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Original research

Biochemical evaluation of mutant genotypes of *Sesamum indicum* for the development of improved varieties

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Abstract

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The nutritional attributes of sesame, *Sesamum indicum*, vary substantially and are primarily influenced by the plant's genetic makeup. This investigation accessed the biochemical composition of sesame mutants from two generations. Seeds of eleven M_3 lines were planted in a Randomized Complete Block Design (RCBD) to raise M_4 lines, which were advanced to M_5 generation. The seeds were harvested at maturity, and their biochemical compositions were quantitatively determined following standard procedures. Results revealed significant differences ($p < 0.05$) in the biochemical composition of the mutants across the two generations. Exactly 27.27% of the mutants showed a substantial increase in oil composition over their parental varieties at the M_4 generation. In comparison, 36.36% of the mutants had higher oil contents than their parental varieties at the M_5 generation. The M_5 mutants showed significantly ($p < 0.05$) higher oil content than the M_4 mutants. Significant differences ($p < 0.05$) were observed in the protein content of M_4 and M_5 mutants, with M_4 mutants having higher protein content. Significant differences ($p < 0.05$) were observed in fibre, carbohydrate, energy, and moisture contents of M_4 and M_5 mutants, with M_5 mutants having significantly higher ($p < 0.05$) carbohydrate and energy contents, while no significant difference ($p > 0.05$) was observed in ash content of M_4 and M_5 mutants. Results also reveal significant differences ($p < 0.05$) in anti-nutrients of M_4 and M_5 mutants with notable reductions in some anti-nutritional properties of some mutant lines. High and stable nutritional attributes are desirable traits in sesame breeding. The identified mutants with desirable traits are potential candidates that could be selected and developed as the first mutant varieties of sesame in Nigeria.

Keywords: anti-nutrients, breeding, desirable traits, genetic variability, seeds.

INTRODUCTION

Sesamum indicum L. (Pedaliaceae) is a vital oilseed crop (Masoudi, 2019). It is primarily grown for its oil-rich seeds, the bulk of which is used for oil extraction, and the rest are used for edible purposes (Ravichandran & Jayakumar, 2018). Although it has been reported that sesame seeds are important sources of oil (44–58%) and contain 18.95% protein, 5.24% moisture, 4.01–4.81% ash, 47.09–47.33% fat and around 4.92–5.61% crude fibre (Ali et al., 2020), the oil content and nutritional compositions of sesame seeds vary substantially. These variations are greatly influenced by genetic makeup and environmental conditions during plant growth and oil accumulation (Savant & Kothekar, 2011).

Sesame plants are considered to have originated in Africa (Rizki et al., 2015), and there are approximately 20,000 available sesame accessions worldwide (Ju et al., 2021). Most of these accessions are wild, with only *S. indicum* being the most commonly cultivated species for seed production (Abejide et al., 2013). Sesame plants suffer from a relatively low genetic variability, limiting the progress of the world's sesame breeding. Lack of elite accessions has been a significant challenge hindering sesame improvement (Ju et al., 2021).

Sesame seeds have been known as an excellent and cheap source of vegetable oil, protein and minerals. Shittu et al. (2007) have reported that sesame seeds also contain a significant amount of anti-nutritional factors (ANFs) such as tannins, oxalate, phytate, flavonoids and alkaloids, which accounts for its anti-bacterial, anti-fungal and anti-oxidant activities and increases the stability of the oil. However, high levels of toxic ANFs interfere with nutrient absorption and causes reduced protein digestibility. Reducing these ANFs to safer limits while increasing the nutrient composition has thus become a part of the significant breeding objectives of sesame for value addition. Numerous attempts have been made to reduce the ANFs of oilseeds to a safe limit, including exposure to high irradiation levels (Kadam et al., 2021).

Genetic variability is a preeminent requirement for crop development regarding variant selection (Kara et al., 2016). Mutation breeding has become an inevitable tool for creating desirable genetic variability (Chaudhary et al., 2019). Gamma rays are more effective than many other irradiations because of their easy availability and high penetration power (Fan & Niemira, 2020). Mutagenesis has been used in developing many sesame varieties, including early maturing varieties with determinate growth and monostem types (Saha, 2018), chlorophyll mutants (Saha & Paul, 2017), oil-rich mutants, and so many other characters. However, no single mutant variety of sesame has been released officially in Nigeria. More also, almost all the existing varieties in Nigeria were released with little or no emphasis on nutritional value rather than the high seed yield and resistance to biotic and abiotic factors.

Considering the background information, the current investigation aimed at determining the biochemical constituents of sesame mutant genotypes from two generations (M_4 and M_5). The objectives of the research were to determine the (i) proximate composition of sesame mutants from two generations, (ii) anti-nutritional properties of two generations of sesame mutants, (iii) the relationship between the M_4 and M_5 mutant genotypes of sesame. The hypotheses of the research were: (i) The two generations of sesame mutants do not show any improvement in their proximate composition; (ii) The two generations of sesame mutants do not possess any anti-nutritional properties; (iii) There is no significant difference in proximate and anti-nutritional factors of the M_4 and M_5 mutant lines of sesame.

MATERIALS AND METHODS

Study area

The fieldwork was conducted at the experimental field of the Department of Plant Biology, the Federal University of Technology Minna, Nigeria (latitude 9.6536351 °N and longitude 6.5278096 °E). In contrast, the laboratory analyses were conducted at the Central Laboratory, National Cereal Research Institute, Badeggi, Nigeria (latitude 9.0652195 °N and longitude 6.0986163 °E).

Planting materials

Seeds of eleven mutant lines of sesame were obtained from breeders' lines in the Department of Plant Biology, Federal University of Technology Minna, Nigeria. They were initially derived from three Nigerian sesame varieties (NCRIBEN-04E (early maturing variety), NCRIBEN-01M (mid-maturing variety), and NCRIBEN-03L (late-maturing variety)) using four doses of gamma irradiation (250 Gy, 350 Gy, 450 Gy, and 550 Gy).

Field experiment

The field experiments were conducted between August and December 2019 and 2021 for the M_4 and

Table 1. Characteristics of experimental materials

Mutant lines	Pedigree	Description	Capsule characteristics
A1	04E-550-G_{1-3}	NCRIBEN-04E exposed to 550 Gy	Multicarpellate single capsule
A2	04E-550-G_{2-3}	NCRIBEN-04E exposed to 550 Gy	Bicarpellate single capsule
A3	04E-550-G_{3-3}	NCRIBEN-04E exposed to 550 Gy	Bicarpellate multicapsule
A	NCRIBEN-04E	Parental variety	Bicarpellate multicapsule
B1	01M-350-G_{1-2}	NCRIBEN-01M exposed to 350 Gy	Multicarpellate single capsule
B2	01M-350-G_{1-2}^{1}	Segregated from NCRIBEN-01M exposed to 350 Gy at M_3	Multicarpellate multicapsule
B3	01M-350-G_{2-2}	NCRIBEN-01M exposed to 350 Gy	Bicarpellate multicapsule
B4	01M-350-G_{2-2}^{2}	Segregated from NCRIBEN-01M exposed to 350 Gy at M_3	Bicarpellate multicapsule
B	NCRIBEN-01M	Parental variety	Bicarpellate single capsule
C1	03L-250-G_{1-1}	NCRIBEN-03L exposed to 250 Gy	Bicarpellate single capsule
C2	03L-250-G_{1-1}^{1}	Segregated from NCRIBEN-03L exposed to 250 Gy at M_3	Multicarpellate single capsule
C3	03L-450-G_{1-2}	NCRIBEN-03L exposed to 450 Gy	Bicarpellate single capsule
C4	03L-450-G_{2-2}	NCRIBEN-03L exposed to 450 Gy	Multicarpellate single capsule
C	NCRIBEN-03L	Parental variety	Bicarpellate single capsule

M_5 generations. Seeds of the eleven mutant lines were planted alongside their parental varieties (checks) in a Randomized Complete Block Design (RCBD) with three replications. Seeds were sown at 10 × 20 cm inter and intra-row spacing on a plot size of 3 × 3 m^2. All the agronomic practices for successfully raising sesame plants recommended by the International Plant Genetic Resource Institute and National Bureau of Plant Genetic Resources (IPGRI & NBPGR, 2004) were followed throughout the experiment until the seeds were harvested for analysis.

Biochemical analyses

At harvest, 100 g of seeds were collected from three randomly selected plants of each mutant line and controlled for proximate and anti-nutritional analysis.

Proximate composition analyses

The oil content, protein, fibre, ash, carbohydrate, energy and moisture contents were determined following the standard methods of the Association of Official Analytical Chemists (AOAC, 2020). Percentage oil was determined by the Soxhlet extraction method with n-hexane as the solvent, and protein content was determined using the Kjeldahl method. The crude fibre content was determined following the AOAC (2020) method by sample digestion, boiling, filtration, drying and burning in a furnace at 500°C for six (6) hours. Ash content was determined by burning the sample in a furnace at 550°C, according to the AOAC (2020). The total carbohydrate content was determined by difference using the method of Muller & Tobin (1980) by subtracting the total sum of the percentage of moisture, ash, crude fibre, and crude protein from hundred (100). The calorific value (energy) was calculated by the Atwater factor method following the method of Osborne & Voogt (1978), while the moisture content was determined by oven-drying at 105 ± 1°C for four (4) hours (AOAC, 2020). All analyses were carried out in triplicates.

Determination of anti-nutrients

Alkaloid content was determined using the method of Harborne (1973). Oxalate content was determined following the method of Nwosu (2011). Tannin content was determined by the titrimetric method following the standard procedure of Khasnabis et al. (2015). Flavonoid content was determined following the aluminium chloride method (Chang et al., 2002), which involved the addition of 0.3 ml of 5% sodium nitrite and 0.3 ml of 10% aluminium chloride at 5 minutes intervals. The mixtures were incubated at room temperature for 6 minutes, and 1 ml of 1 M sodium hydroxide was added to each mixture. A V-750 UV-Visible spectrophotometer was used to measure the absorbance of the sample against the blank at 510 nm. The phytate content was determined following Lucas (1975), while hydrocyanide content was determined following AOAC (2020) method. All analyses were carried out in triplicates.

Data analyses

Data obtained were subjected to the Shapiro-Wilk W test to test for the normality of the data using SPSS version 23.0. and non-normal data were transformed into customarily distributed data using the normal inverse distribution function by first generating the fractional rank variables. The transformed data were subjected to a one-way analysis of variance (ANOVA), and Duncan's Multiple Range Test (DMRT) was used to separate the means where there were significant differences between the mutant lines. Paired T-test was used to test for the significant difference between the two generations. All results were considered important at a 95% confidence level.

RESULTS

Notable variations were observed in the oil composition of mutant lines across the two generations (Table 2). Statistical analysis revealed a significant increase ($p < 0.05$) in the oil composition of B3, B4 and C2 over their parental varieties at the M_4 generation and in A1, A2, A3, and C4 at the M_5 generation. In addition, results revealed significant differences in the oil composition of M_4 and M_5 mutants except in B3 and B4, with M_5 mutants showing significantly higher oil percentages (Table 2).

Significant differences were observed in the protein contents of mutant lines across the two generations (Table 3). A significant increase was observed in C1 over its parental variety in the M_4 generation, while B2 and C3 revealed a substantial increase over their parental varieties in the M_5 generation. Significant differences ($p < 0.05$) were observed in the protein content of the M_4 and M_5 mutants, with the M_5 mutants having significantly lower protein contents.

No significant increase ($p > 0.05$) was observed in the fibre contents of all the mutants over their parental variety in the M_4 generation. In contrast, in the M_5 generation, a significant increase was observed in the fibre contents of all the mutants except in C1, C2, C3 and C4 (Table 4). Results revealed no significant difference ($p > 0.05$) in fibre contents of M_4 and M_5 mutants except in A2, A, B3 and C3 (Table 4).

A significant increase ($p < 0.05$) was observed in the ash content of B1 at the M_4 generation and A1, A2, A3 and B3 at the M_5 generation over their parental varieties (Table 5). No significant difference ($p > 0.05$) was observed in the ash content of M_4 and M_5 mutants except in the parental varieties A and B (Table 5).

Significant ($p < 0.05$) increase was observed in carbohydrate contents of A1, B1 and C3 at the M_4

Table 2. Oil composition (%) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	23.41 ± 2.00^{a}	51.93 ± 0.22^{d}	0.002*
A2	40.32 ± 3.02^{d}	50.05 ± 0.48^{c}	0.032*
A3	33.71 ± 0.43^{b}	56.41 ± 3.90^{g}	0.013*
A	40.05 ± 0.96^{d}	47.50 ± 0.75^{b}	0.003*
B1	35.65 ± 1.40^{bc}	53.58 ± 0.12^{ef}	0.002*
B2	34.73 ± 3.70^{bc}	52.12 ± 0.20^{d}	0.014*
B3	46.31 ± 2.70^{e}	47.61 ± 0.73^{b}	0.494
B4	48.28 ± 1.00^{e}	48.07 ± 0.10^{b}	0.750
B	38.76 ± 1.61^{cd}	52.84 ± 0.73^{de}	0.003*
C1	36.74 ± 1.70^{bcd}	45.76 ± 1.42^{a}	0.011*
C2	45.10 ± 1.90^{e}	51.82 ± 0.29^{d}	0.024*
C3	34.48 ± 3.20^{bc}	48.73 ± 0.06^{b}	0.016*
C4	37.50 ± 1.17^{bcd}	55.62 ± 1.01^{g}	0.004*
C	36.97 ± 2.11^{bcd}	54.13 ± 0.73^{f}	0.002*
Mean	38.00 ± 0.28	51.16 ± 0.27	

Table 3. Protein contents (%) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	22.83 ± 1.70^{abc}	14.68 ± 0.54^{cdef}	0.010*
A2	28.63 ± 2.70^{e}	14.49 ± 0.34^{cdef}	0.010*
A3	27.41 ± 1.90^{de}	16.54 ± 1.60^{def}	0.026*
A	25.51 ± 2.17^{cde}	16.86 ± 0.75^{efg}	0.030*
B1	20.31 ± 2.60^{ab}	13.00 ± 0.20^{bc}	0.037*
B2	23.72 ± 0.15^{bc}	19.46 ± 0.97^{g}	0.015*
B3	22.85 ± 1.90^{abc}	15.79 ± 3.41^{cdef}	0.048*
B4	23.41 ± 2.50^{cd}	15.33 ± 3.37^{cdef}	0.018*
B	23.73 ± 0.00^{cd}	15.78 ± 0.18^{cdef}	0.010*
C1	32.83 ± 2.60^{f}	11.49 ± 0.34^{ab}	0.006*
C2	19.93 ± 0.06^{a}	13.96 ± 1.96^{bcde}	0.034*
C3	23.73 ± 1.40^{bc}	17.19 ± 0.25^{fg}	0.013*
C4	24.99 ± 2.25^{cd}	10.06 ± 0.72^{a}	0.010*
C	25.31 ± 0.80^{cde}	13.76 ± 0.15^{bcd}	0.001*
Mean	24.66 ± 0.27	14.89 ± 0.27	

generation and in B3, B4, C1 and C3 at the M_5 generation over their parental varieties (Table 6). Results revealed significant differences ($p < 0.05$) in carbohydrate contents of M_4 and M_5 mutants except in A1, A3, B1, B2 and C, with M_5 mutants having significantly higher carbohydrate content (Table 6).

A significant increase ($p < 0.05$) was observed in the energy value of mutants B3, B4 and C2 at the M_4 generation and A1, A3 and C4 at the M_5 generation over their parental varieties. The M_4 and M_5 mutants showed significant differences in energy values except in B3 and B4. The M_5 mutants were observed to have significantly higher energy values (Table 7).

In the M_4 generation, a significant increase ($p < 0.05$) was observed in the moisture content of all the mutants over their parental varieties except in C2 and

Table 4. Fibre contents (%) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	4.01 ± 0.08^{b}	3.71 ± 0.21^{cd}	0.113
A2	3.23 ± 0.21^{a}	3.88 ± 0.14^{d}	0.038*
A3	3.50 ± 0.06^{ab}	3.89 ± 0.53^{d}	0.336
A	4.08 ± 0.30^{b}	2.22 ± 0.17^{a}	0.014*
B1	3.44 ± 0.14^{ab}	3.22 ± 0.37^{bcd}	0.482
B2	3.65 ± 0.08^{ab}	3.20 ± 0.30^{bcd}	0.156
B3	4.02 ± 0.13^{b}	3.55 ± 0.14^{cd}	0.039*
B4	3.54 ± 0.80^{ab}	3.12 ± 0.37^{bc}	0.552
B	3.15 ± 0.49^{ab}	2.65 ± 0.22^{ab}	0.125
C1	3.33 ± 0.09^{a}	3.17 ± 0.70^{bc}	0.711
C2	4.00 ± 0.12^{b}	3.09 ± 0.34^{bc}	0.068
C3	4.04 ± 0.07^{b}	3.22 ± 0.19^{bcd}	0.014*
C4	3.72 ± 0.43^{ab}	2.82 ± 0.17^{ab}	0.116
C	3.65 ± 0.05^{ab}	3.77 ± 0.56^{cd}	0.767
Mean	3.67 ± 0.05	3.26 ± 0.05	

Table 5. Ash contents (%) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	5.85 ± 0.90^{e}	5.58 ± 0.46^{def}	0.582
A2	5.77 ± 0.50^{e}	5.85 ± 0.41^{f}	0.863
A3	5.33 ± 0.70^{de}	5.72 ± 0.86^{ef}	0.667
A	5.88 ± 0.12^{e}	3.47 ± 0.22^{a}	0.005*
B1	5.85 ± 0.01^{e}	5.01 ± 0.50^{bcdef}	0.105
B2	4.33 ± 0.50^{bc}	4.74 ± 0.37^{bcde}	0.415
B3	5.29 ± 0.09^{de}	5.27 ± 0.23^{cdef}	0.932
B4	4.85 ± 0.02^{cd}	4.73 ± 0.43^{bcde}	0.700
B	4.90 ± 0.04^{cd}	4.08 ± 0.32^{ab}	0.038*
C1	3.89 ± 0.06^{ab}	5.26 ± 1.18^{cdef}	0.180
C2	5.97 ± 0.20^{e}	4.65 ± 0.39^{bcd}	0.050
C3	4.78 ± 0.02^{cd}	4.81 ± 0.21^{bcde}	0.836
C4	3.34 ± 0.62^{a}	4.27 ± 0.24^{abc}	0.149
C	5.51 ± 0.66^{de}	5.26 ± 0.55^{cdef}	0.671
Mean	5.11 ± 0.08	4.91 ± 0.08	

Table 6. Carbohydrate contents (kg/g) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	38.44 ± 1.00^{i}	35.43 ± 1.33^{e}	0.127
A2	20.39 ± 1.76^{bc}	36.62 ± 0.92^{ef}	0.002*
A3	25.49 ± 3.50^{def}	29.50 ± 1.00^{ab}	0.175
A	22.59 ± 1.10^{cd}	44.55 ± 0.79^{h}	0.002*
B1	31.17 ± 2.10^{h}	34.72 ± 1.51^{de}	0.179
B2	28.17 ± 1.50^{fg}	31.25 ± 0.43^{abc}	0.058
B3	17.10 ± 1.15^{a}	38.80 ± 0.93^{fg}	0.001*
B4	15.98 ± 2.03^{a}	40.34 ± 1.19^{g}	0.004*
B	26.66 ± 1.40^{ef}	36.45 ± 2.39^{ef}	0.008*
C1	18.32 ± 1.09^{ab}	36.92 ± 2.52^{ef}	0.005*
C2	21.74 ± 1.17^{c}	28.45 ± 3.22^{a}	0.044*
C3	29.59 ± 1.74^{gh}	41.61 ± 1.24^{gh}	0.017*
C4	27.033 ± 1.07^{efg}	33.81 ± 1.28^{cde}	0.032*
C	24.85 ± 0.46^{de}	32.08 ± 2.77^{bcd}	0.054
Mean	24.82 ± 0.27	35.75 ± 0.26	

Table 7. Energy values (kcal/g) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	480.80 ± 8.34^{a}	609.20 ± 8.34^{d}	0.002*
A2	522.89 ± 15.05^{bcd}	594.90 ± 6.17^{bc}	0.009*
A3	502.48 ± 9.64^{b}	626.53 ± 3.02^{f}	0.003*
A	546.34 ± 11.49^{e}	599.60 ± 1.11^{c}	0.015*
B1	521.15 ± 17.51^{bc}	622.31 ± 3.78^{f}	0.009*
B2	511.86 ± 15.15^{b}	601.25 ± 2.90^{c}	0.007*
B3	578.84 ± 9.71^{f}	580.47 ± 3.15^{a}	0.828
B4	581.86 ± 14.98^{f}	591.03 ± 2.75^{b}	0.339
B	550.07 ± 12.86^{e}	621.22 ± 0.06^{ef}	0.011*
C1	$543.29 \pm 1.98d^{e}$	578.90 ± 2.01^{a}	0.003*
C2	570.96 ± 10.18^{f}	600.11 ± 3.26^{c}	0.023*
C3	518.05 ± 3.37^{bc}	601.96 ± 5.86^{c}	0.004*
C4	546.56 ± 14.96^{e}	633.39 ± 2.36^{g}	0.012*
C	534.94 ± 11.10^{cde}	615.44 ± 7.74^{de}	0.003*
Mean	536.05 ± 1.38	605.45 ± 1.36	

C3, while in the M_5 generation, a significant increase was observed in the moisture content of B2, B3, B4, C1 and C2 over their parental varieties (Table 8).

Significant differences ($p < 0.05$) were observed in moisture contents of the M_4 and M_5 mutants in A1, B2, B3, B4, C2 and C4.

A Significant increase ($p < 0.05$) was observed in the alkaloid content of mutants over their parental varieties except in A1, C3 and C4 at M_4 generation and mutants A1, B1, C2, C3 and C4 at M_5 generation (Table 9). Results of the t-test revealed significant differences ($p < 0.05$) in alkaloid contents of M_4 and M_5 mutants, with M_5 mutants having significantly higher alkaloid contents (Table 9).

A significant increase was observed in oxalate content A2 and C3 at the M_4 generation and in mu-

Table 8. Moisture content (%) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	5.46 ± 0.09^{j}	4.16 ± 0.08^{abcd}	0.004*
A2	5.12 ± 0.02^{i}	4.47 ± 0.57^{bcd}	0.192
A3	4.56 ± 0.08^{g}	4.09 ± 1.36^{abc}	0.607
A	4.21 ± 0.05^{f}	3.45 ± 1.33^{ab}	0.421
B1	3.76 ± 0.06^{d}	4.24 ± 0.68^{bcd}	0.373
B2	5.39 ± 0.02^{j}	6.15 ± 0.18^{fg}	0.021*
B3	3.79 ± 0.05^{d}	5.71 ± 0.12^{efg}	0.001*
B4	3.90 ± 0.04^{e}	$5.35 \pm 0.15d^{ef}$	0.005*
B	3.67 ± 0.04^{c}	4.21 ± 0.85^{bcd}	0.379
C1	4.89 ± 0.08^{h}	4.94 ± 0.08^{cde}	0.392
C2	3.26 ± 0.04^{a}	6.79 ± 0.20^{g}	0.001*
C3	3.38 ± 0.02^{b}	3.38 ± 0.36^{ab}	0.988
C4	4.97 ± 0.03^{h}	3.48 ± 0.41^{ab}	0.026*
C	3.45 ± 0.05^{b}	2.94 ± 0.67^{a}	0.323
Mean	4.27 ± 0.07	4.56 ± 0.07	

Table 9. Alkaloid content (mg/g) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	0.18 ± 0.08^{a}	2.80 ± 0.15^{b}	0.000*
A2	1.42 ± 0.19^{f}	4.20 ± 0.06^{f}	0.001*
A3	0.71 ± 0.17^{bc}	3.73 ± 0.05^{de}	0.001*
A	0.58 ± 0.14^{b}	3.51 ± 0.10^{cd}	0.002*
B1	0.74 ± 0.03^{bc}	2.33 ± 0.22^{a}	0.008*
B2	1.03 ± 0.07^{de}	3.80 ± 0.18^{de}	0.001*
B3	0.61 ± 0.08^{b}	4.38 ± 0.07^{fg}	0.000*
B4	0.55 ± 0.05^{b}	4.91 ± 0.57^{h}	0.006*
B	0.35 ± 0.03^{a}	3.17 ± 0.13^{bc}	0.000*
C1	1.44 ± 0.06^{f}	4.83 ± 0.34^{h}	0.002*
C2	1.34 ± 0.17^{f}	3.07 ± 0.19^{b}	0.013*
C3	1.11 ± 0.07^{e}	3.55 ± 0.25^{cd}	0.002*
C4	0.90 ± 0.06^{cd}	4.03 ± 1.72^{ef}	0.000*
C	0.97 ± 0.09^{de}	4.72 ± 0.15^{gh}	0.001*
Mean	0.85 ± 0.03	3.79 ± 0.03	

Table 10. Oxalate content (mg/g) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	1.00 ± 0.07^{a}	8.79 ± 0.42^{cd}	0.002*
A2	6.00 ± 0.18^{ghi}	8.84 ± 0.41^{cd}	0.011*
A3	1.57 ± 0.07^{a}	11.55 ± 1.49^{g}	0.150
A	5.48 ± 0.17^{fgh}	11.39 ± 0.33^{g}	0.002*
B1	6.58 ± 0.21^{hi}	8.84 ± 0.41^{cd}	0.008*
B2	1.92 ± 0.05^{ab}	9.57 ± 0.30^{de}	0.042*
B3	4.45 ± 0.25^{def}	10.79 ± 0.24^{fg}	0.001*
B4	3.85 ± 0.17^{cde}	5.96 ± 0.99^{a}	0.048*
B	9.55 ± 0.59^{k}	7.15 ± 0.71^{b}	0.214
C1	3.33 ± 0.18^{cd}	6.96 ± 0.61^{ab}	0.005*
C2	2.75 ± 0.21^{bc}	8.08 ± 0.13^{bc}	0.000*
C3	8.20 ± 0.38^{jk}	7.13 ± 0.64^{b}	0.110
C4	4.99 ± 0.16^{efg}	9.65 ± 0.37^{de}	0.004*
C	7.27 ± 0.26^{ij}	10.27 ± 0.15^{ef}	0.002*
Mean	4.84 ± 0.09	8.93 ± 0.09	

Table 11. Tannin content (mg/g) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	0.23 ± 0.01^{d}	0.14 ± 0.01^{g}	0.001*
A2	0.28 ± 0.01^{g}	0.07 ± 0.02^{c}	0.001*
A3	0.25 ± 0.01^{ef}	0.09 ± 0.01^{cde}	0.004*
A	0.32 ± 0.02^{i}	0.07 ± 0.00^{c}	0.001*
B1	0.25 ± 0.01^{ef}	0.03 ± 0.01^{ab}	0.028*
B2	0.22 ± 0.00^{c}	0.11 ± 0.00^{ef}	0.041*
B3	0.27 ± 0.01^{g}	0.18 ± 0.01^{h}	0.005*
B4	0.24 ± 0.00^{de}	0.08 ± 0.01^{cd}	0.000*
B	0.19 ± 0.01^{a}	0.04 ± 0.02^{b}	0.010*
C1	0.21 ± 0.01^{b}	0.10 ± 0.00^{def}	0.001*
C2	0.28 ± 0.01^{g}	0.12 ± 0.01^{fg}	0.000*
C3	0.26 ± 0.00^{f}	0.14 ± 0.03^{g}	0.016*
C4	0.30 ± 0.01^{h}	0.06 ± 0.03^{c}	0.002*
C	0.30 ± 0.01^{h}	0.01 ± 0.00^{a}	0.000*
Mean	0.26 ± 0.00	0.09 ± 0.00	

tants B1, B2 and B3 at the M_5 generation over their parental varieties (Table 10). Significant differences were also observed in oxalate contents of M_4 and M_5 mutants except in A3, B and C3, with M_5 mutants revealing significantly higher oxalate contents.

Mutants B1, B2, B3 and B4, revealed a significant decrease ($p < 0.05$) in tannin contents over their parental variety at the M_4 generation, while at the M_5 generation, no significant decrease was observed in tannin contents of the mutants over their parental varieties. The M_4 and M_5 mutants showed significant differences ($p < 0.05$) in tannin contents, with M_5 mutants having significantly higher tannin contents (Table 11).

A significant decrease was observed in the flavonoid content of B1, B2, B3, B4 and C3 at the M_4 generation and in mutants C1, C2, C3 and C4 at the M_5 generation over their parental varieties. Significant differences ($p < 0.05$) were observed in flavonoid contents of M_4 and M_5 mutants except in A3, B2 and B3 (Table 12).

Significant reductions ($p < 0.05$) were observed in phytic acid contents of B1, B2, B4, C1, C2, C3 and C4 at the M_4 generation and in mutants A1, A2, A3, B1, B2, B4, C1 and C3 at the M_5 generation over their parental varieties. Results revealed significant differences ($p < 0.05$) in the phytic acid content of M_4 and M_5 mutants except in A2, B1, B2 and B4, with M_5 mutants having significantly higher phytic acid content (Table 13).

Mutants A1, A2, A3, C1 and C2 revealed a significant decrease ($p < 0.05$) in hydrocyanide content at the M_4 generation, while at the M_5 generation, mutants A1, A3, B3 and B4 revealed a significant decrease in hydrocyanide content over their parental varieties. Significant differences ($p < 0.05$) were observed in

Table 12. Flavonoid content (mg/g) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	2.62 ± 0.12^{d}	4.97 ± 0.16^{j}	0.005*
A2	2.01 ± 0.01^{c}	5.78 ± 0.32^{k}	0.002*
A3	2.99 ± 0.12^{e}	3.67 ± 0.19^{def}	0.062
A	1.48 ± 0.02^{b}	2.54 ± 0.11^{b}	0.004*
B1	1.52 ± 0.01^{b}	4.65 ± 0.14^{ij}	0.001*
B2	3.51 ± 0.24^{f}	3.81 ± 0.25^{efg}	0.398
B3	4.03 ± 0.03^{g}	4.40 ± 0.86^{hi}	0.532
B4	1.45 ± 0.12^{b}	3.91 ± 0.17^{fgh}	0.004*
B	4.64 ± 3.26^{h}	3.26 ± 0.21^{cde}	0.002*
C1	5.12 ± 0.33^{i}	1.73 ± 0.32^{a}	0.012*
C2	5.39 ± 0.68^{i}	1.95 ± 0.46^{a}	0.036*
C3	0.46 ± 0.02^{a}	3.18 ± 0.08^{cd}	0.000*
C4	3.50 ± 0.03^{f}	2.84 ± 0.09^{bc}	0.003*
C	1.99 ± 0.03^{c}	4.29 ± 0.13^{ghi}	0.001*
Mean	2.91 ± 0.04	3.64 ± 0.04	

Table 13. Phytic acid content (mg/g) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	1.28 ± 0.00^{h}	1.67 ± 0.05^{de}	0.005*
A2	1.65 ± 0.00^{j}	1.48 ± 0.07^{bcd}	0.052
A3	1.05 ± 0.01^{e}	1.54 ± 0.07^{cde}	0.009*
A	0.95 ± 0.02^{d}	2.11 ± 0.06^{h}	0.001*
B1	0.97 ± 0.02^{d}	1.04 ± 0.14^{a}	0.454
B2	1.07 ± 0.09^{ef}	1.08 ± 0.16^{a}	0.878
B3	1.32 ± 0.00^{h}	1.88 ± 0.11^{fg}	0.014*
B4	1.11 ± 0.01^{f}	1.09 ± 0.18^{a}	0.843
B	1.22 ± 0.01^{g}	1.91 ± 0.06^{g}	0.002*
C1	0.69 ± 0.02^{b}	1.31 ± 0.03^{b}	0.000*
C2	0.52 ± 0.02^{a}	1.73 ± 0.21^{efg}	0.011*
C3	0.86 ± 0.00^{c}	1.43 ± 0.09^{bc}	0.007*
C4	0.96 ± 0.03^{d}	2.32 ± 0.07^{i}	0.000*
C	1.53 ± 0.01^{i}	1.70 ± 0.05^{ef}	0.021*
Mean	1.08 ± 0.01	1.59 ± 0.01	

Table 14. Hydrocyanide content (mg/g) of two generations of sesame mutants (mean ± standard error). Values along the same column with different superscripts are significantly different at $p < 0.05$. * – significant differences at $p < 0.05$ for values across the same row

Mutants	M_4	M_5	*p*-value
A1	39.04 ± 0.52^{cd}	42.82 ± 1.61^{f}	0.045*
A2	48.00 ± 1.20^{h}	55.98 ± 3.31^{h}	0.071
A3	40.49 ± 0.46^{de}	30.61 ± 8.41^{e}	0.178
A	56.33 ± 1.10^{j}	48.65 ± 2.40^{g}	0.154
B1	42.23 ± 0.85^{ef}	29.61 ± 4.12^{e}	0.022*
B2	45.20 ± 1.13^{g}	37.86 ± 1.87^{f}	0.085
B3	44.76 ± 0.43^{fg}	20.00 ± 2.23^{bc}	0.002*
B4	37.36 ± 0.62^{bc}	11.79 ± 1.48^{a}	0.002*
B	31.59 ± 1.00^{a}	31.63 ± 1.05^{ef}	0.983
C1	35.22 ± 0.85^{b}	27.54 ± 1.00^{de}	0.005*
C2	42.66 ± 0.84^{efg}	38.51 ± 1.30^{f}	0.060
C3	49.10 ± 1.30^{hi}	36.01 ± 2.47^{f}	0.002*
C4	50.40 ± 3.65^{hi}	22.99 ± 1.86^{cd}	0.013*
C	50.96 ± 2.19^{i}	15.82 ± 1.23^{ab}	0.001*
Mean	43.88 ± 0.38	27.55 ± 0.37	

hydrocyanide contents of M_4 and M_5 mutants except in A2, A3, A4, B2, B and C2 (Table 14).

The percentage increment in proximate composition of the sesame mutants over their parental varieties at M_4 and M_5 generations are presented in Fig. 1 and Fig. 2, respectively. At M_4 generation, the highest percentage increase in oil composition was recorded in B4, and the highest percentage increase in protein content was recorded in C1. B3 had the highest percentage increase in fibre content, and the highest percentage increase in ash content was recorded in B1. A1 revealed the highest percentage increase in carbohydrate content. The highest percentage increase in energy value was recorded in C2, while B2 showed the highest percentage increase in moisture content (Fig. 1).

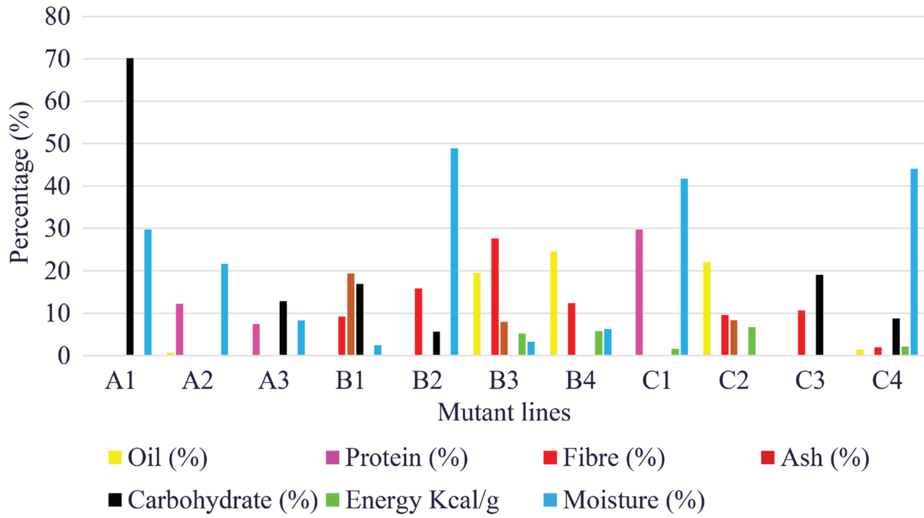

Fig. 1. Changes in proximate composition of the M_4 mutants over their parental varieties. This figure reveals the percentage increments observed in the proximate composition of the M_4 mutants over their respective parental varieties from which they were derived. The full description of the mutant lines is presented in Table 1

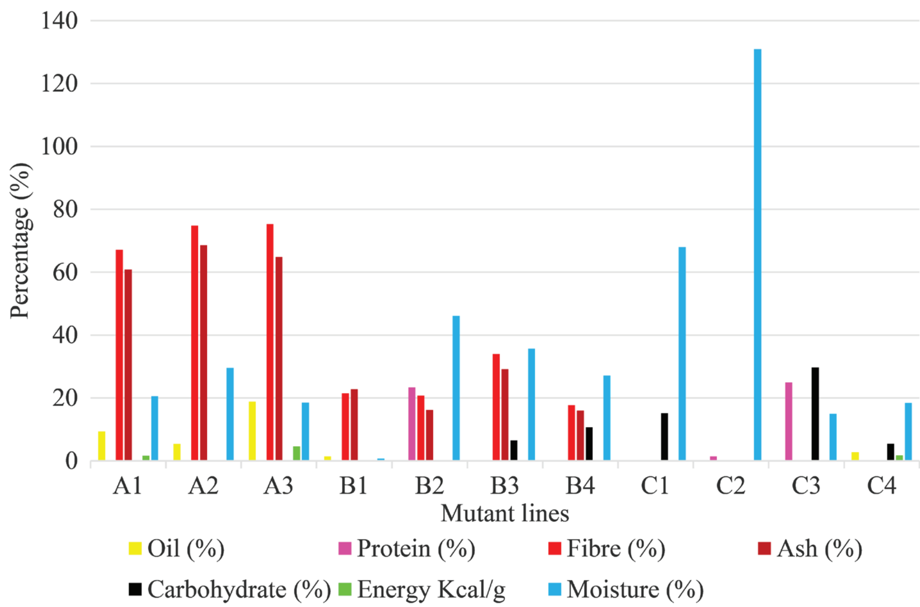

Fig. 2. Changes in proximate composition of the M_5 mutants over their parental varieties. This figure reveals the percentage increments observed in the proximate composition of the M_5 mutants over their respective parental varieties from which they were derived. The full description of the mutant lines is presented in Table 1

At M_5 generation, A3 had the highest percentage increase in oil, fibre and energy content, and C3 had the highest percentage increase in protein and carbohydrate content. In addition, highest percentage increase in ash content was recorded in A3, while C2 had the highest percentage increase in moisture content (Fig. 2).

The percentage decrease in anti-nutritional contents of the mutants over their parental varieties at M_4 and M_5 generations are presented in Figures 3 and 4, respectively. At M_4 generation, the highest percentage decrease in alkaloid and oxalate contents was recorded in A1, and the highest percentage decrease in tannin content was recorded in C1. C3 and C2 had the highest percentage decrease in flavonoid and phytic acid contents, respectively, while B4 had the highest percentage decrease in hydrocyanide content (Fig. 3).

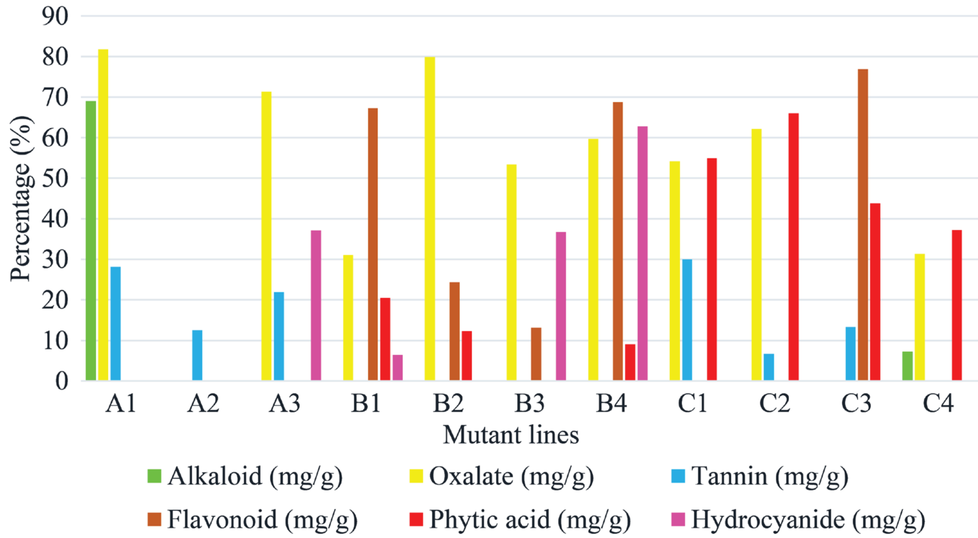

Fig. 3. Changes in the anti-nutritional composition of the M_4 mutants over their parental varieties. This figure reveals the percentage decrease observed in anti-nutritional factors of the M_4 mutants over their respective parental varieties from which they were derived. The full description of the mutant lines is presented in Table 1

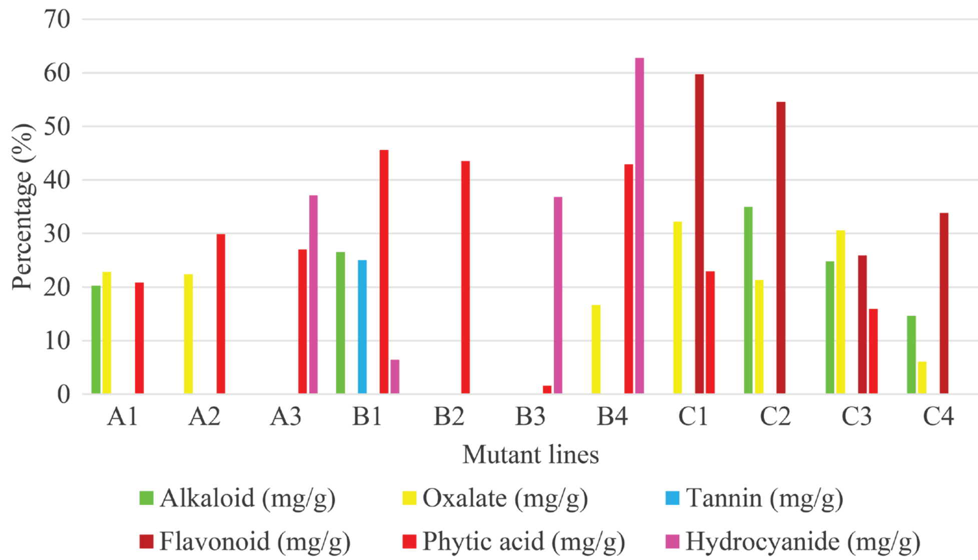

Fig. 4. Changes in the anti-nutritional composition of the M_5 mutants over their parental varieties. This figure reveals the decrease observed in anti-nutritional factors of the M_5 mutants over their respective parental varieties from which they were derived. The full description of the mutant lines is presented in Table 1

At M_5 generation, the highest percentage decrease in alkaloid content was recorded in C2. C1 had the highest percentage decrease in oxalate and flavonoid contents. B1 had the highest percentage decrease in tannin and phytic acid contents, while B4 revealed the highest percentage decrease in hydrocyanide content (Fig. 4).

DISCUSSION

The proximate composition and anti-nutritional analyses of sesame seeds (*Sesamum indicum*) varied among the mutants and across the two generations (M_4 and M_5). However, the results revealed the presence of significant quantities of oil, protein, moisture, ash, fibre, carbohydrate, alkaloid, oxalate, tannin, flavonoid, phytic acid, and hydrocyanide contents in the two generations. The presence of these constituents indicates the nutritional qualities obtainable from sesame seeds.

In this study, the crude oil extract of some mutant lines was positively affected, while others were negatively affected. This could indicate that gamma irradiation can result in positive and negative genetic variability in sesame seeds. This is in line with the work of Abdul et al. (2018), who have reported that gamma rays can either result in genomic damage or gene reshuffling corresponding to healthy outcomes. The significant increase in oil composition in some of the mutants over their parental varieties is similar to the findings of Chowdhury et al. (2009). A similar report of an increase in oil content as a result of gamma irradiation has been reported by Munda et al. (2022) in *Cymbopogon winterianus*. The significant decrease in oil content in some of the mutant lines may be attributed to the negative effect of gamma irradiation on biochemical pathways involved in oil synthesis. This could be corroborated by the report of Gudkov et al. (2019), who have opined that ionising radiation affects physiological and molecular processes in plants.

Significant variations observed in the seed oil content of the mutants are likely due to variability in their genetic makeup. This conforms with the report of Wei et al. (2016), who have attributed variability in seed oil contents of sesame varieties to their genomic variation. Variations observed in the two generations' oil composition likely reflect differences in environmental factors. A similar variability has been reported in the oil content of sesame accessions examined over three years by Were et al. (2006).

The significant increase observed in crude protein contents of the mutant lines, especially at M_4 generations, could be attributed to the ionising effect of gamma irradiation. Similar findings have been reported by Rizki et al. (2015) in sesame. Ogbonna & Ukaan (2013) have opined that protein is a vital part of human nutrition and a nutrient primarily low in plant products. The significant differences observed in protein content across the two generations could be due to the fluctuations in environmental factors that influence seed composition. Environmental factors have been reported to affect seed compositions, especially oil and protein content. A similar discovery of significant variability has been reported in the protein content of soybean species under different climatic conditions (Kara et al., 2016).

The significant decrease observed in crude fibre content in most of the mutants could be attributed to the depolymerisation and delignification of the plant matrix due to the irradiation (Tresina & Mohan, 2011). A similar discovery of reduction in crude fibre contents following electron beam irradiation has been reported in cottonseed by Salari & Poorazadi (2018). This is also in line with reports of Sharma & Thakur (2021), who have reported lowered crude fibre content in gamma-irradiated ginger. On the contrary, Beheshti-Moghadam et al. (2019) have reported no significant difference in the crude fibre content of gamma-irradiated flaxseed. This differential response could be attributed to varietal differences. The crude fibre observed in this study (2.22–4.04) falls within the range reported by Awopetu & Oyerinde (2022) in fifteen sesame cultivars (2.00–9.00). The presence of significant levels of crude fibre in the diet helps to maintain human health by lowering cholesterol levels in the body (Bello et al., 2013) and could also be beneficial in producing livestock feed.

The significant decrease in the percentage of ash in some of the mutants across the two generations could be attributed to the physiological disturbances caused by irradiation. This result conforms with the reports of Abu et al. (2020) on two Nigerian peppers, but contrary to the reports of Lima et al. (2019) and Liu et al. (2018). They have reported no significant difference in the ash content of gamma-irradiated common bean and gamma-irradiated peanut, respectively. These differential responses could be due to differences in the plant's genetic makeup.

The significant increase in carbohydrate content and energy value in some mutants over their parental varieties could be due to improved physiological activities due to gamma irradiation (Mounir et al., 2015). This could be corroborated by the work of Devi et al. (2018), who have reported an increase in the carbohydrate content of *Citrus jambhiri* fruits due to gamma irradiation.

The significant increase observed in the moisture content of all the mutants over their parental varieties in both generations could be due to the adverse effects of gamma irradiation. A similar finding of an increase in moisture content has been reported by Maraei & Hammoud (2019) in gamma-irradiated date seeds. The mutant lines with higher moisture content can be used to obtain higher cake recovery of up to 74% (Ishola et al., 2020), while those with lower moisture content would be suitable for conventional threshing (Nobre et al., 2019). In addition, a high moisture content encourages the growth of microbes (Afolabi, 2008), which implies that mutant lines with lower moisture content will be better preserved and have a longer shelf life.

Results on anti-nutritional factors reveal significant variations in alkaloid, oxalate, tannin, flavonoid, phytic acid and hydrogen cyanide contents. Some mutants show a decrease over their parental varieties, while others show a significant increase over their parental varieties. These variations conform with reports on the effects of gamma-irradiation on seed anti-nutritional contents of crop plants and further show that gamma irradiation effects depend on species, genotypes, time and irradiation dose.

The significant decrease observed in anti-nutritional factors of some mutants could be attributed to the breakage in trypsin inhibitor (TI) or chymotrypsin inhibitor (CTI) structure as a result of the irradiation (Vagadia et al., 2017). Similar reports of reductions in some anti-nutritional factors such as tannin, oxalate, phytic acid and hydrogen cyanide have been reported in two landraces of *Vigna unguiculata* 'Fiofio' and 'Olaudi' by Udensi et al. (2012), in maize by Hassan et al. (2009), in Faba bean by Osman et al. (2014) and have all been attributed to the oxidative damages caused by gamma-irradiation.

The significant increase observed in anti-nutrients in some of the mutants over their parental varieties could be due to elevated reactions from gamma-irradiation. Similar findings of an increase in anti-nutrients as a result of gamma irradiation have been reported by several authors. Tresina & Mohan (2011) have reported a significant rise in the tannin contents of *Vigna unguiculata* following gamma-irradiation. Rizki et al. (2015) have also reported enhancement in alkaloid and flavonoid contents of sesame as a result of gamma-irradiation.

High amounts of some anti-nutritional factors could impair flavour in plants and could also have detrimental effects on humans. For example, a high concentration of oxalates and phytates negatively affects mineral absorption and protein digestibility, and eradicating these anti-nutrients to the maximum extent will enhance sesame's nutritional and economic value.

Yadav & Bhatnagar (2017) have opined that plants release anti-nutritional factors for their defence which means that they may also have positive effects on plants. Tannin in sesame accounts for its anti-fungal and anti-bacterial properties (Shittu et al., 2007), and the significant increase observed in some tannin contents could increase their anti-fungal and anti-bacterial activities. Also, the presence of high alkaloids and flavonoids accounts for sesame's anti-oxidant properties and increases the stability of sesame oil (Ramesh et al., 2005).

The significant variations observed in anti-nutritional factors of the two generations could be attributed to the fluctuations in environmental factors that influence seed composition. This agrees with the report of Carlsson et al. (2009), who have reported that environmental factors, along with genetic factors, influence seed compositions in sesame.

CONCLUSIONS

Results from this study revealed that proximate compositions were increased in some of the mutants, especially in the M_5 generation. However, some mutants witnessed significant reductions over their parental varieties. The results also revealed notable declines in some mutant lines' toxic anti-nutritional properties. Additionally, significant variations were recorded between the proximate and anti-nutritional factors of the M_4 and M_5 mutants, which could indicate that these mutants are not yet stable and could be further examined in later generations. The existence of these variabilities broadens the scope for improving nutritional attributes through the selection of mutants with high and stable desirable traits.

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