

## Morphogenetic improvement of false sesame (*Ceratotheca sesamoides* Endl.) induced by fast neutron irradiation

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### ABSTRACT

This study was designed to evaluate the beneficial effect of fast neutron irradiation towards improving the agromorphological traits of false sesame (*Ceratotheca sesamoides*). False sesame seeds were subjected to four doses (0.16sv, 0.32sv, 0.48sv and 0.64sv) of fast neutron. Untreated seeds served as control. The treatments were laid out in a completely randomized block design with three replications. Fast neutron induced significant ( $p < 0.05$ ) variabilities on most of the agronomic traits evaluated both at the first and second mutant generations. At a fast neutron dose of 0.16sv, false sesame recorded better growth in both morphological and yield traits evaluated. Broad sense heritability estimates for the agronomic traits ranged from 1.47% in seedling height to 83.75% in germination percentage. High genetic heritability estimates in yield traits were also recorded for number of pod per plant (50.24%), number of seed per pod (54.77%) and thousand seed weight (66.67%). Hence, the high genetic variance recorded indicates that false sesame has a great potential for genetic improvement and provides adequate genetic background for false sesame improvement.

**Keywords:** False sesame, heritability, traits, variability, mutation

### INTRODUCTION

Mutation breeding has attained importance in recent years and has produced an increasing number of desirable cultivars in different crops by generating variability which is an important requirement for the creation of genetic variability for further selection and hybridization (Adamu *et al.*, 2004). Several achievements in crop improvement through mutation breeding have resulted in two major outcomes: improved varieties that are directly used for commercial cultivation and new genetic stocks with improved characters or with better combining ability of traits (Rowchowdry and Tah, 2013). Efforts have been made to genetically enhance crops

through induced mutations (Ranalli, 2012). The widespread use of induced mutants in plant breeding programmes throughout the world has led to the official release of more than 2700 plant mutant varieties (FAO, 2009) and during the past seven decades, more than 2252 mutant varieties have been officially released in the world (Kharkwal *et al.*, 2004). Application of fast neutrons has generated a vast amount of genetic variability and has played a significant role in plant breeding and genetic studies. Fast neutron irradiation (FNI) is widely used in increasing the yield of major crops (Swaminathan, 1998; Zhang *et al.*, 2002). Development of high-yielding varieties requires a thorough knowledge of the existing genetic variation and heritability of

agronomic traits and their interrelationship which helps in understanding yield components and yield potential in crops (Wani and Khan, 2006). Among the locally grown leafy vegetable is false sesame (Bedigian, 1988). It is one of the most important crops in the world according to some archaeological findings (Bedegian and Harlan, 1986). However, the architectures of the crops are poorly adapted to modern farming system because of their indeterminate growth habit and seed shattering at maturity (Uzun and Cagiran, 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).

Therefore, there is an urgent need for new higher yielding varieties with improved agronomic traits. This study was therefore, designed to evaluate the beneficial effect of fast neutron irradiation towards improving the agromorphological traits of false sesame and to provide adequate genetic background for its improvement.

## MATERIALS AND METHODS

### Study Location

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).

### Sources of Materials

Twenty five grams (25g) each of sesame seeds were obtained from local farmers within Danja metropolis of Katsina State, Nigeria and identified by Jigawa State Agricultural and Rural Development Authority (JARDA), Ringim, Jigawa State. The fast neutron source, which is a miniature neutron source reactor (MNSR) designed by the China Institute of Atomic Energy (CIAE) and licensed to operate at maximum power of 31kw (SAR, 2005) is located at the radiology Department, CERT, ABU, Zaria.

### Treatment and Experimental Design

The seeds of false sesame were divided into 5 groups. The first group was not exposed to FNI and served as the control, while the remaining four groups were irradiated with fast neutrons for 2 hours, 4 hours, 6 hours and 8 hours (resulting in 0.16, 0.32, 0.48 and 0.64Sv respectively) at the Centre for Energy and Research Training (CERT), Ahmadu Bello University, Zaria, Kaduna State. For each dose 5g of seeds were treated. The experiments were laid out in a completely randomized block design (CRBD) with three replications and a total of 30 pots per block. Each replicate was sown on a 1.5m by 0.75m field space. Within row and between plants, distances of 30 cm and 15 cm respectively, were maintained (Mensah and Tope, 2007). Harvested seeds from first mutant ( $M_1$ ) generation were sown to raise the second mutant ( $M_2$ ) generation. All cultural practices such as planting, weeding, thinning as well as harvesting methods were conducted as described by Bedigian and Adetula (2004).

### Data Collection

Data were collected at both  $M_1$  and  $M_2$  generations for germination Percentage at 7 and 14 Days after sowing, seedling height (cm), number of days to 50% flowering, height at maturity (cm), survival rates (%), number of leaves per plant, internodes length (cm), leaf area (cm<sup>2</sup>), number of pods per plant, number of seeds per pod, thousand seeds weight (g) and dry weights (g) following the methods described by Nura *et al.* (2014).

### Chlorophyll Deficient Mutants

**Determination of 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

Morphological data on growth parameters were subjected to analysis of variance (ANOVA) to determine significant differences amongst the

parameters evaluated. Where significant means were separated by Duncan's Multiple Range Test (DMRT) with the statistical analytical software (SAS, 2004) version: 9.1.

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

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

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

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

Broad sense heritability ( $H_B$ ) was computed at the second mutant ( $M_2$ ) 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

### Mutagenic Frequency, Efficiency and Effectiveness of Fast Neutron on False Sesame

Lethality in false sesame was dose dependent (table 1). Lethality increased with increasing fast neutron dose. Mutagenic frequency, effectiveness and efficiency of fast neutron was 0.00 at all dose of fast neutron evaluated in this study.

**Table 1:** Mutagenic Frequency, Efficiency and Effectiveness of Fast neutron *Ceratotheca sesamoides* at the second mutant Generation.

Conc/ dose	MF (%)	LT(%)	ME(%)	Me(%)
0.16sv	0.00	12.47	0.00	0.00
0.32sv	0.00	31.22	0.00	0.00
0.48sv	0.00	35.40	0.00	0.00
0.64sv	0.00	29.15	0.00	0.00

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

## **Mutagenic Effects of Fast neutron doses on the Agronomic traits of False sesame at M<sub>1</sub> and M<sub>2</sub> Generation**

The mean performance of traits evaluated at M<sub>1</sub> generation of false sesame is presented in Table 2. Fast neutron induced significant ( $p < 0.05$ ) variabilities on the agronomic traits evaluated. Seeds treated at a fast neutron dose of 0.16sv reduced days to flowering (70.00), had best survival rates (43.75%) and good leaf area (15.40cm<sup>2</sup>). Yield attributes such as number of pod per plant (56.80), number of seed per pod (55.00) and thousand seed weight (3.90g) were also highest at this dose. At an exposure of 0.32sv higher numbers of leaf per plant (37.83) and dry weight (54.13g) were recorded. There was no significant variation in internode lengths and height at maturity between mutants and control. Germination percentage at 7 and 14 DAS were significantly higher ( $p < 0.05$ ) in the control than in the mutants. Seeds treated with 0.48sv and 0.64sv dose of fast neutron were in some cases comparable to the control treatment.

At M<sub>2</sub> generation, there was no significant variation in seedling heights and number of leaves per plant between mutants and control (Table 3). Similar to the observations at M<sub>1</sub>, germination percentages at 7 and 14 DAS were significantly higher ( $p < 0.05$ ) in the control than in the mutants. Seeds treated with 0.16sv showed better improvements in morphological and yield traits compared to other treatments. These improvements reflected on their reduced days to flowering (71.25), survival rate (38.50%), larger leaf area (33.95cm<sup>2</sup>), increased number of pod per plant (39.00), higher numbers of seed per plant (54.50), thousand seed weight (3.40g) and dry weight (43.33g). The comparative effect of fast neutron at M<sub>1</sub> and M<sub>2</sub> generation in false sesame showed significant difference only in the survival rate and leaf area (Table 4).

**TABLE 2:** Mean Performance of False sesame at M<sub>1</sub> Generation upon exposure to Fast neutron

PLANTS	DOSE	GPSD(%)	GPFD(%)	SH(cm)	DF	HM(cm)	SR(%)	NLPP	LA(cm <sup>2</sup> )	IL(cm)	NPOD	NSPP(cm)	THSWT(g)	DW(g)
<i>Ceratotheca sesamoides</i>	0.0sv	97.93 <sup>a</sup>	85.43 <sup>a</sup>	8.73 <sup>ab</sup>	79.25 <sup>ab</sup>	52.00 <sup>a</sup>	22.95 <sup>b</sup>	29.00 <sup>c</sup>	15.45 <sup>b</sup>	4.80 <sup>a</sup>	34.00 <sup>d</sup>	51.75 <sup>bc</sup>	3.01 <sup>d</sup>	29.33 <sup>b</sup>
	0.16sv	64.60 <sup>b</sup>	64.60 <sup>b</sup>	13.48 <sup>a</sup>	70.00 <sup>c</sup>	55.25 <sup>a</sup>	43.75 <sup>a</sup>	37.00 <sup>ab</sup>	15.4 <sup>b</sup>	4.60 <sup>a</sup>	56.50 <sup>a</sup>	55.00 <sup>a</sup>	3.90 <sup>a</sup>	42.20 <sup>ab</sup>
	0.32sv	62.48 <sup>b</sup>	62.48 <sup>b</sup>	13.25 <sup>a</sup>	71.00 <sup>b</sup>	50.75 <sup>a</sup>	29.15 <sup>ab</sup>	37.83 <sup>a</sup>	15.3 <sup>b</sup>	4.80 <sup>a</sup>	47.75 <sup>b</sup>	53.25 <sup>ab</sup>	3.60 <sup>b</sup>	54.13 <sup>a</sup>
	0.48sv	60.43 <sup>b</sup>	60.43 <sup>b</sup>	8.73 <sup>ab</sup>	72.50 <sup>b</sup>	48.75 <sup>a</sup>	29.15 <sup>ab</sup>	35.00 <sup>ab</sup>	26.7 <sup>a</sup>	5.20 <sup>a</sup>	43.00 <sup>c</sup>	53.00 <sup>ab</sup>	3.60 <sup>b</sup>	20.68 <sup>c</sup>
	0.64sv	52.05 <sup>b</sup>	47.90 <sup>b</sup>	8.80 <sup>ab</sup>	75.00 <sup>a</sup>	52.50 <sup>a</sup>	25.03 <sup>b</sup>	30.00 <sup>bc</sup>	20.5 <sup>ab</sup>	4.50 <sup>a</sup>	40.50 <sup>c</sup>	50.00 <sup>c</sup>	3.30 <sup>c</sup>	41.45 <sup>ab</sup>
	SE	4.34	5.35	0.42	0.61	5.13	4.86	0.02	5.72	0.29	0.83	0.82	0.01	8.94

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

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 plan

**TABLE 3:** Mean Performance of False sesame at M<sub>2</sub> Generation upon exposure to Fast neutron

Plants	Dose	GPSD(%)	GPFD(%)	SH(cm)	DF	HM(cm)	SR(%)	NLPP	LA(cm <sup>2</sup> )	IL(cm)	NPOD	NSPP	THSWT(g)	DW(g)
<i>Ceratotheca sesamoides</i>	0.0sv	95.85 <sup>a</sup>	93.75 <sup>a</sup>	12.63 <sup>a</sup>	75.25 <sup>a</sup>	82.50 <sup>ab</sup>	29.15 <sup>b</sup>	32.75 <sup>a</sup>	20.05 <sup>b</sup>	4.38 <sup>b</sup>	27.00 <sup>c</sup>	48.50 <sup>b</sup>	3.03 <sup>d</sup>	28.08 <sup>b</sup>
	0.16sv	58.3 <sup>b</sup>	54.15 <sup>b</sup>	13.38 <sup>a</sup>	71.25 <sup>b</sup>	66.75 <sup>c</sup>	41.68 <sup>a</sup>	38.50 <sup>a</sup>	33.95 <sup>a</sup>	5.08 <sup>a</sup>	39.00 <sup>a</sup>	54.50 <sup>a</sup>	3.40 <sup>a</sup>	43.33 <sup>a</sup>
	0.32sv	54.15 <sup>b</sup>	54.15 <sup>b</sup>	14.03 <sup>a</sup>	72.00 <sup>ab</sup>	75.5 <sup>bc</sup>	22.93 <sup>b</sup>	36.00 <sup>a</sup>	29.35 <sup>ab</sup>	5.08 <sup>a</sup>	36.25 <sup>ab</sup>	54.5 <sup>a</sup>	3.30 <sup>b</sup>	38.65 <sup>ab</sup>
	0.48sv	58.35 <sup>b</sup>	56.25 <sup>b</sup>	13.00 <sup>a</sup>	73.25 <sup>ab</sup>	85.75 <sup>ab</sup>	20.85 <sup>b</sup>	34.25 <sup>a</sup>	31.20 <sup>a</sup>	5.03 <sup>ba</sup>	31.50 <sup>bc</sup>	51.50 <sup>ab</sup>	3.20 <sup>c</sup>	35.1 <sup>ba</sup>
	0.64sv	45.85 <sup>b</sup>	45.85 <sup>b</sup>	13.78 <sup>a</sup>	74.50 <sup>ab</sup>	89.25 <sup>b</sup>	16.70 <sup>c</sup>	33.75 <sup>a</sup>	26.00 <sup>ab</sup>	4.50 <sup>ab</sup>	32.5 <sup>abc</sup>	50.00 <sup>b</sup>	3.20 <sup>c</sup>	31.83 <sup>b</sup>
	SE	3.32	4.34	0.46	1.06	4.51	2.76	3.39	4.81	0.18	2.22	0.80	0.01	9.52

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

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, SE- Standard error

**Table 4:** Combined Performance of False sesame treated with Sodium Azide at M<sub>1</sub> and M<sub>2</sub> Generation

PLANTS	GENS	GPSD	GPFD(%)	SH(cm)	DF	HM (cm)	SR(%)	NLPP	LA(cm <sup>2</sup> )	IL(cm)	NPOD	NSPP	THSWT(g)	DW(g)
<i>Ceratotheca sesamoides</i>	M <sub>1</sub>	67.50 <sup>a</sup>	64.16 <sup>a</sup>	11.51 <sup>a</sup>	73.55 <sup>a</sup>	52.65 <sup>a</sup>	30.00 <sup>a</sup>	33.77 <sup>a</sup>	19.70 <sup>b</sup>	4.78 <sup>a</sup>	44.35 <sup>a</sup>	52.60 <sup>a</sup>	3.48 <sup>a</sup>	37.56 <sup>a</sup>
	M <sub>2</sub>	62.50 <sup>a</sup>	60.83 <sup>a</sup>	13.36 <sup>a</sup>	73.25 <sup>a</sup>	79.95 <sup>a</sup>	26.26 <sup>b</sup>	35.05 <sup>a</sup>	28.11 <sup>a</sup>	4.81 <sup>a</sup>	33.2 <sup>a</sup>	51.80 <sup>a</sup>	3.23 <sup>a</sup>	35.40 <sup>a</sup>

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

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

**Genetic Variance and Heritability  
Expressed by False sesame at M<sub>2</sub>  
Generation**

The estimation of genotypic, environmental, phenotypic variance and broad sense heritability ( $H^2$ ) for traits evaluated at M<sub>2</sub> generation of false sesame treated with fast neutron are presented in table 5. The genetic contributions derived from the estimate of the genetic variance compared to the estimate derived from the environmental

variance at the second mutant generation were 83.75%, 80.70%, 70.56%, 63.86%, 50.24%, 54.77%, 66.67% in germination percentage at 7DAS, 14 DAS, survival rate, leaf area, number of pod per plant, number of seed per pod and thousand seed weight respectively in false sesame (Table 5).

Table 5: Variance Component Estimates for Fast neutron at M<sub>2</sub> Generation of False sesame

**Table 5:** Variance Component Estimates for Fast neutron at M<sub>2</sub> Generation in False sesame

Traits	$\delta^2g$	$\delta^2e$	$\delta^2ph$	$h^2$ (%)
GPSD (%)	356.23	69.11	425.34	83.75
GPFD (%)	334.59	80.01	414.59	80.70
SH(cm)	0.02	1.34	1.36	1.47
DF	1.65	4.53	6.18	26.70
HM (cm)	69.82	41.41	111.23	37.23
SR (%)	85.52	35.68	121.20	70.56
NLPP	2.83	31.76	34.59	8.18
LA (cm <sup>2</sup> )	274.95	155.59	430.54	63.86
IL (cm)	0.08	0.16	0.24	33.33
NPOD	16.98	16.82	33.80	50.24
NSPP	5.97	4.93	10.90	54.77
THSWT(g)	0.02	0.01	0.03	66.67
DW(g)	71.32	425.18	496.5	14.36

$\delta^2g$  - Genetic Variance,  $\delta^2e$  - Environmental Variance,  $\delta^2ph$  - 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.

## DISCUSSION

The improvement in agronomic traits induced by fast neutron in false sesame over the unirradiated control at M<sub>1</sub> generation suggests the modification of DNA caused by the mutagen. Gill and Tuteja (2010) reported similar results that fast neutron mutagenesis causes deletions (Rogers *et al.*, 2009) of DNA fragment of various sizes (Naito *et al.*, 2005). The inability of fast neutron to improve traits such as percentage germination at 7 and 14 DAS, height at maturity and internode length suggests that genes that confer these traits were not significantly affected by fast neutron irradiation. Higher germination percentages at 7 and 14 DAS obtained in the control could be due to mutant lethality with increasing dose of fast neutron. Amjad *et al.* (2008) reported similar negative effects of irradiation on chickpea seed germination. Fast neutron must have triggered the genes or hormones responsible for flowering, stimulated rapid expansion of the cells of the lamina, thereby increasing photosynthesis. These improvements must have reflected on yield characters recorded. Waghmare and Mehra (2000) have reported similar improvements induced by fast neutron on number of seeds per pod and thousand seed weight.

The improvements in yield as well as in some morphological traits of the M<sub>2</sub> generation at 0.16sv fast neutron dose indicated that FNI has bio-positive effects on false sesame as corroborated by Haliem *et al.* (2013) that FNI had bio-positive effects at specific dose probably by increasing number of amino acid produced in a protein. Furthermore, the lack of improved variations observed in percentage germination and seedling height suggests the repairs of some induced damages to the DNA by certain biochemical mechanisms present in the plants. This is in agreement with the work Festus *et al.* (2016) who observed no significant variations in seed germination

in all treatments across eight cowpea accessions. The improvement in the vegetative and yield characters at M<sub>1</sub> generation upon comparison to the M<sub>2</sub> generation shows little or no segregation in traits at the M<sub>2</sub> generation. This is partly in agreement with the work of Falusi *et al.* (2013) who reported that African Wrinkled Pepper irradiated with fast neutron showed lower improvements in morphological and yield characters in the M<sub>2</sub> generation.

The high genetic variance coupled with high heritability in germination percentage at 7 and 14 DAS, height at maturity, survival rates, leaf area, number of pod per plants, number of seed per pod and thousand seed weight in false sesame mutants could be as a result of high additive gene effects with less environmental actions. Similar findings have been reported by Dwivedi *et al.* (2002). Falusi *et al.* (2013) reported that the local germplasm collection had wide range of variability for different traits coupled with high heritability and high genetic advance for improvement of yield traits. In conclusion, fast neutron at a dose of 0.16sv efficiently induced beneficial agromorphological improvements in false sesame. High genetic variance in most traits evaluated indicates that false sesame has a great potential for genetic improvement.

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