassu, M., & Endale, G. (2016). Variability heritability and genetic advance in Ethiopian Okra [*Abelmoschus esculentus* (L.) Monech] collections for tender fruit yield and other agro-morphological traits. *Journal of Apllied Life Sciences International, 4*(1), 1-12. https://doi.org/10.9734/jalsi/2016/19483

Naaz, N., Choudhary, S., Sharma, N., Hasan, N., Al Shaye, N. A., & El-Moneim, A. D. (2023). Frequency and spectrum of M2 mutants and genetic variability in cyto-agronomic characteristics of fenugreek induced by caffeine and sodium azide. *Fontiers in Plant Science, 13*, 1-21. https://doi.org/10.3389/fpls.2022.1030772

NGuessan, K., Martine, B. M., Jacob, K. N., & Justin, Y. K. (2021). Réponse à la fertilisation organique et minérale de deux variétés de gombo

(*Abelmoschus esculentus* (L.) Moench, *Malvacea*) À Daloa, Côte D'ivoire. *International Journal of Advanced Research, 9*(6), 51-60. https://doi.org/10.21474/IJAR01/12987

Nizamani, M. M., Rafiq, M., Noor-ul-Ain, N., Naqvi, S. H. A., Kaleri, A. H., & Gul, J. (2020). Effect of chemical mutagens on growth of Okra (*Abelmoschus esculentus* L. Moench). *Pure and Applied Biology, 9*(1), 1110-1117. https://doi.org/10.19045/bspab.2020.90116

Njock, R. S., Likeng-Li-Ngue, C. B., Ndjaga, J. M., Ngalle, H. B., & Bell, J. M. (2024). Caractérisation phytochimique et application de l'azoture de sodium (mutagène chimique) sur les graines de trois variétés de gombo (*Abelmoschus esculentus*) cultivées au Cameroun. *Afrique Science, 24*(6), 108-119.

Ogino, K., Kodama, N., Nakajima, M., Yamada, A., Nakamura, H., Nagase, H., Sadamitsu, D., & Maekawa, T. (2001). Catalase catalyzes nitrotyrosine formation from sodium azide and hydrogen peroxide. *Free Radical Research, 35*(6), 735-747. https://doi.org/10.1080/10715760100301241

Okey, E. N., Duncan, E. J., Sirju-charran, G., & Sreenivasan, T. N. (1997). *Phytophthora* canker resistance in cacao: Role of peroxidase, polyphenoloxidase and phenylalanine ammonia-lyase. *Journal of Phytopathology, 145*(7), 295-299. https://doi.org/10.1111/j.1439-0434.1997.tb00404.x

Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. A., Miah, G., & Usman, M. (2016). Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnology & Biotechnological Equipment, 30*(1), 1-16. https://doi.org/10.1080/13102818.2015.1087333

Omeke, J. O. (2021). Effect of sodium azide induction on morphological traits of *shombo* and *tatase* (*Capsicum annuum* L.). *Nigerian Agricultural Journal, 52*(1), 111-117.

Panigrahi, K. K., Mohanty A. & Baisakh, B. (2014). Genetic divergence variability and charácter association in landraces of black gram (*Vigna mungo* (L.) Hepper) from Odisha. *Journal of Crop and Weed, 10*(2), 155-165.

Patil, B. S., Ahamed, L. M., Babu D. R., & Rani Y. A. (2015). Studies on genetic variability, heritability and genetic advance estimates in maize (*Zea mays* L.). *The Andhra Agricultural Journal, 62*(4), 821-824.

Pour, A. H., Tosun, M., & Haliloglu, K. (2023). Effect of Sodium Azide Treatment at Different Duration and Concentration on germination and Seedling Growth Characters in Wheat (*Triticum aestivum* L.). *Journal of Science and Technology, 16*(2), 406-422. https://doi.org/10.18185/erzifbed.1058149

Ramya, A. R., Ahamed, L., Rakesh, K., & Srivastava. (2017). Genetic diversity analysis among inbred lines of Pearl millet [*Pennisetum glaucum* (L.) R. Br.] based don grain yield and yield component characters. *International Journal of Current Microbiology and Applied Science, 6*(6), 2240-2250.

Rodriguez, R., & Tames, R. S. (1982). Peroxidase and IAA oxidase in germinating seeds of *Cicer arientum* L. *Revista Espanola de Fisiologia, 38*, 183-188.

Roychowdhury, R., & Tah, J. (2013). Mutagenesis - a potential approach for crop improvement. In K. Hakeem, P. Ahmad, & M. Ozturk (Eds.), *Crop Improvement* (pp. 149-187) Boston, MA: Springer. https://doi.org/10.1007/978-1-4614-7028-1_4

Saad-Allah, K. M., Hammouda, M., Kasim, W.A. (2014). Effect of sodium azide on growth criteria, some metabolites, mitotic index and chromosomal abnormalities in *Pisum sativum* and *Vicia faba*. *International Journal of Agronomy and Agricultural Research, 4*(4), 129-147.

Sable, A. D., Sable, A. D., Shegokar, S. P., Gore, N. S., & Harke, S. (2018). Effect of Sodium Azide Induction on Germination Percentage and Morphological Growth in Two Varieties of Okra. *International Journal of Current Microbiology and Applied Sciences, 7*(6), 3586-3593. https://doi.org/10.20546/ijcmas.2018.706.422

Seck, F., Covarrubias-Pazaran, G., Gueye, T., & Bartholomé J. (2023). Realized Genetic Gain in rice: achievements from breeding programs. *Rice,* 16, 61. https://doi.org/10.1186/s12284-023-00677-6

Sheikh, S. A., Wani, M. R., Lone, M. A., Tak, M. A., & Malla, N. A. (2012). Sodium Azide Induced Biological Damage and Variability for Quantitative Traits and Protein Content in Wheat [*Triticum aestivum* L.]. *Journal of Plant Genomics, 2*(1), 34-38.

Singh, B., Upadhyay, P. K., & Sharma K. C. (2014). Genetic variability, correlation and path analysis in Pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Indian Research Journal of Genetics & Biotechnology, 6*(3), 491-500.

Srivastava, P., Marker, S., Pandey, P., & Tiwari, D. K., (2011). Mutagenic effects of sodium azide on the growth and yield characteristics in wheat (*Triticum aestivum* L. em. Thell.). *Asian Journal of Plant Sciences, 10*(3), 190-201. https://doi.org/10.3923/ajps.2011.190.201

Srivastava, R., Agarwal, J., Pareek, M., & Verma, A. (2019). Mutagenic Effect of Sodium Azide (NaN_3) on Seed Germination and Chlorophyll Content of *Spinach oleracea*. *Indian Journal of Pure Applied Biosciences, 7*(4), 366-370. https://doi.org/10.18782/2320-7051.7733

Sundari, S., Vasline, A. Y. & Saravanan, K. (2022). Selection of traits for seed yield improvement through variability parameters in sesame (*Sesamum indicaum* L.) genotypes. *Journal of Applied and Natural Science, 14*(3), 829-834. https://doi.org/10.31018/jans.v14i3.3507

Türkoğlu, T., Haliloğlu, K., Tosun, M., Szulc, S., Demirel, F., Eren, B., Bujak, H., Karagöz, H., Selwet, M., Özkan, G., & Niedbała, G. (2023). Sodium Azide as a Chemical Mutagen in Wheat (*Triticum aestivum* L.): Patterns of the Genetic and Epigenetic Effects with iPBS and CRED-iPBS Techniques. *Agriculture, 13*(6), 1242. https://doi.org/10.3390/agriculture13061242

Viani, V. E., Pegoraro, C., Busanello, C., & de Oliveira, A. C. (2019) Mutagenesis in rice: the basis for breeding a new super plant. *Frontiers in Plant Science, 10*, 1326. https://doi.org/10.3389/fpls.2019.01326

Vijay, K. G., Vanaja, M., Jyothi, L. N., & Maheswari, M. (2015). Studies on variability, heritability and genetic advance for quantitative traits in black gram (*Vigna mungo* L.). *Agricultural Research Journal, 52*(4), 28. https://doi.org/10.5958/2395-146X.2015.00056.3

Yafizham, & Herwibawa, B. (2018). The effects of sodium azide on seed germination and seedling growth of chili pepper (*Capsicum annum* L. cv. Landung). *IOP Conference Series: Earth and Environmental Science, 102*, 1-6. https://doi.org/10.1088/1755-1315/102/1/012052

Yahaya, Y. (2015). Correlation and heritability in Pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *African Journal of Agronomy, 3*(3), 257-258.

Zeinullina, A., Rysbekova, A., Dyussibayeva, E., Zhirnova, I., Zhanbyrshina, N., Zhunusbayeva, Z., & Yancheva, S. (2023). Application of sodium azide for chemical induced mutagenesis of proso millet culture (*Panicum miliaceum* L.). *Bulgarian Journal of Agricultural Science, 29*(4), 623-631.