AGRICULTURAL AND BIOLOGICAL SCIENCES

PHENOTYPIC VARIABILITY OF FALSE SESAME (*CERATOTHECA SESAMOIDES* ENDL.) TREATED WITH SODIUM AZIDE

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Date Manuscript Received: 19/12/2016 Accepted: 25/12/2016 Published: December, 2016

ABSTRACT
The mutagenic efficiency and effectiveness of sodium azide to induce genetic variabilityin false sesame (*Ceratotheca sesamoides*) was evaluated with the aim of obtaining beneficial mutants. The seeds of false sesame were subjected to four concentrations (0.5mM, 1.0mM, 1.5mM and 2.0mM) of sodium azide. Treated and untreated seeds were sown on the field. Harvested M1 false sesame seeds were sown to raise the M_2seedlings. The sodium azide concentration of 1.0mM significantly ($p<0.05$) induced benefitial variabilities on the agronomic traitsevaluated at M_1 and M_2 generation of false sesame. The mutagenic effectiveness, efficiency and mutation frequency of sodium azide were not obtained due to the absence of chlorophyll deficient mutants. Lethality due to mutagen was observed not to be dose dependent. Broad sense heritability estimates for the agronomic traits evaluated ranged from 2.14% to 92.01%. High heritability values recorded for and days to flowering (92.10%), thousand seed weight (75.00%), height at maturity (63.84%) and leaf area (60.35%) broadens the scope for improving false sesame via selection. Results are further discussed to validate the potential of this mutagenic treatment on false sesame breeding and selection.

Keyword: *False sesame, Heritability, Mutation, Mutagens, Sodium azide*

INTRODUCTION

Across the world, most indigenous plant species that are cultivated for food are neglected and underutilized. These plants play a crucial role in the food security, nutrition, and income generation of the rural farmers (Magbagbeola *et al*., 2010). One of such underutilized crop is false sesame (*Ceratotheca sesamoides*). It has a chromome number of 2n=32 (Bedigen, 2004), belong to the family *Pedaliaceae* which has 16 genera and 60 species predominantly in Africa, Indo-Malayan region, and tropical Australia (Falusi *et al*., 2002). It is known by various names such as Eku (Yoruba-Western Nigeria), Tchaba-laba (Guinea Bissau), Lalu-caminho (Senegal) and False Sesame (English) (Adegoke *et al*., 1968) and is colloquially referred to as false sesame owing to its marked similarities with common sesame (Sesamum indicum). In northern Nigeria it is commonly called karkashi. Itis one of the Traditional Leafy Vegetables (TLVs) whose leaves, young shoots and flowers are acceptable for use as vegetable (FAO, 2006). It is a source of protein, vitamins and minerals (Dansi *et al.*, 2009) with the dry seed yielding 35 percent oil with characteristics practically identical to those of sesame oil (LePelly, 1959). False sesame shows strong antibacterial activity (Maikere-Faniyo *et al.*, 1989). Its leaf maceration also facilitates delivery in both humans and animals (Bedigian and Adetula, 2004), the leaves when ground with the rhizome of '*Anchomanes difformis*' can be applied in cases of leprosy (Bedigian and Adetula, 2004). It has also been reported to be used as an aphrodisiac, against jaundice, snakebites and skin diseases (Bedigian, 2003).

Despite the medical, pharmaceutical, cultural and commercial values of false sesame, they have been poorly researched because the architectures of false sesame are poorly adapted to modern farming system due to their indeterminate growth habit, sensitivity to wilting under intensive management and seed shattering at maturity (Uzun and Cagirgan, 2006). 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).This has led to the deterioration in the vast reservoir of wealth of this plant (Attere,1999) with the danger of continued genetic erosion and disappearance. Hence, this research was aimed at determining the phenotypic and genetic variabilities induces by sodium azide towards producing beneficial mutation in false sesame and utilization of these mutants in breeding programmes.

MATERIALS AND METHODS

This study was conducted at the Botanical garden of the Department of Botany, Ahmadu Bello University, Zaria, (lat. 11°12'N, long 7° 33'E and on altitude 660m above sea level).

Twenty five grams (25g) of false sesame seeds wereobtained and identified from Jigawa State Agricultural and Rural Development Authority (JARDA), Ringim, Jigawa. Sodium azide (made in Kem light laboaratory PVT, LTD Mumbai, India) wasobtained from the store of Department of Biological Sciences, Ahmadu Bello University, Zaria. False sesame seeds were treated (5g for each mutagenic treatment) with four concentrations (0.5, 1.0, 1.5 and 2.0mM) of Sodium azide for 4 hours. The control (0.0Mm) was not exposed to the mutagen. Exposed seeds were thoroughly washed with distilled water and were left to dry for 24 hours. The experiments were laid in a randomized complete block design (RCBD) with 4 replications. Each replication was laid out on a field size of 1.5m by 0.75m 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 were collected at both M_1 and M_2 generations for the following growth parameters according to the method described by Nura *et al*. (2014)

Germination percent was determined on the seventh and fourteen days after planting (7 and 14 DAS) 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.

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 was taken per treatment when 50% of the plants in each treatment produced flowers.Height at Maturity (cm) was recorded 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. Plants were considered matured after the emergence of the first flower. Survival Rates (%) was recorded by counting the number of plants that survived per treatment and recordedafter the plants have attained 50% flowering.Number of Leaves per Plant was determined by the number of leaves per plant in each treatment and recorded after the plants have attained 50% flowering and averaged over three

(3) plants.Internodes Length (cm) was measured as the lengths between two successive leaves per plant in each treatment by the use of meter-rule after the plants produced podsand averaged over three (3) plants.

Leaf Area (cm^2)was determined by measuring the length and width of randomly selected leaves and applying the formula outlined by Pearce *et al*. (1979):

$$A = [(L)(W)(0.75)] \times 2$$

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

The number of pods produced per plant per treatment was counted and and averaged over three (3) plants. The Number of Seeds per Pod 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) was measured by counting 1000 seeds per treatment and measuring the weight using a Sartorius electronic weighing balance (model: cp8201).

Dry Weights (g) was determined for all the treatments after the plants are uprooted and dried in the oven for three days at 70 0C. Their dry weights were taken using a Sartorius electronic weighing balance (model: cp8201) per treatment.The number of seedlings that showed chlorophyll deficiency was identifiedat M_2based on the foliar coloration and recorded (Giri and Apparao, 2011).

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

where:

$$\text{Mutation frequency (\%)} = \frac{\text{Chlorophyll mutants at } M_2 \times 100}{\text{Total number of plants studied}}$$

$$\text{Mutagenic effectiveness(\%)} = \frac{\text{Mutation frequency} \times 100}{\text{Dosage or time x concentration}}$$

$$\text{Mutagenic efficiency(\%)} = \frac{\text{Mutation frequency}}{\text{Percentage lethality}}$$

Morphological data on growth biometrics were analyzed statistically by analysis of variance (ANOVA) using SAS statistical software version 9.1 and treatment effects were compared using Fisher's protected least signigicant difference (LSD) test at $p<0.05$.

Broad sense heritability (HB) was computed at M^2as specified by the method of Singh and Chaudhary (1985) and Moll *et al*., (1960):

$$HB = (\delta^2 g)/\delta^2 p$$

Where:H_B=Broadsenseheritability,$\delta^2 g$=Genotypicvariance, $\delta^2 p$=Phenotypicvariance

RESULTS AND DISCUSSION

The mutagenic efficiency, effectiveness, lethality percentage of sodium azide on false sesame is presentedin Table 1. Mutation frequency, mutagenic efficiency and effectivnes were not evaluated due to the absence of chlorophyll deficient mutants. Lethality in the mutants was not dose dependent. However, lethality induced by sodium azide in false sesame was highest (14.60%) at a concentration of 0.5mMand lowest (8.25%) at a concentration of 1.5mM.

The treatment of false sesame with sodium azide at M_1 improved its agronomic traits (Table 2) except for the number of seeds per pod which was not significantly different across treatments. Seeds treated with 1.0mM sodium azide showed better improvement in morphological traits compared to other treatments. Most M_2 mutants showed better improvements in agronomic traits compared to the control (Table 3). However, there was no significant improvement in seedling heights, internode length and number of seeds per pod between mutants and control. The germination percentage at 7 DAS (77.10%) was highest in the control treatment. At 14 DAS, germination percentage was highest in seeds treated with 0.5mM sodium azide. At this concentration traits like survival rate, number of leaf per plant, number of pod per plant, thousand seed weight and dry weight were highest compared to other treatments. Height at maturity and leaf area were significantly higher ($p<0.05$) in mutants treated with 1.0mM of sodium azide, while days to flowering was lowest (70.25) at this concentration. The agronomic traits of mutants treated with 1.5mM and 2.0mM concentration of sodium azide were in most cases comparable to the control treatment.

The comparative responses of *Ceratotheca sesamoides* treated with sodium azide at M_1 and $M^2$are presented in Table 4. There was no comparative advantage of the M^1 false sesame mutants over the M_2 mutants.however, the germination percentages at 7 and 14 days after seeding were significantly higher in M1 mutants (Table 4).

Table 1: Mutagenic Frequency, Efficiency and Effectiveness of Sodium Azide in False sesame

Conc/ dose	MF (%)	LT(%)	ME(%)	Me(%)
0.5mM	0.00	14.60	0.00	0.00
1.0mM	0.00	10.45	0.00	0.00
1.5mM	0.00	8.25	0.00	0.00
2.0Mm	0.00	10.65	0.00	0.00

TABLE 2: Mean Performance of Ceratotheca sesamoidesat M_1 Generation induced by Sodium Azide

Plants	CON	GPSD(%)	GPFD(%)	SH(cm)	DF	HM(cm)	SR(%)	NLPP	LA(cm2)	IL(cm)	NPOD	NSPP	THSWT(g)	DW(g)
Ceratotheca sesamoides	**0.0mM**	**70.83**	**62.48**	**10.75**	**80.75**	**59.75**	**37.48**	**44.00**	**23.33**	**5.23**	**52.00**	**47.50**	**3.01**	**38.78**
	0.5mM	**70.83**	**66.28**	**14.38**	**70.00**	**76.00**	**37.50**	**53.25**	**44.75**	**5.70**	**54.75**	**54.00**	**3.20**	**64.60**
	1.0mM	**81.28**	**75.03**	**11.38**	**76.50**	**66.86**	**41.68**	**50.50**	**44.75**	**5.85**	**103.00**	**57.75**	**3.30**	**54.30**
	1.5mM	**70.83**	**62.50**	**12.13**	**75.00**	**64.50**	**39.60**	**45.50**	**29.10**	**4.98**	**64.25**	**56.00**	**3.04**	**59.15**
	2.0mM	**66.67**	**68.73**	**11.13**	**73.75**	**72.25**	**39.60**	**46.75**	**24.03**	**5.25**	**58.00**	**55.00**	**3.05**	**48.60**
	LSD (P<0.05)	**7.24**	**7.55**	**2.21**	**7..50**	**5.70**	**4.12**	**3.85**	**8.00**	**0.77**	**26.34**	**11.01**	**0.11**	**9.62**

Key: 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 False sesame at M_2 Generation induced by Sodium Azide

PLANTS	CON	GPSD(%)	GPFD(%)	SH(cm)	DF	HM(cm)	SR(%)	NLPP	LA(cm2)	IL(cm)	NPOD	NSPP	THSWT(g)	DW(g)
Ceratotheca sesamoide	**0.0Mm**	**77.10**	**50.00**	**13.43**	**72.25**	**83.75**	**37.50**	**42.75**	**43.15**	**4.90**	**35.25**	**56.75**	**3.00**	**38.83**
	0.5Mm	**64.58**	**62.50**	**13.78**	**73.25**	**86.25**	**47.90**	**52.25**	**62.80**	**5.38**	**48.25**	**64.00**	**3.35**	**58.08**
	1.0mM	**58.35**	**54.20**	**14.68**	**70.25**	**100.75**	**43.75**	**50.00**	**77.60**	**5.63**	**42.25**	**59.50**	**3.30**	**51.13**
	1.5Mm	**58.35**	**52.00**	**13.63**	**73.75**	**85.75**	**43.75**	**44.75**	**43.98**	**5.38**	**44.75**	**62.75**	**3.24**	**43.90**
	2.0Mm	**58.35**	**52.00**	**13.55**	**75.00**	**85.00**	**41.65**	**45.00**	**58.15**	**5.25**	**40.25**	**57.00**	**3.20**	**47.80**
	LSD p<0.05)	**11.24**	**10.80**	**3.60**	**12.50**	**13.50**	**6.20**	**9.33**	**15.56**	**2.81**	**7.32**	**14.44**	**0.11**	**13.26**

Key: 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 False sesame treated with Sodium Azide at M_1 and M_2 Generation

PLANTS	GENS	GPSD(%)	GPFD(%)	SH(cm)	DF	HM(cm)	SR(%)	NLPP	LA(cm2)	IL(cm)	NPOD	NSPP	THSWT(g)	DW(g)
Ceratotheca sesamoide	**M1**	**67.08**	**67.08**	**11.95**	**74.40**	**67.88**	**39.17**	**48.00**	**28.43**	**5.40**	**66.40**	**55.25**	**3.14**	**53.09**
	M2	**63.35**	**53.64**	**13.81**	**72.9**	**88.30**	**42.91**	**46.95**	**57.14**	**5.31**	**42.15**	**60.00**	**3.18**	**47.95**
	LSD (p<0.05)	**3.94**	**13.10**	**2.60**	**10.67**	**28.65**	**9.48**	**11.05**	**30.00**	**2.12**	**15.34**	**8.87**	**0.73**	**7.42**

Keys: 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 Errorof Mean, Gens: Generation

Genetic Variation and Heritability of M^2False sesame induced by Sodium azide

The estimation of genotypic, environmental, phenotypic variance and broad sense heritability (H^2) for traits evaluated at M_2 generation in *Ceratotheca sesamoides* 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.Broad sense heritability was height for days to flowering (92.10%). High heritability values werealso recorded for and thousand seed weight (75.00%), height at maturity (63.84%) and leaf area(60.35%).

Table 5: Variance Component Estimates for Sodium Azide at M_2 Generation of *Ceratotheca sesamoides*

Traits	δ^2g	δ^2e	δ^2ph	h^2 (%)
GPSD(%)	32.35	394.96	427.31	7.57
GPFD(%)	51.61	313.43	365.04	14.14
SH(cm)	0.01	1.0	1.00	1.00
DF	3.11	0.27	3.38	92.01
HM(cm)	43.21	24.47	67.68	63.84
SR(%)	32.39	186.71	219.10	14.78
NLPP	10.58	21.38	31.96	33.10
LA (CM2)	176.23	115.77	292.00	60.35
IL(cm)	0.01	0.30	0.30	33.3
NPOD	17.24	26.23	43.47	39.66
NSPP	1.04	47.54	48.58	2.14
THSWT(g)	0.03	0.01	0.04	75.00
DW(g)	16.96	144.44	161.40	10.51

δ^2g- Genetic Variance, δ^2e- Environmental Variance, δ2ph- 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 absence of chlorophyll deficient mutants in false sesame may be due to the fact that oil seed crops are resistant to induced chlorophyll mutations as also

reported by Bharathi *et al,* (2014). It may be further attributed to the probable reasons of the elimination of gametes carrying mutations or zygote inviability. This also explains the lethality variations observed with increasing concentration of the mutagen. High lethality at low concentration might be due to greater damage caused by the mutagens, thus prohibiting the plants to express the induced mutation successfully. This result is not in conformity with the work of Konzak *et al*,(1965)who reported that injuries are low when concentration of mutagenic treatment is low.

The improvement in morphological traits of M_1 false sesame at low concentration ofsodium azide could be due to the alteration of their genome integrated by environmental signals as reported by Uno *et al,* (2001) probably by increasing the rates of cellular division and expansion at their meristematic regions. This was in agreement with the findings of Hoballah (1999) who reported increase in morphological traits such as plant height due to mutagenesis. The improvement in number of leaves per plant, leaf area, number of pods per plants and thousand seed weight compared to the control at M^2 at 1.0mMconcentration of sodium azide could be as a result of environmental factors (Udensi, 2012).The reduced germination percentage at second mutant generation could be as a result of toxic nature of the mutagen that cause damage on the embryo of the seeds (Devi and Mullainathan, 2012). This finding agrees with the work of Samiullah *et al.* (2004). who reported that all the mutagenic treatments brought reduction in seed germination when two mungbean varieties, K-851 and Pusa Baisakhi were treated with sodium azide. Meanwhile the improvement in number of leaf per plants, number of pod per plant, thousand seed weight and dry weight at lowconcentration of sodium azide could be due to promotion of physiological and biological processes necessary for growth of the plant which includes enzyme activity. This is in conformity with the work of Bind *et al*.(2016) who reported that low concentration of mutagen improve biological parameters.The higher germination percentage of the M_1 mutants over the M_2 mutant generation might be because mutations are recessive therefore variations can only be expressed in the M_2 generation of the false sesame after segregation might have 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

Phenotypic heritability induced by environmental variance for most traits indicates the influence of environmental effect on the mutants. Grace *et al*. (2014) in their work on *Fadherbia albida* reported that phenotypic variance was greater than genotypic variance for seed length and width. The high genetic variance for thousand seed weight and days to flowering in sesame indicates a highly significant effect of the genotype on phenotypicexpression for these traits with very little effect of environment. However, high heritability for these traitsshows that variation for these characters is due to high additive gene effects and consequently increases the scope for the improvement of false sesame through selection.

In conclusion, sodium azide best improved the agronomic traits of false sesame at a sodium azide concentration of 1.0mM. High genetic heritabilities recorded for days to 50% flowering and thousand seeds weight, height at maturity and leaf area broadens the scope for improving false sesame via selection.

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