INDUCING PHENOTYPIC VARIANTS IN SESAME *(SesamunindicumL.)* WITH FAST NEUTRON IRRADIATION

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ABSTRACT

The mutagenic efficiency and effectiveness of fast neutron irradiation on sesame was evaluated at M_1 and M_2 generations with the aim of deserning its abiity to induce beneficial mutants with improved agronomic traits that could facilitate selection within local genotypes. Five grames (5g) each of sesame seeds were exposed to four concentrations of sodium azide(0.5mM, 1.0mM, 1.5mM and 2.0mM).Untreated sesame seeds (5g) served as control. The treatments were laid out in a randomized complete block design with three replications. Fast neutron significantly ($p<0.05$) induced benefitialvariabilities on the agronomic traits evaluated. Mutation frequency, mutagenic efficiency and lethalithy induced by fast neutron were not dose dependent. However, the mutagenic effectiveness decreased with increasing dose. Seeds exposed to 0.16sv fast neutron dose showed taller seedling height (15.70cm), reduced days to flowering (45.00), best height at maturity (32.43cm), highest survival rate (35.43%), highest number of leaf per plants (12.50), highest internode length (16.50cm) and best dry weight (12.70g). However, sesame mutants had the best number of seeds per plant (4.60) and thousand seed weight (5.00g) at 0.32sv fast neutron dose. Broad sense heritability estimates for the agronomic traits ranged from 5.96% to 89.73%. Days to flowering recorded the highest heritability value of 89.73%. High heritabilities were also recorded for height at maturity (86.71%) and thousand seed weight (80.00%). Variability in phenotypic characters reflected the existence of genetic diversity among the genotypes.

Keywords: Mutation, Irradiation, Sesame, Heritability, Traits.

INTRODUCTION

The importance of traditional vegetables is often discussed in the context of wild and collected crops (Ogle *et al.,* 2001) that contribute to food safety in times of hunger. In Africa traditional Leafy Vegetables (TLVs) are locally known crops that occur as cultivated, semi-cultivated, weedy and wild plants, with ecological, social and cultural values, playing a significant role in the day to day food and nutritional requirements of local people mainly in rural areas (Chweya and Eyzaguire, 1999). Despite the medical, pharmaceutical, cultural and commercial roles of leafy vegetables like false sesame, they have been poorly researched and fall within the neglected and underutilized species (NUS) of Africa as reported by 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.The neglect of traditional vegetables is unfortunate since these crops are usually better adapted to the environment than the introduced exotic vegetables. Presently, false sesame isfaced 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). Development of high-yielding varieties requires a thorough knowledge of the existing genetic variationand heritability of agronomic traits and their interrelationship which helps in understanding yield components and yield potential in crops (Wani and Khan, 2006). The variability thus created enhances opportunities for selection of new genotypes with the desired characteristics.

Mutation induction with fast neutron radiation is the most frequently used method to develop direct mutant varieties (Ahloowalia and Maluszynski, 2001).They cause mutations by breaking chemical bonds in the DNA molecule, deleting a nucleotide, or substituting it with a new one (Acquaah, 2006).Induced mutation can play a momentous role in the restructuring of the plant, leading to yield improvement. It could create additional genetic variability to supplement conventional crop breeding. The induced variability can be exploited to develop new varieties of sesame with improved agronomic traits(Begum and Dasgupta, 2011).The usefulness of mutagens in mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagen), but also on its mutagenic efficiency in relation to undesirable changes like sterility, lethality and injury (Girija and Dhanavel, 2009). The selection of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations (Solanki*et al.,* 1994).

Selection within the local genotypes had not resulted in considerable achievements towards sesame improvement. Knowledge of the availability and extent to which the genetic diversity is heritable is essential for effective selection among sesame population. Hence, exploiting the available source of germplasm for desired characters would provide adequate genetic background for sesame 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^{o} 12^{1}N, long 7^{o} 33^{1}E and on altitude 660m above sea level).

Sources of Materials

Twenty five grams (25g) of sesame seeds wereobtained and identified from 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 sesame (*Sesamumindicum*)were irradiated for 2hrs, 4hrs, 6hrs and 8 hours at 0.16, 0.32, 0.48 and 0.64Sv doses of fast neutron which was derived from Americium-beryllium source with flux of $1.5x10^{4}ncm^{-2}s^{1}$. For each dose 5g of seeds were treated. The control treatment was not irradiated. The experiments were laid out in a randomized complete block design (RCBD) with 4 replications. Each replicate was sown on a 1.5m by 0.75mfield space. Within row and between plant distance of 30 cm and 15 cm respectively were maintained (Mensah and Tope, 2007).Harvested seeds from M_1 generation were sown to raise 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, seedlings 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^{2}), 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 sesame: The number of seedlings that showed chlorophyll deficiency was identifiedat 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:

$$\text{Mutation frequency (\%)} = \frac{\text{Chlorophyll mutants at } M_2}{\text{Total number of plants studied}} \text{ x } 100 \text{ ---------------Eqn1}$$

$$\text{Mutagenic effectiveness(\%)} = \frac{\text{Mutation frequency}}{\text{Dosage or time x concentration}} \text{ x } 100 \qquad \text{-------------Eqn2}$$

$$\text{Mutagenic efficiency}(\%) = \frac{\text{Mutation frequency}}{\text{Percentage lethality}} \text{-----------------------------Eqn3}$$

Morphological data on growth biometrics were analyzed statistically by analysis of variance (ANOVA) and were 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_2as specified by the method of Singh and Chaudhary (1985) and Moll *et al*., (1960):

$$H_B = \frac{\delta^2 g}{\delta^2 p} \text{--------------Eqn4}$$

Where: H_B= Broad sense heritability,

$\delta^2{}_g$=Genotypicvariance,

$\delta^2{}_p$=Phenotypicvariance

RESULTS

Mutagenic Frequency, Efficiency and Effectiveness of Fast neutron in Sesame

Mutagenic effectiveness was dose dependent and declined with increasing dose of fast neutron (Table1). There was no dosedependent relationship between the concentrations of mutagens with mutagenic frequency, lethality and mutagenic efficiency. The mutagenic frequency of fast neutron was highest (2.70%) at 0.64sv and lowest. At this dose, lethality observed was also highest (13.30%). At a dose of 0.32sv, mutagenic efficiency was highest (0.53%) with minimal lethality of 4.15% (Table 1).

Table 1: Mutagenic Frequency, Efficiency and Effectiveness of Fast neutron in Sesame

Dose	MF (%)	LT(%)	ME(%)	Me (%)
0.16sv	0.92	8.32	2.87	0.23
0.32sv	2.22	4.15	1.73	0.53
0.48sv	2.17	0.00	0.75	0.00
0.64sv	2.70	13.30	0.53	0.20

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 Sesame at $M_1$and M_2Generation

Fast neutron significantly ($p<0.05$) induced beneficial variabilities on the agronomic traits evaluated at M_1 generation (Table 2). These variabilities were an improvement over the control treatment. Seeds exposed to 0.16sv had the longest seedling height (15.70cm), reduced days to flowering (45.00), best height at maturity (32.43cm), highest survival rate (35.43%), highest number of leaf per plants (12.50), good leaf area (29.08cm^2), highest internode length (16.50cm) and best dry weight (12.70g). Seeds exposed to 0.32sv fast neutron dose showed comparable growth in height at maturity, survival rate, number of leaf per plant and dry weight with seed treated with 0.16sv. However, sesame mutants had the best number of seeds per plant (4.60) and thousand seed weight (5.00g) at 0.32sv fast neutronirradiation dose.

The mean performances of false sesame and sesame at M_2 generation are presented in table 3. The mutants showed significant ($p<0.05$) improvement in agronomic traits compared to the control. However, there was no significant improvement in seedling height and number of seed per plant. Germination percentage at 7 and 14 DAS were highest in seeds treated with 0.16sv of fast neutron. At this dose (0.16sv) survival rate, number of leaf per plant, leaf area, internode length, number of pod per plant, thousand seed weight and dry weight of the plants were highest. Seeds exposed to fast neutron at a dose of 0.32sv showed comparable growth in height at maturity, number of pod per plant and dry weight with the seeds treated at 0.16sv.

The comparative effect of fast neutron at M_1 and M_2 generation in sesame showed that the germination percentages at 7 DAS, seedling height andnumber of pod per plant were significantly ($p<0.05$) higher in M_1 generation than in M^2 generation. However, the survival rate, leaf area and dry weight were most prolific in M^2 generation (Table 5).

TABLE 3: Mean Agronomic Performance of Sesame at M_1 Generation induced by Fast neutron Irradiation

PLANTS	**DOSE**	**GPSD(%)**	**GPFD(%)**	**SH(cm)**	**DF**	**HM(cm)**	**SR(%)**	**NLPP**	**LA(cm2)**	**IL(cm)**	**NPOD**	**NSPP(cm)**	**THSWT(g)**	**DW(g)**
Sesamumindicum	0.0sv	60.40^{a}	54.15^{a}	10.50^{b}	52.00^{a}	20.63^{b}	20.85^{b}	9.75^{a}	11.55^{b}	5.15^{b}	11.25^{b}	47.25^{b}	4.10^{e}	7.80^{b}
	0.16sv	62.50^{a}	58.35^{a}	15.70^{a}	45.00^{c}	32.43^{a}	35.43^{a}	12.50^{a}	29.08^{a}	6.40^{a}	16.50^{a}	51.25^{ab}	4.60^{b}	12.70^{a}
	0.32sv	60.40^{a}	54.18^{a}	14.13^{ab}	47.50^{b}	28.75^{a}	35.43^{a}	12.25^{a}	14.70^{b}	6.08^{ab}	13.25^{ab}	52.50^{a}	5.00^{a}	10.20^{a}
	0.48sv	60.40^{a}	50.03^{a}	11.75^{b}	49.00^{ab}	27.75^{ab}	29.18^{ab}	12.25^{a}	25.45^{ab}	5.73^{ab}	13.00^{ab}	48.75^{ab}	4.20^{d}	9.25^{ab}
	0.64sv	58.33^{a}	43.78^{a}	11.83^{b}	49.00^{ab}	24.98^{ac}	25.03^{ab}	9.75^{a}	22.68^{ab}	5.30^{ab}	12.25^{ab}	49.00^{ab}	4.30^{c}	8.98^{ab}
	SE	12.94	12.17	1.06	0.27	2.20	6.09	0.88	4.56	0.28	2.56	1.03	0.01	1.76

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 4: Mean Agronomic Performance of Sesame at M_2 Generation Induced by Fast neutron

Plants	**Dose**	**GPSD(%)**	**GPFD(%)**	**SH(cm)**	**DF**	**HM(cm)**	**SR(%)**	**NLPP**	**LA(cm^2)**	**IL(cm)**	**NPOD**	**NSPP**	**THSWT(g)**	**DW(g)**
Sesamumindicum	0.0sv	62.50^{a}	62.50^{a}	8.70^{a}	49.25^{a}	24.50^{b}	43.78^{b}	12.00^{b}	17.63^{c}	4.13^{a}	4.75^{a}	47.75^{a}	4.00^{d}	13.25^{b}
	0.16sv	66.65^{a}	66.65^{a}	9.03^{a}	42.25^{d}	29.75^{a}	58.33^{a}	13.50^{a}	29.63^{a}	4.73^{a}	6.50^{a}	51.00^{a}	4.50^{a}	15.33^{a}
	0.32sv	25.00^{b}	25.00^{b}	8.70^{a}	45.25^{b}	29.75^{a}	20.85^{c}	12.00^{b}	25.63^{ab}	4.50^{a}	6.50^{a}	49.50^{a}	4.40^{b}	15.38^{a}
	0.48sv	$58.00^{a.}$	54.15^{a}	9.00^{a}	43.50^{c}	30.13^{a}	54.15^{ab}	12.00^{b}	27.60^{ab}	4.48^{a}	5.50^{a}	50.00^{a}	4.30^{c}	14.65^{ab}
	0.64sv	58.00^{a}	54.15^{a}	9.00^{a}	44.75^{b}	29.13^{a}	45.85^{b}	12.00^{b}	23.63^{b}	4.13^{a}	5.00^{a}	49.5^{a}	4.30^{c}	14.48^{ab}
	SE	9.21	8.10	0.73	1.29	0.39	8.77	0.26	4.86	0.23	0.16	0.04	0.01	0.73

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 5: Combined Performance of Sesametreated with Fast Neutron 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)**
Sesamumindicum	M_1	60.41^{a}	52.50^{a}	12.78^{a}	48.50^{a}	$26..9^{a}$	29.18^{b}	11.30^{a}	20.69^{b}	5.73^{a}	13.25^{a}	49.75^{a}	4.40^{a}	9.79^{b}
	M_2	54.16^{a}	52.50^{a}	8.89^{b}	44.40^{a}	28.65^{a}	44.59^{a}	12.30^{a}	24.85^{a}	4.39^{a}	5.75^{b}	48.55^{a}	4.30^{a}	14.62^{a}

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 Variation and Heritability of the M$_2$Sesame

Broad sense heritability estimates for the agronomic traits ranged from 5.96% to 89.73% (Table 6). Days to flowering recorded the highest heritability value of 89.73%. High heritabilities were also recorded for height at maturity (86.71%) and thousand seed weight (80.00%).

Table 6: Variance Component Estimates for Fast neutron at M$_2$ Generation in Sesame

Traits	**δ^2g**	**δ^2e**	**δ^2ph**	**h^2 (%)**
GPSD(%)	215.27	249.50	464.77	46.32
GPFD(%)	206.18	237.92	444.10	46.43
SH(cm)	0.25	1.10	1.35	18.52
DF	2.36	0.27	2.63	89.73
HM(cm)	5.35	0.64	6.17	86.71
SR(%)	166.35	250.07	416.42	39.95
NLPP	0.27	0.73	1.00	27.00
LA (cm^2)	8.36	71.78	80.14	10.43
IL(cm)	0.03	0.23	0.26	11.54
NPOD	0.18	1.55	1.73	10.40
NSPP	5.76	28.58	34.34	5.96
THSWT(g)	0.04	0.01	0.05	80.00
DW(g)	1.35	3.00	4.35	31.01

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.

DISCUSSION

The decline in mutagenic effectiveness with increasing dose of fast neutron 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. The dose independent relationship of fast neutron to mutagenic frequency, lethality and mutagenic efficiency could be attributed to non-proportional increase of mutation frequency with increase in the dose of the mutagen. Furthermore, these fluctuations might have been influenced by DNA repair after damage.Britt (1995) hypothesized therefore that plants normally evolved particularly efficient mechanisms for the elimination of irradiation DNA damages and mutations.

The improvement in agronomic traits induced by fast neutron mutants over the control suggests that fast neutron is efficient in inducing beneficial mutation in sesame.Fast neutron action must have been effected by the induction of structural changes in DNA, such as chromosomal rearrangement, strand breaks, base deletions, pryrimidine dimers, cross-links and base modifications, mutations, and other genotoxic effects (Gill and Tuteja, 2010). These DNA damages may influence the expression of a number of genes leading to alteration in proteins that control many metabolic processes like plant development, cell cycle, fertilization, and seed formation (Agrawal, 2009). The improvement recorded might also have been due to the antioxidant defense system of sesame plant which allows the toxic-free radical oxygen intermediates to perform useful biological functions without too much damage as corroborated by Karuppanapandian(2011). Similar results were reported by Falusi*et al.,* (2012) on pepper who reported that FNI generally increased the vegetative and yield traits of two pepper species. The stimulatory effects of the low dose of fast neutron on the morphological traits could be due to improved cell division rates as well as an activation of growth hormone. Zaka*et al.* (2004) and Luckey(1980) reported that low doses of irradiation stimulated cell division, growth, and development in various plants, this phenomenon is termed “hormesis”. The improvement in the yield traits such as number of seeds per plants and thousand seed weight at low dose of FNI have also been reported by Rubaihayo (1976) who obtained high yielding mutants and found significant genetic variability in yield and maturity in soybean plants, grown from seeds irradiated with gamma rays.

The differences in agronomic performances observed at M_1 and M_2 could be due to variation cause by the cumulative effects of the mutagen and hybridization by the plants as reported by Gregory (1956), that the variation induced by irradiation should be cumulative with that from hybridization.

The greater estimate of phenotypic variance over genotypic and environmental variance for all the traits indicates the influence of environmental effect on the fast neutron mutants. This agrees with the report by Grace *et al.* (2014) in their work on *Fadherbiaalbida* that phenotypic variance was greater than genotypic variance for seed length and width. The high genetic variance for thousand seed weight and height at maturity could be as a result of high additive gene effects with less environmental effects. Similar findings have been reported by Dwivedi*et al.* (2002. They reported that the local germplasms had wide range of variability for different traits coupled with high heritabilities. Conclusively, fast neutron significantly ($p<0.05$) induced benefitialvariabilities on the agronomic traits of sesame. Mutagenic effectiveness was dose dependent; however, mutation frequency, mutagenic efficiency and lethalithy induced by fast neutron were not dose dependent. Variability in phenotypic characters reflects the existence of genetic diversity and suggests potential genetic improvements for sesame.

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