INDUCTION OF GENETIC VARIABILITY IN SESAME (*Sesamum indicum* L.) WITH SODIUM AZIDE

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Abstract: An evaluation of the mutagenic efficiency and mutagenic effectiveness of fast neutron and sodium azide to induce genetic variability and beneficial mutation on *Sesamum indicum* was carried out. The seeds of sesame were subjected to four treatments (0.5, 1.0, 1.5 and 2.0 mM) of sodium azide. The treatments were laid out in a completely randomized block design. Harvested seeds from M_1 generation were sown to raise the M_2. The mutagenic effectiveness and efficiency of sodium azide in sesame was 2.27 and 0.72%, respectively. Mutagenic effectiveness was dose dependent. A nonlinear decline in mutagenic efficiency was observed with increasing concentration and dose of sodium azide. Upon comparism with the control, sodium azide induced beneficial variabilities on the agronomic traits evaluated at M_2 generation. Best growth recorded in most of the morphological and yield traits evaluated was at 0.5 mM concentration of sodium azide concentration. Broad sense heritability estimates for the agronomic traits evaluated ranged from 1.11 to 97.82%. High heritabilities obtained in days to flowering (97.82%) and thousand seed weight (87.50%) indicates that variation for these characters are due to high additive gene effects and consequently the scope for improving yield through selection for these traits in sesame is high.

Keywords: Genetic, heritability, mutagen, sesame, variance

Introduction

Sesamum indicum is a very ancient crop and one of the earliest domesticated oil crops in the world dated back to 2130 BC (Weiss, 1983). It acquired importance as a source of cheap vegetable oil, proteins and natural oxidants (Sesamin and sesamolin) which are unique for sesame and present in the oil (Ashri, 2007). In some poor countries it is used as a meat substitute (Mohammad *et al.*, 2009). The crop is described as the "Queen of oil seeds" because of its high oil content (38-54%), protein (18-25%), calcium (Ca), phosphorous (P), oxalic acid and excellent qualities of the seed oil and meal (Prasad, 2002). Sesame seeds contains slightly low amount of lysine but is rich in other amino acids like methionine, cystine, arginine and leucine (Moazzami *et al*., 2006).

However, despite the medical, pharmaceutical, cultural and commercial roles of sesame, they have been poorly researched and fall within the neglected and underutilized species (NUS) of Africa (Chweya and Eyzaguire, 1999). This led to the deterioration in the vast reservoir of wealth of this plant (Attere, 1999) and placed them in danger of continued genetic erosion and disappearance which has further restricted developmental options. A number of factors affecting sesame improvement programmes have been identified. Firstly, the germplasm of sesame is not as large as that of some other crops (Ashri, 2007). Secondly, the architectures of the crops are poorly adapted to modern farming system because of their indeterminate growth habit, sensitivity to wilting under intensive management and seed shattering at maturity (Uzun and Cagirgan, 2009). They are also faced with low seed yield which has been attributed to lack of agricultural inputs such as improved varieties, poor management and lack of appropriate breeding programmes (Pham *et al.*, 2010).

One of the most profound multifaceted challenges which the global population is facing in the present millennium is the need to ensure not just adequate quantity but also high quality of food (Godfray *et al*., 2010). Increasing crop yields to ensure food security is a major challenge. Amongst the obstacles against this, are the changing climate (increasing temperatures and more erratic rainfall) which most often compromise crop productivity (Parry *et al.*, 2005). With the onset of the market economy and modernization of agriculture in Africa, conventional agronomy has, to a large extent, concentrated on conserving the genetic resources of exotic rather than indigenous or traditional vegetables.

Consequently, the latter are threatened with extinction as they have to compete for attention with the much more popular exotic vegetables (Maundu *et al.*, 1999). Indigenous knowledge on production methods, preservation, use and nutritive value is no longer transmitted systematically from one generation to another (Weinberger and Msuya, 2004). The neglect of traditional vegetables is unfortunate since these crops are usually better adapted to the environment than the introduced exotic vegetables. Furthermore, traditional vegetables provide low-cost quality nutrition for large parts of the population in both rural and urban areas (Chweya and Eyzaguirre, 1999). There is, also an urgent requirement for new higher yielding varieties (Reynolds *et al.*, 2009) with improved nutrient and water use efficiency (Richards, 2000). As well as exploration of the hidden treasures conserved within the leafy vegetables to protect them from genetic erosion (Mahmoud *et al.*, 1995). This paper reports the effectiveness and efficiency of Sodium azide in inducing beneficial mutation on sesame.

Materials and Methods

Study location

This study was conducted at the botanical garden of the Department of Botany, Ahmadu Bello University, Zaria, (lat. 11º 12ᴵN, long 7º 33ᴵE and on altitude 660 m above sea level).

Sources of materials

Twenty five grams (25 g) of sesame (*Sesamum indicum* L) seeds were obtained and identified from Jigawa State Agricultural and Rural Development Authority (JARDA), Ringim, Jigawa. Sodium azide (made in Kem light laboaratory PVT, LTD Mumbai, India) was obtained from the store of Department of Biological Sciences, Ahmadu Bello University, Zaria.

Treatment and experimental design

Sesame seeds were treated (5 g for each mutagenic treatment) with four concentrations (0.5, 1.0, 1.5 and 2.0 mM) of Sodium azide for 4 h. The control was not exposed to the mutagen. Exposed seeds were thoroughly washed with distilled water and were left to dry for 24 h. The experiments were laid in a completely randomized block design (CRBD) with 4 replications. Each replication was laid out on a field size of 1.5 m by 0.75 m with a row to row and plant to plant distance of 30 and 15 cm, respectively (Mensah and Tope, 2007). Harvested seeds from M_1 generation were sown to raise the M_2

generation. All cultural practices were done as described by Bedigian and Adetula (2004).

Data collection

Data were collected at both M_1 and M_2 generations for the following growth parameters:

Germination percentage at 7 and 14 days after sowing: Germination percents were determined on the seventh and fourteen days after planting (7 and 14 DAP) when the plumule completely emerged out of the soil by counting the number of plants that germinated per treatment divided by the six seeds planted and multiply by hundred.

Seedlings height (cm): Seedling height was taken 30 days after sowing using meter rule in centimeters per treatment. The height was determined by holding the highest leaves erect and recording the highest point of the highest leaf from the soil level or base of the shoot and averaged over three (3) plants.

Number of days to 50% flowering: These were taken per treatment when 50% of the plants in each treatment produced flowers.

Height at maturity (cm): Plants were considered matured after the emergence of first flower. The heights were taken in centimeters using meter rule by recording the height from soil level or base of the shoot to the tip of the highest leaf and averaged over three (3) plants.

Survival rates (%): The number of plants that survived per treatment was counted after the plants have attained 50% flowering.

Number of leaves per plant: The number of leaves per plant in each treatment were counted and recorded after the plants have attained 50% flowering and averaged over three (3) plants.

Internodes length (cm): The lengths between two successive leaves per plant in each treatment were measured by the use of meter-rule after the plants produced pods and averaged over three (3) plants.

Leaf area (cm^2): The leaf area was determined by measuring the length and width of randomly selected leaves and applying the formula outlined by Pearce *et al.* (1979): A= [(L) (W) (0.75)] × 2

Where: A=Leaf Area per plant, L=Length of Leaves and W=Width of leaves

Number of pods per plant: The number of pods produced per plant per treatment was counted and averaged over three (3) plants.

Number of seeds per pod: This was done by breaking four (4) pods from four (4) plants per treatment and the number of seeds produced per pod counted and averaged over the three (4) plants.

Thousand seeds weight (g): Weight of 1000 seeds per treatment was measured using a Sartorius electronic weighing balance (model: cp8201).

Dry weights (g): Dry weights of all the treatments were determined after the plants are uprooted and dried in the oven for three days at 70 ^{0}C. Their dry weights were taken using a Sartorius electronic weighing balance (model: cp8201) per treatment.

Chlorophyll deficient mutants determination of sesame and false sesame: The number of seedlings that showed chlorophyll deficiency was identified at M_2 based on the foliar coloration and recorded (Giri and Apparao, 2011).

Statistical analyses

The mutagenic efficiency and effectiveness were calculated by adopting the formulae recommended by Konzak *et al.* (1965), where;

$$Mutation\ freq.(\%) = \frac{chlorophyll\ mutants\ at\ M_2}{total\ number\ of\ plants\ studied}\ x\ 100$$

$$Mutagenic efficiency.(\%) = \frac{mutation\ frequency}{dosage or\ time\ x\ conc.}\ x\ 100$$

$$Mutagenic efficiency.(\%) = \frac{mutation\ frequency}{percentage lethality}\ x\ 100$$

Morphological data on growth biometrics were analyzed statistically by analysis of variance (ANOVA) and where significant; means were separated by Duncan's Multiple Range Test (DMRT) with the statistical analytical software (2004) version: 9.1.

Broad sense heritability (H_B) was computed at M_2 as specified by the method of Singh and Chaudhary (1985) and Moll *et al.* (1960):

$$H_B = \frac{\delta^2 g}{\delta^2 p}$$

Where: H_B= Broad sense heritability, $\delta^2 g$=Genotypic variance, $\delta^2 p$=Phenotypic variance

Results and Discussion

Mutagenic frequency, efficiency and effectiveness of fast neutron and sodium azide in sesame (Sesamum indicum)

The mutagenic efficiency, effectiveness, lethality percentage of the two mutagens on sesame is presented in Table 1. There was no linear relationship between concentrations of mutagens, mutagenic frequency, lethality and mutagenic efficiency. However, mutagenic effectiveness was dose dependent. Mutagenic frequency was highest at 0.5 mM concentration of sodium azide. At this concentration, mutagenic efficiency (0.72%) and effectiveness (2.27%) were highest. Lethality in sesame was highest (24.1%) at 1.5 mM concentration of sodium azide.

Table 1: Mutagenic frequency, efficiency and effectiveness of sodium azide in *Sesamum indicum*

Conc.	MF (%)	LT(%)	ME(%)	Me (%)
0.5 mM	4.54	6.25	2.27	0.72
1.0 mM	3.75	6.20	0.94	0.60
1.5 mM	3.13	24.1	0.52	0.13
2.0 Mm	4.00	16.6	0.50	0.24

MF-Mutagenic frequency, LT-Lethality, ME- Mutagenic effectiveness, Me-Mutagenic efficiency, Conc: Concentrations.

Mutagenic effects of sodium azide concentrations on the agronomic traits of Sesamum indicum at M_1 generation

The Mean performances of *Sesamum indicum* treated with four concentrations of sodium azide at M_1 generation are presented in Table 2. Sodium azide induced variabilities on the agronomic traits evaluated. These variabilities were an improvement over the control treatment (Table 2). Seeds treated with 0.5 mM had longest seedling height (17.75 cm), best height at maturity (31.25 cm), highest number of leaf per plant (15.75), good leaf area (38.13 cm^2) and highest number of pods per plant (20.50). The germination percentage at 7 and 14 days after sowing were best in seeds treated with 1.0 mM sodium azide. At this concentration, days to flowering was lowest (44.75), survival rate was highest (46.68%), the number of seeds per plant (56.50) and thousand seed weight (4.50 g) were highest. Morphological traits like internode lengths and dry weight in sesame recorded better growth compared to other treatments in seeds treated at a sodium azide concentration of 1.50 mM.

Mutagenic Effects of Sodium Azide concentrations on the Morphological and Agronomic traits of Sesame at M_2 Generation

The Mean performances of sesame traits evaluated at M_2 generation with four concentrations of sodium azide are presented in Table 3. These mutants showed better agronomic improvements compared to the control. However, there was no significant improvement in seedling heights in the mutants and control. There was also no significant variation in the dry weight of all the plants from the seeds treated with sodium azide. However, their dry weights were significantly ($p< 0.05$) higher than the control treatment. Seeds treated with 0.5 mM sodium azide showed better percentage germination (7 and 14 DAS). Days to 50% flowering were shortest (42.00) at this concentration. In addition, survival rates (72.93%), number of leaf per plant (14.00), leaf area (50.38 cm^2), number of seeds per pod (53.25) and thousand seed weight (4.45 g) were highest. Seeds treated with 1.0 mM sodium azide showed comparable growth in germination percentage (7 DAS), number of pod per plants and number of seeds per pod to seeds treated with 0.5 mM concentration of sodium azide. However, the best height at maturity (28.63 cm) and internode length (5.15 cm) was recorded at 1.0 mM concentration of sodium azide.

Comparative Response of Sesamum indicum Treated with Sodium Azide at M_1 and M_2 Generations

The comparative effect of sodium azide at M_1 and M_2 generation in sesame (Table 4) showed that seedling survival rate, leaf area, number of seed per plants, internode length and number of pod per plant, which are mostly vegetative growth parameters, were most prolific at the M_1 generation in sesame. However, germination percentages (7 and 14 DAS) were highest at the M_2 generation.

Table 2: Mean performance of *Sesamum indicum* and *Ceratotheca sesamoides* at M_1 generation upon exposure to sodium azide

Plant	CON	GPSD (%)	GPFD (%)	SH (cm)	DF	HM (cm)	SR (%)	NLPP	LA (cm2)	IL (cm)	NPOD	NSPP	THSWT (g)	DW (g)
Sesamum indicum	0.0Mm	60.40^{b}	56.25^{b}	13.78^{b}	49.00^{a}	23.12^{c}	29.15^{b}	10.25^{c}	22.50^{ab}	5.28^{ab}	11.25^{c}	38.75^{b}	4.10^{d}	9.50^{c}
	0.5mM	70.85^{ab}	60.43^{b}	17.75^{a}	44.75^{b}	31.25^{a}	35.35^{ab}	15.75^{a}	38.13^{a}	5.47^{ab}	20.50^{a}	57.25^{a}	4.40^{b}	15.85^{ab}
	1.0mM	83.35^{a}	66.68^{a}	16.03^{ab}	44.75^{b}	29.00^{ab}	46.68^{a}	12.00^{bc}	33.58^{ab}	5.83^{ab}	15.00^{b}	56.50^{a}	4.50^{a}	14.20^{b}
	1.5Mm	64.58^{b}	57.00^{b}	16.75^{ab}	46.00^{ab}	29.25^{ab}	31.25^{ab}	14.50^{ab}	35.60^{a}	6.28^{a}	16.00^{b}	46.25^{b}	4.44^{a}	16.35^{a}
	2.0Mm	62.50^{b}	58.33^{b}	14.38^{b}	45.25^{ab}	27.50^{b}	37.50^{ab}	14.00^{ab}	27.50^{ab}	5.30^{ab}	15.25^{bc}	56.25^{a}	4.36^{c}	14.03^{b}
	S.E	13.88	11.18	0.74	0.10	0.69	4.85	0.49	3.67	0.49	1.17	2.23	0.01	2.74

Means with the same letter within a column are on significantly different at $P \leq 0.05$

GPSD- Germination % 7 days after sowing, GPFD- Germination % 14 days after sowing SH- Seedling height, DF- Days to 50% flowering , HM- Height at maturity, SR- Survival rate, NLPP- Number of leaves per plant, LA- Leaf Area, IL- Internode length , NPOD- Number of pod per plant, NSPP- Number of seeds per pod, THSWT- Thousand seeds weight , DW- Dry weight of the plants, CON- Concentration of sodium azide

Table 3: Mean performance of *Sesame indicum* at M_2 generation upon exposure to sodium azide

Conc.	GPSD (%)	GPFD (%)	SH (cm)	DF	HM (cm)	SR (%)	NLPP	LA (cm^2)	IL (cm)	NPOD	NSPP	THSWT (g)	DW (g)
0.0Mm	58.35^{b}	54.20^{bc}	9.30^{a}	55.00^{a}	24.25^{ab}	50.00^{b}	10.75^{b}	30.93^{b}	5.00^{ab}	5.25^{b}	51.00^{a}	4.00^{e}	11.63^{b}
0.5Mm	83.35^{a}	79.18^{a}	9.85^{a}	42.00^{e}	26.25^{ab}	72.93^{a}	14.00^{a}	50.38^{a}	4.50^{b}	9.00^{a}	53.25^{a}	4.45^{a}	13.78^{a}
1.0Mm	87.50^{a}	64.60^{ab}	9.98^{a}	44.00^{c}	28.63^{a}	58.4^{ab}	12.50^{ab}	43.13^{ab}	5.15^{a}	9.75^{a}	53.00^{a}	4.30^{c}	13.73^{a}
1.5Mm	64.60^{ab}	45.85^{c}	9.45^{a}	43.00^{d}	25.88^{ab}	40.50^{ab}	13.00^{ab}	38.28^{ab}	5.05^{ab}	6.50^{ab}	53.00^{a}	4.40^{b}	13.25^{a}
2.0Mm	75.00^{ab}	58.40^{bc}	9.68^{a}	45.75^{b}	25.55^{ab}	58.4^{ab}	13.00^{ab}	39.28^{ab}	4.68^{ab}	5.75^{b}	52.50^{a}	4.20^{d}	13.40^{a}
S.E	5.02	8.03	0.59	2.96	1.48	7.80	0.714	6.25	0.19	1.6	2.42	0.01	0.41

Means with the same superscript within a column are not significantly different at $P \leq 0.05$

GPSD- Germination % 7 days after sowing, GPFD- Germination % 14 days after sowing SH- Seedling height, DF- Days to 50% flowering , HM- Height at maturity, SR- Survival rate, NLPP- Number of leaves per plant, LA- Leaf Area, IL- Internode length, NPOD- Number of pod per plant, NSPP- Number of seeds per pod, THSWT- Thousand seeds weight of plants, DW- Dry weight of the plants, S.E- Standard error, Con: concentration of siodium azide.

Table 4: Combined performance of *Sesame indicum* treated with sodium azide at M_1 and M_2 generation

Plants	GENS	GPSD (%)	GPFD (%)	SH (cm)	DF	HM (cm)	SR (%)	NLPP	LA (cm^2)	IL (cm)	NPOD	NSPP	THSWT (g)	DW (g)
Sesamum indicum	M_1	68.33^{a}	58.33^{b}	15.74^{a}	45.35^{a}	28.03^{a}	34.99^{a}	13.30^{a}	31.46^{a}	5.63^{a}	15.60^{a}	51.00^{a}	4.40^{a}	13.99^{a}
	M_2	73.76^{a}	65.02^{a}	9.64^{a}	45.95^{a}	26.11^{a}	60.02^{a}	12.65^{a}	40.40^{b}	4.88^{b}	7.25^{b}	52.55^{a}	4.26^{a}	13.16^{a}

Means with the same superscript within a column are not significantly different at $P \leq 0.05$

GPSD- Germination % 7 days after sowing, GPFD- Germination % 14 days after sowing SH- Seedling height, DF- Days to 50% flowering ,HM- Height at maturity, SR- Survival rate, NLPP- Number of leaves per plant, LA- Leaf Area, IL- Internode length , NPOD- Number of pod per plant, NSPP- Number of seeds per pod, THSWT- Thousand seeds weight , DW- Dry weight of the plants , S.EM-Standard Error Mean, Gens: Generation

Broadsense heritabilities induced by sesame at M_2 generation

The estimation of genotypic, environmental, phenotypic variance and broad sense heritability (H^2) for traits evaluated at M_2 generation in *Sesamum indicum* treated with sodium azide are presented in Table 5. The results indicated that the estimates of most of the environmental variance were greater in magnitude compared to the corresponding genotypic variance. In sesame mutants, broad sense heritability was 87.50% for thousand seed weight. High heritability was also recorded for days to 50% flowering (97.84%). Leaf area was the least heritable (1.11%).

Table 5: Variance component estimates for sodium azide at M_2 generation of *Sesamum indicum*

Plant Traits	*Sesamum indicum*			
	δ^2g	δ^2e	δ^2ph	h^2 (%)
GPSD(%)	98.75	208.70	307.45	32.11
GPFD(%)	225.85	331.36	557.21	40.53
SH(cm)	0.27	1.37	1.64	16.46
DF	2.25	0.05	2.30	97.82
HM(cm)	0.82	6.94	7.76	10.57
SR(%)	6.83	245.40	252.23	2.71
NLPP	0.80	2.50	3.30	24.24
LA (CM2)	2.17	193.33	195.5	1.11
IL(cm)	0.05	0.12	0.17	29.41
NPOD	1.37	10.66	12.03	12.85
NSPP	5.93	27.23	33.16	17.88
THSWT(g)	0.07	0.01	0.08	87.50
DW(g)	0.64	0.54	1.18	54.24

δ^2g- Genetic Variance, δ^2e- Environmental Variance, δ^2ph- Phenotypic Variance, h^2- Heritability, GPSD- Germination % 7 days after sowing, GPFD- Germination % 14 days after sowing SH- Seedling height, DF- Days to 50% flowering , HM- Height at maturity, SR- Survival rate, NLPP- Number of leaves per plant, LA- Leaf Area, IL- Internode length , NPOD- Number of pod per plant, NSPP- Number of seeds per pod, THSWT- Thousand seeds weight , DW- Dry weight, S.EM-Standard Error Mean.

Mutagenic effectiveness is an index of the response of a genotype to the increasing doses of the mutagen, whereas mutagenic efficiency indicates the extent of genetic damage recorded (Wani, 2009). The decline in mutagenic effectiveness with increasing concentration of sodium azide might be attributed to increasing chromosome damage with increasing concentration of the mutagen. This result is in conformity with the report of Kulthe *et al*. (2013) that at higher concentration of mutagen, mutagenic effectiveness declined considerably. High mutagenic frequency, mutagenic effectiveness and mutagenic efficiency at low concentration of the mutagen could also be as a result of low injuries caused with low concentration of mutagens. In this study, the non-linear relationship between the concentration of mutagen, lethality and mutagenic efficiency could be attributed to non-proportional increase of mutation frequency with increase in the concentration of the mutagen. Furthermore, low effects of the mutagen on mutants lethality at the low concentration might be as a result of less damage caused by the low concentration thus, enabling the plants to express the induced mutation successfully. A similar result was reported by Konzak *et al.* (1965) that injuries are low when concentration of mutagenic treatment is low.

The improvement in agronomic traits in sesame treated with sodium azide over untreated sesame could be due to induced modification of the DNA caused by the mutagen. Lee *et al.* (2003) and Falusi *et al*. (2012) reported that mutagenic treatment improved the morphological and yield traits of pepper. The improvement in traits such as seedling height, height at maturity, leaf area and number of leaf per plants at the 0.5mM sodium azide concentration could be attributed to increased cell division rates as well as activation of growth regulators at low concentration of the mutagen. This report also agrees with the works of Adamu *et al*. (2004) who reported increased seedling height and height at maturity at lower doses of gamma rays treated Popcorn and Zaka *et al.* (2004). Monica and Seetharaman (2016) also reported that the germination percentage of garden bean was improved at low concentration/ dose of gamma rays and EMS and decreased at high concentration. Bind *et al.* (2016) also reported that low concentration of mutagen improve biological parameters.

Higher improvements in evaluated traits at M_2 generation might be attributed to the fact that some mutations are recessive therefore variations can only be expressed at the M_2 generation after segregation has occurred during meiosis in the M_1 generation. This is in agreement with the work of Samiullah *et al.* (2004) who reported that the two varieties of mung bean showed significant shift in mean values for quantitative characters in M_2 and M_3 generations and genetic parameters were recorded higher for all the treatments in both the generations than M_1 generation.

The greater estimate of environmental variance over genotypic variance for most traits indicates active environmental influence on the mutants. Grace *et al*. (2014) in their work on *Fadherbia albida* reported that phenotypic variance was greater than genotypic variance for some seed traits. The high genetic variance for thousand seed weight and days to flowering in sesame indicates a highly significant effect of the genotype on phenotypic expression for these traits with very little effect of environment. However, high heritability for these traits shows that variation for these characters is due to high additive gene effects and consequently the scope for improving yield through selection is more.

In conclusion, sodium azide best improved the agronomic traits of sesame at low concentration of sodium azide (0.5 mM). The mutagenic effectiveness and efficiency of sodium azide in sesame is 2.27 and 0.72%, respectively. High genetic heritabilities recorded for days to 50% flowering and thousand seeds weight broadens the scope for improving yield through selection for these traits in sesame

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