CHILEAN JOURNAL of AGRICULTURAL RESEARCH
RESEARCH ARTICLE

Enhancing productivity and fatty acid profiles in sesame through induced mutagenesis with gamma irradiation and sodium azide

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Received: 31 July 2025; Accepted: 4 November 2025, doi:10.4067/S0718-58392026000100061

ABSTRACT

Enhancing seed and oil productivity using gamma irradiation and sodium azide provides a promising approach for developing superior genotypes. This study employed induced mutation breeding using gamma irradiation (100, 200, 400 Gy) and sodium azide (NaN_3, 0.001 M, 0.002 M, 0.003 M) on three sesame (*Sesamum indicum* L.) genotypes (Red sesame, White sesame, and 'Shandaweel3') to develop superior lines with enhanced seed and oil productivity. From the M_1 generation, 89 morphologically distinct mutants were identified in the M_2 generation, leading to the selection of 14 promising M_3 mutants based on days to maturity, plant height, capsule number, seed count per capsule, seed weight, and overall yield. Early-maturing mutants (VER1, EW9, EW12, EW3) were identified as potential candidates for short-season or drought-prone areas, while shorter genotypes (White-Seeds, EW11, HNCW8) could facilitate mechanized harvesting and dense planting. Taller mutants (ER2, VESh1, Red-Seeds, HNCR4, ESh3, EW9) showed potential for higher yields. The promising mutants VESh1, ESh4, ER2, and HNCR4 combined high capsule counts, seed setting, and seed weight, indicating yield improvement potential. Fatty acid profiles revealed significant diversity: Red genotype had high linoleic acid (60.27%), but mutant ER2 showed reduced linoleic acid (5.34%) and unique presence of palmitoleic and lignoceric acids. HNCR4 had the highest linoleic acid (70.30%) and contained these fatty acids. White seeds also had high linoleic acid (60.50%), with mutant EW12 showing a large decrease (8.78%). HNCW8 had the lowest linoleic acid (5.53%) but uniquely contained cerotic acid. 'Shandaweel3' and its mutants showed varied fatty acid contents. These results demonstrated the effectiveness of mutation breeding for improving sesame yield and quality.

Key words: Fatty acid content, genotypic variation, induced mutagenesis, multivariate analysis, mutation breeding.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an ancient oilseed crop cultivated for its high-quality edible oil, protein-rich meal and lignans with antioxidant properties. It has significant economic and nutritional importance globally, particularly in tropical and subtropical regions (Gonzalez et al., 2025). As the demand for vegetable oils is growing, improving the oil content of sesame seeds is still the top breeding objective to increase its economic

viability and satisfy consumer demands (Islam et al., 2016). While the traditional breeding methods are successful in many crops, they often face limitations in producing novel genetic variations for complex traits like oil content, which are affected by multiple genes and environmental factors (John Martin et al., 2022). This necessitates the exploration of alternative approaches to accelerate the breeding process and expand the genetic base for oil improvement in sesame.

Employing physical or chemical mutagens to create genetic variations in plants is a powerful tool to overcome the limitations of conventional breeding (Islam et al., 2016). Gamma irradiation is an important physical mutagen due to its high penetration power, simplicity of use, and capacity to cause a variety of mutations, such as deletions, point mutations, and chromosomal rearrangements (Penna et al., 2023). Similarly, chemical mutagens such as sodium azide (NaN_3) are effectively used in a variety of crops to improve desired traits because of their effectiveness in causing gene mutations (Khursheed and Khan, 2023).

The application of induced mutagenesis in sesame breeding demonstrated promising results in producing variability for various agronomic traits, including plant height, flowering time, capsule number, and seed yield (Ju et al., 2021). However, specifically focusing on significantly enhancing oil content through mutagenesis necessitates methodical research and mutagenic treatment optimization (Sharma et al., 2025). An effective mutation breeding program for oil improvement in sesame requires an understanding of the dose-response relationship of various mutagens, identification of effective screening methods for high oil mutants, and an assessment of the stability and heritability of induced variations (Gadri et al., 2024).

Fatty acid composition of sesame oil is crucial in determining its nutritional value, stability, and suitability for various applications, even beyond its sheer volume. The fatty acid profile of sesame oil is primarily unsaturated, with oleic acid (omega-9) and linoleic acid (omega-6) being the most prevalent parts, usually making up more than 80% of the total fatty acids (Gouveia et al., 2017). Sesame oil typically contains 35%-43% oleic acid (C18:1), 37%-47% linoleic acid (C18:2), 9%-11% palmitic acid (C16:0), 5%-10% stearic acid (C18:0), and approximately 0.7% arachidic acid (C20:0) (Kurt, 2018). Palmitic and stearic acids are the primary saturated fatty acids, whereas oleic and linoleic acids are the most common unsaturated fatty acids. The health benefits of consuming sesame oil, such as its ability to lower cholesterol and lower the risk of cardiovascular diseases, are attributed to this advantageous ratio of unsaturated to saturated fatty acids (Oboulbiga et al., 2023). Smaller amounts of minor fatty acids, like stearic and palmitic acid, are also present and contribute to the oil properties (Yol et al., 2015). The specific proportions of these fatty acids in sesame oil can be affected by a range of factors, including genetic makeup, growing geographical location, harvesting stage and the climatic conditions during seed development (Kurt, 2018).

While mutation breeding using physical and chemical mutagens is a proven strategy for crop improvement, the specific efficacy and optimal induction parameters of gamma irradiation and sodium azide for significantly enhancing oil content in Egyptian sesame genotypes remain underexplored. This study addresses this critical gap by rigorously investigating the potential of gamma irradiation and sodium azide to induce beneficial, high-oil-content mutations in three distinct Egyptian sesame genotypes. The primary objective was to screen and evaluate the resulting mutant populations across generations to accurately quantify the increase in oil content. Ultimately, this research sought to identify promising, stable mutant lines that possess significantly enhanced oil yields, providing immediate, valuable germplasm for accelerating sesame breeding programs and contributing essential data to optimize future mutation strategies aimed at improving the nutritional and economic value of this vital oilseed crop.

MATERIALS AND METHODS

Plant materials

Seeds of three sesame (*Sesamum indicum* L.) genotypes, Red sesame, White sesame, and 'Shandaweel3', were obtained from Ismailia Governorate, El-Sharkia Governorate, and the Field Crops Research Institute (FCRI) in Egypt, respectively. The selection of the studied genotypes was based on a combination of genetic diversity, agronomic relevance, and specific traits of interest relevant to productivity and fatty acid content.

Field trial

The trial was conducted at the Experimental Farm (30°35' N, 31°30' E) of the Faculty of Agriculture, Zagazig

University, Egypt, during the summer seasons of 2021, 2022, and 2023. The weather at the experimental site is hot and dry, with no rainfall during the sesame growing season. The soil was classified as clay containing 50.82% clay, 29.73% silt, and 19.45% sand. It was mildly alkaline and moderate salinity with pH of 7.78 and electrical conductivity (EC) of 1.75 ds m^{-1}. Mid-April is the recommended sowing period for sesame in the area; therefore, sowing was applied at this period during the two seasons. Three replicates of a randomized complete block design (RCBD) were used in the experiment. Three 4 m-long rows, separated by 70 cm and with 20 cm between plants within rows, were used for each genotype. Superphosphate (15.5% P_2O_5) was used to apply P fertilizer at 70 kg P_2O_5 ha^{-1} before sowing. After thinning, 110 kg K_2O ha^{-1} were applied as potassium sulfate (48% K_2O). Ammonium sulfate (20.5% N) was used to apply N fertilizer at 170 kg N ha^{-1} in three separate doses spaced 12 d apart, beginning soon after planting.

Mutagenesis and M_1 generation

In 2021, seeds from each genotype were subjected to chemical and physical mutagenesis. For chemical mutagenesis, 200 seeds per genotype were pre-soaked in distilled water for 12 h and then treated with sodium azide (NaN_3, SA) solutions at concentrations of 0.001 M, 0.002 M, and 0.003 M for 4 h under continuous shaking (120 rpm) at 18-24 °C. Following treatment, seeds were thoroughly rinsed with running tap water and air-dried. For physical mutagenesis, in June 2021, 200 seeds per genotype were irradiated with gamma rays using a radioisotope Co^{60} source (Gamma chamber Model-900 supplied by Nuclear Research Center, Inshas, Egypt) at doses of 100, 200, and 400 Gy. Exposure times were precisely calibrated to ensure accurate dosage. Treated and untreated (control) seeds were sown in triplicates to raise the M_1 generation.

M_2 Generation and phenotypic evaluation of M_3 generation

In the M_2 generation, a rigorous selection process was implemented to identify viable mutants/macromutants based on morphological characteristics, following the methodology described by Swaminathan (1965). Standard agricultural practices, including irrigation and weeding, were consistently applied throughout the growing seasons. In the M_3 generation, agronomic traits were measured to evaluate the performance of sesame genotypes. Days to maturity were recorded as the number of days from sowing until plants reached physiological maturity. At maturity, plant height was determined by measuring from the soil surface to the tip of the main stem on ten randomly selected plants per plot. The number of capsules per plant was counted on ten randomly chosen plants from each plot. Seeds per capsule were counted from ten randomly selected capsules per genotype in each plot. The 1000-seed weight was measured by weighing a representative sample of 1000 seeds. Seed weight per capsule was determined by weighing seeds extracted individually from ten randomly selected capsules from each plot. Seed yield per plant was calculated as the total seed weight harvested from ten randomly selected plants per replicate.

Analysis of seed oil and protein contents

The oil content (%) in seeds from selected M_3 mutants was determined using the Soxhlet extraction method with a Soxtec System-HT (1043). The Marguard method was used to etherify sesame oil (Marquard, 1987).

The micro-Kjeldahl method was used to estimate the total N content following the procedures provided by AOAC (2000). The total N value was then multiplied by a conversion factor of 6.25 to determine the total protein content (%).

Fatty acid composition analysis

Gas-liquid chromatography (GC) was utilized to determine the fatty acid composition of oil samples (1 mL) from selected M_3 mutants.

Briefly, each oil sample was mixed with 1 mL sodium methylate, and the mixture was left to react at room temperature overnight. Then, 0.25 mL isooctane was added and 0.5 µL aliquot of the resultant mixture was injected into Fison GC system equipped with a flame ionization detector (FID) and a fused capillary column (FFAP-DF, 25 m × 0.25 mm ID). The temperatures of the injector and detector were set at 250 and 260 °C, respectively. The temperature of the column was programmed to increase at a rate of 5 °C min^{-1} from 150 to 200 °C. At a steady pressure of 0.15 MPa and a flow rate of 1 mL min^{-1}, helium was employed as the carrier gas. Individual fatty acids were identified by comparing the peak areas and retention times with those of authentic standards (Sigma, St. Louis, Missouri, USA).

Statistical analysis

The R software (version 4.2.1) (R Foundation for Statistical Computing, Vienna, Austria). was used to perform all statistical analyses. The collected data were analyzed using ANOVA followed by the least significant difference (LSD) test at a significance level of $p \leq 0.05$ to assess significant differences among genotypes. Multivariate analysis techniques, including hierarchical clustering and principal component analysis (PCA), were performed to classify genotypes based on agronomic performance and to identify traits contributing to genotypic variation.

RESULTS

Characterization of M_2 populations

In the M_2 generation, the types of mutants observed across three sesame genotypes: Red sesame, White sesame, and 'Shandaweel3', are presented in Table 1. White sesame had the most mutants (46), followed by 'Shandaweel3' (27), and Red sesame (16) out of the 89 total that were found. High capsules number (33), early (29), and very early (10) were the most common mutant types in the M_2 population, whereas mono calm, dwarf, and semi dwarf were less common (4, 1, and 2, respectively). Ten mutants were found to have high seed weight. The overall number of M_2 mutants in each of the three sesame genotypes exposed to different concentrations of sodium azide and gamma rays is presented in Table 2. Considering gamma rays treatment, the 100 Gy dose produced the highest number of mutants (17), with White sesame had five and Red sesame and 'Shandaweel3' each had six. The number of mutants decreased with increasing gamma ray doses, reaching 14 at 200 Gy and 13 at 400 Gy.

The 0.001 M dose of sodium azide produced the most mutants (24), with White sesame exhibiting 16 mutants, 'Shandaweel3' had 7, and Red sesame had 1. With 11 mutants at 0.002 M (7 in White sesame, 3 in 'Shandaweel3', and 1 in Red sesame) and 10 mutants at 0.003 M (8 in White sesame and 2 in 'Shandaweel3', with no mutants in Red sesame), the number of mutants decreased with higher sodium azide doses (0.002 M and 0.003 M). Overall, it seemed that sodium azide treatment, especially at 0.001 M, was more successful than gamma rays at producing mutants in the M_2 generation.

Table 1. Number and types of mutants in the M_2 generation induced by gamma rays and sodium azide.

	Genotypes			
Types of mutants	Red sesame	White sesame	Shandaweel3	Total
Very early	-	3	7	10
Early	4	17	8	29
High capsules number	9	16	8	33
Mono calm	-	3	1	4
Dwarf	-	-	1	1
Semi dwarf	-	2	-	2
High seed weight	3	5	2	10
Total	16	46	27	89

Table 2. Number of mutants in the M_2 generation following treatment with different doses of gamma rays and sodium azide.

		Genotypes			
Mutagen	Dose	Red sesame	White sesame	Shandaweel3	Total
Gamma rays	100 Gy	6	5	6	17
	200 Gy	3	5	6	14
	400 Gy	5	5	3	13
Sodium azide	0.001 M	1	16	7	24
	0.002 M	1	7	3	11
	0.003 M	-	8	2	10
Total		16	46	27	89

Characterization of M_3 mutants

In the M_2 generation, a total of 14 mutants in addition to their three parents; White-Seed and Red-Seed ecotypes, and 'Shandaweel3', were selected for evaluation in the M_3 generation based on their morphological characteristics: Days to maturity, plant height, capsule number, seeds per capsule, seed weight, and seed yield. The evaluation of 17 sesame genotypes revealed substantial variation across morphological and agronomic traits in the M_3 generation. These differences reflect the efficacy of gamma irradiation and sodium azide treatments in generating genetic variability aimed at improving yield-related attributes.

Maturity and plant stature

Days to maturity displayed significant variation among the evaluated genotypes, ranging from 104.7 d in mutant VER1 to 125 d in EW11, VESh1, and ESh3. The mutants VER1, EW9, EW12, and EW3 exhibited early maturity (≤ 105 d), which is valuable for breeding programs targeting short-season and stress-prone environments (Figure 1A). In contrast, EW11, VESh1, and ESh3 recorded late maturity (125 d), which could provide the benefit of extended vegetative growth and greater biomass accumulation under favorable conditions. In addition, intermediate maturity genotypes such as ER10, HNCW8, 'Shandaweel3', VESh5, White-Seeds, and HNCW11 exhibited balance between earliness and delayed development.

The genotypes exhibited substantial variation in plant height, varied from 112.3 cm in White-Seeds to 293.7 cm in ER2. The genotypes White-Seeds, EW11, and HNCW8 were short-statured (< 145 cm) (Figure 1B). In contrast, tall genotypes such ER2, VESh1, Red-Seeds, HNCR4, ESh3, and EW9 (≥ 210 cm). Genotypes with intermediate plant height, such as VESh5, EW12, 'Shandaweel3', EW3, ESh4, ER10, and VER1 (160-185 cm).

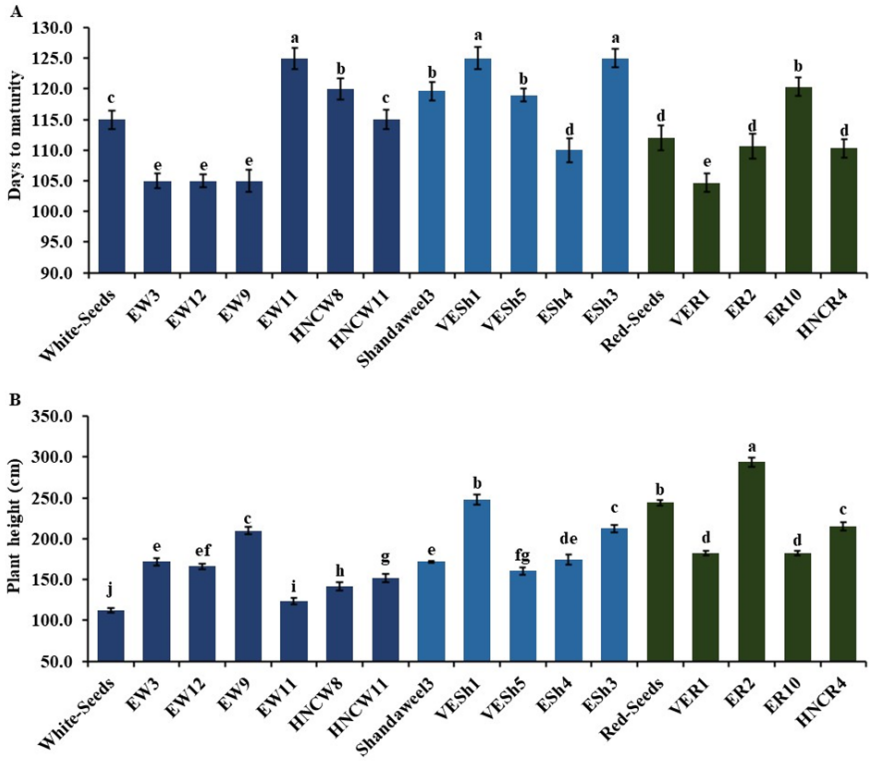

Figure 1. Comparative performance of days to maturity (A) and plant height (B) among the evaluated seventeen sesame genotypes. Dark blue columns represent the White-Seed ecotype and its derived mutants, light blue columns indicate mutants derived from 'Shandaweel3', and green columns correspond to the Red-Seed ecotype and its mutants. Error bars represent standard error, with different letters denoting significant differences (LSD test, $p \leq 0.05$).

Seed yield component

Considerable differences were observed in the number of capsules per plant, ranging from 22.67 in White-Seeds control to 129.3 in the mutant VESh1. The mutants VESh1, HNCW8, ER2, ESh4, VER1, EW9, and ER10 recorded high capsule numbers (≥ 85) (Figure 2A). On the other hand, White-Seeds, EW12, EW11, 'Shandaweel3', and HNCW11 exhibited reduced capsule formation (≤ 48). Intermediate capsule genotypes, including ESh3, Red-Seeds, VESh5, EW3, and HNCR4 (60-75 capsules). The number of seeds per capsule varied significantly among the assessed genotypes, ranged from 20.33 in Red-Seeds to 50.67 in EW12. The genotypes EW12, HNCW11, ESh4, White-Seeds, VESh1, HNCR4, and ER2 showed strong seed setting ability (≥ 44 seeds) (Figure 2B). In contrast, lower seed counts were recorded in Red-Seeds, VER1, EW9, ESh3, VESh5, and HNCW8 (≤ 30). Genotypes with intermediate seed numbers, including EW3, ER10, 'Shandaweel3', and EW11 (36-40 seeds). The 1000-seed weight ranged from 2.56 g in ER10 to 4.93 g in the mutant ESh4. Genotypes ESh4, Red-Seeds, VESh1, ESh3, ER2, and VER1 showed the highest seed index (≥ 4.0 g) (Figure 2C). In contrast, lower seed weights were observed in ER10, EW12, VESh5, and EW3 (2.5-3.0 g). Intermediate genotypes, EW11, White-Seeds, HNCW8, EW9, 'Shandaweel3', HNCW11, and HNCR4 (3.1-3.8 g).

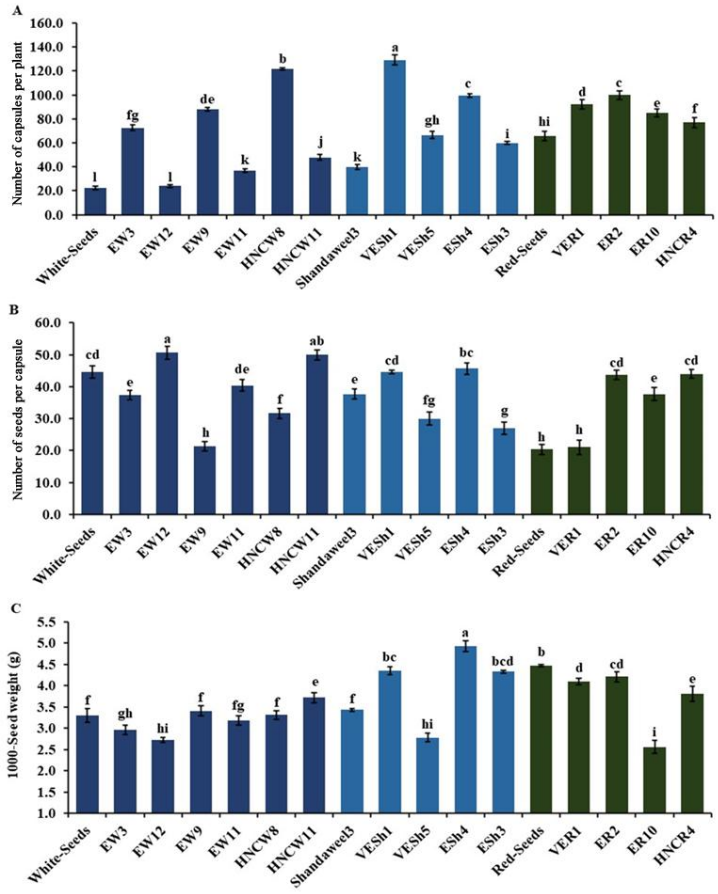

Figure 2. Comparative performance of number of capsules per plant (A), number of seeds per capsule (B) and 1000-seed weight (C) among the evaluated seventeen sesame genotypes. Dark blue columns represent the White-Seed ecotype and its derived mutants, light blue columns indicate mutants derived from 'Shandaweel3', and green columns correspond to the Red-Seed ecotype and its mutants. Error bars represent standard error, with different letters denoting significant differences (LSD test, $p \leq 0.05$).

Seed weight per capsule and plant

Seed weight per capsule ranged from 63.97 mg in EW9 to 201.73 mg in ESh4, displaying significant genotypic variation in seed development. The mutants ESh4, VESh1, HNCR4, HNCW11, and ER2 (≥ 155 mg) exhibited the highest seed weight per capsule (Figure 3A). In contrast, EW9, VESh5, Red-Seeds, VER1, EW3, ER10, and ESh3 had relatively low seed weights (< 105 mg). Moderate seed weight genotypes such as HNCW8, EW12, White-Seeds, EW11, and 'Shandaweel3' (116-144 mg).

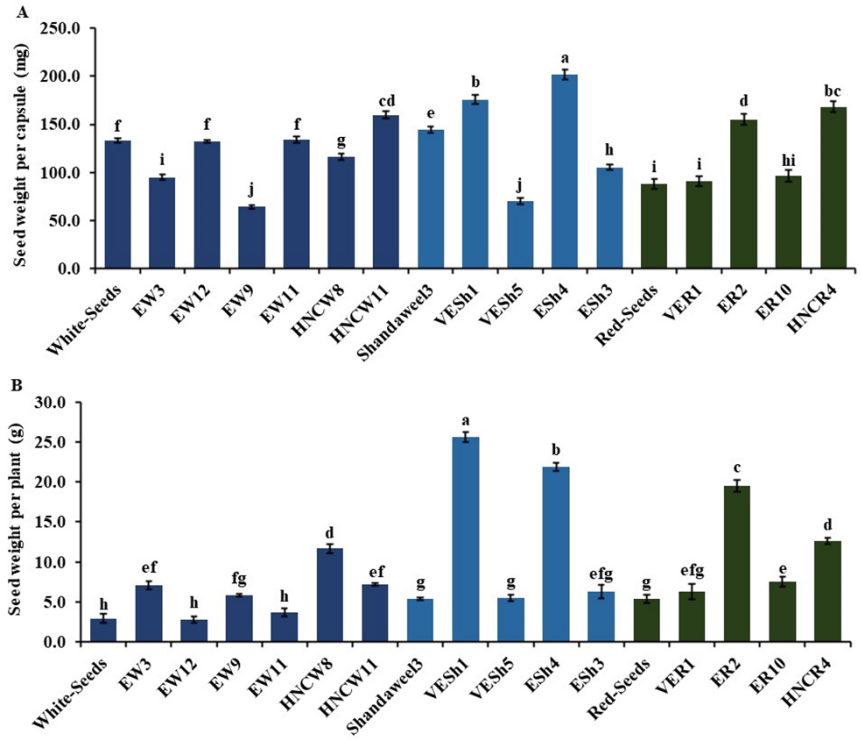

Figure 3. Comparative performance of seed weight per capsule (A) and seed weight per plant (B) among the evaluated seventeen sesame genotypes. Dark blue columns represent the White-Seed ecotype and its derived mutants, light blue columns indicate mutants derived from 'Shandaweel3', and green columns correspond to the Red-Seed ecotype and its mutants. Error bars represent standard error, with different letters denoting significant differences (LSD test, $p \leq 0.05$).

Seed yield per plant

Seed yield per plant ranged from 2.77 g in EW12 to 25.63 g in the top-yielding mutant VESh1. The genotypes VESh1, ESh4, ER2, HNCR4, and HNCW8 recorded the highest seed yield per plant (11.66-25.63 g) as the most promising candidates for yield improvement (Figure 3B). In contrast, EW3, White-Seeds, and EW11 (2.77-3.73 g) produced significantly less, highlighting limitations in reproductive efficiency. Genotypes with intermediate yield performance, including 'Shandaweel3', Red-Seeds, VESh5, EW9, ESh3, VER1, EW3, HNCW11, and ER10 (5.37-7.50 g), showed good yield potential and could be employed as stable donors for yield-related traits in future improvement programs.

Oil and protein content

Significant variation was detected among the 17 sesame genotypes for both oil and protein content (Figure 4). Oil content ranged from 21.7% in VESh5 to 47.5% in EW12, while protein content varied from 6.9% in EW3 to 32.6% in VESh5. The mutants EW12, HNCR4, VESh1, and ER2 exhibited the highest oil content (38.4%-47.5%),

significantly surpassing all other genotypes (Figure 4A). The genotypes EW11, 'Shandaweel3', ESh3, EW3, VER1, HNCW11, EW9, and HNCW8 showed moderate oil content values (32.5%-35.6%). Whereas VESh5, ER10, White-Seeds, Red-Seeds, and ESh4 displayed the lowest oil content (21.7%-32.1%).

Protein content showed significant genotypic differences (Figure 4B). The highest protein content was detected in VESh5 (32.6%), followed by HNCR4 (26%), ESh4 (20.3%), VER1 (19.1%), and 'Shandaweel3' (18.5%). Several genotypes displayed intermediate protein content (13.8%-17.2%), including EW9, ER2, VESh1, EW11, ESh3, White-Seeds and HNCW8. In contrast, EW3, EW12, HNCW11, Red-Seeds, and ER10 exhibited the lowest protein content (6.9%-10.3%).

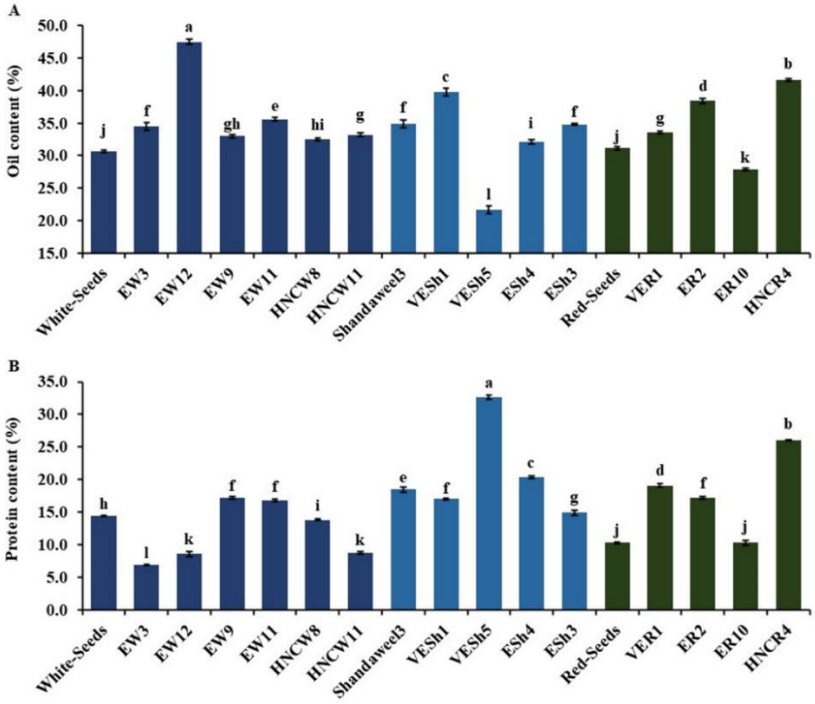

Figure 4. Comparative performance of oil content (A) and protein content (B) among the evaluated seventeen sesame genotypes. Dark blue columns represent the White-Seed ecotype and its derived mutants, light blue columns indicate mutants derived from 'Shandaweel3', and green columns correspond to the Red-Seed ecotype and its mutants. Error bars represent standard error, with different letters denoting significant differences (LSD test, $p \leq 0.05$).

Genotypic classification

The cluster dendrogram based on the evaluated traits classified the 17 sesame genotypes into three major clusters, reflecting genetic and phenotypic dissimilarities (Figure 5). Cluster A included four genotypes (VESh1, ER2, ESh4, and HNCR4) that are grouped due to their high agronomic and seed quality performance. These genotypes are characterized by superior agronomic traits such as high seed yield per plant, improved oil content, increased number of capsules per plant, and enhanced seed weight parameters. These genotypes demonstrated consistent excellence across multiple yield and quality-related traits. Cluster B included five genotypes, which are EW12, EW11, 'Shandaweel3', White-Seeds, and HNCW11. These genotypes exhibited moderate performance across several agronomic traits. Cluster C contained eight genotypes: VESh5, EW3, HNCW8, ER10, EW9, VER1, ESh3, Red-Seeds. This cluster was characterized by lower to moderate performance in most productivity-related traits.

The heatmap combined with hierarchical clustering presented in Figure 6 shows the detected variation among the 17 genotypes based on their performance in studied traits: Days to maturity, number of seeds per

capsule, seed weight per capsule, seed weight per plant, number of capsules per plant, plant height, and 1000-seed weight. The color from red (low values) to blue (high values) indicates the relative performance of each genotype across these traits. Cluster 1 included the mutants VESh1, ER2, ESh4, and HNCR4 that exhibit high performance for most productivity-related traits. This group represents elite, high-yielding genotypes. Other genotypes, such as EW3, VER1, EW9, Red-Seeds, and ESh3, exhibited more red, indicating lower values for the evaluated traits.

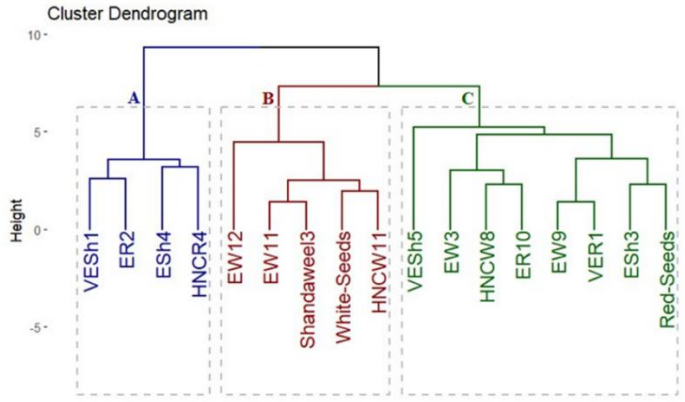

Figure 5. Dendrogram illustrates the genetic relationships among the evaluated 17 sesame genotypes, grouping them into three major clusters A, B, and C according to their agronomic and seed quality performance.

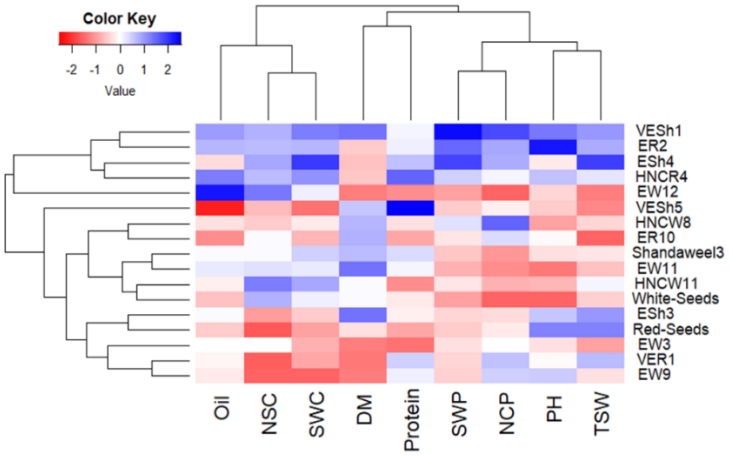

Figure 6. Heatmap and clustering of sesame genotypes based on studied traits. DM: Days to maturity; Oil: oil content (%); Protein: protein content; NSC: number of seeds per capsule; SWC: seed weight per capsule; SWP: seed weight per plant; NCP: number of capsules per plant; PH: plant height; and TSW: 1000-seed weight.

Principal component analysis

The PCA biplot summarizes the variability among the 17 sesame genotypes based on studied traits (Figure 7). The first two principal components accounted for 58.25% of the total variation. The genotypes with high performance, such as VESh1, ER2, ESh4, and HNCR4, were presented on the positive side of PC1 and associated with high values for seed weight per plant, 1000-seed weight, number of capsules per plant, and oil content. In contrast, VESh5, EW9, VER1, and Red-Seeds were located in the lower left quadrant. Trait vectors reveal strong

positive correlations among plant height, number of capsules per plant, 1000-seed weight, and seed weight per plant, which are the primary contributors to genotypic differentiation along PC1. The length of the trait vectors suggests that seed weight per plant, number of capsules per plant, seed weight per capsule, number of seeds per capsule and 1000-seed weight are major discriminating factors in genotype differentiation.

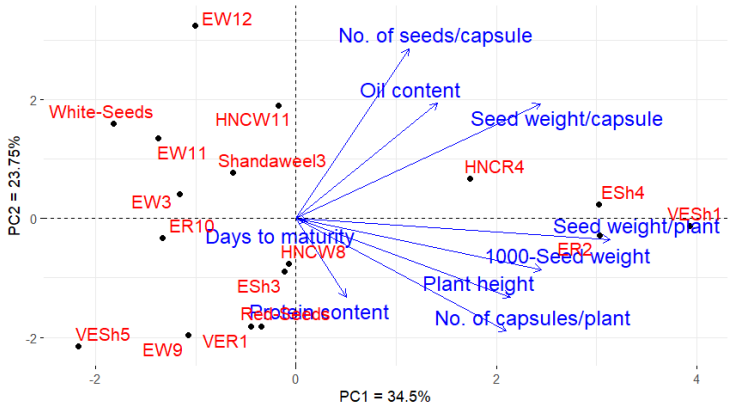

Figure 7. Biplot of principal component analysis revealing genotypic variation of the assessed seventeen sesame genotypes and associations of studied traits.

Fatty acid composition analysis

The fatty acid compositions of three sesame genotypes (Red seeds, White seeds, and 'Shandaweel3') and their respective five promising mutants (ER2, HNCR4, EW12, HNCW8, and VESh1) were evaluated. Eight fatty acids were detected: Palmitic acid (C16:0), palmitoleic acid (C16:1), linoleic acid (C18:2), arachidic acid (C20:0), gondoic acid (C20:1), behenic acid (C22:0), lignoceric acid (C24:0), and cerotic acid (C26:0). These findings are detailed in Table 3 and Figure 8.

Table 3. Fatty acid content of selected sesame mutant lines and their parents. [ND]Not detected.

Genotypes	Palmitic C16:0	Palmitoleic C16:1	Linoleic C18:2	Arachidic C20:0	Gondoic C20:1	Behenic C22:0	Lignoceric C24:0	Cerotic C26:0
				%				
Red seeds (Control)	15.062	ND	60.269	1.432	0.588	0.289	ND	ND
ER2	14.143	0.374	5.345	1.446	0.651	0.279	0.185	ND
HNCR4	14.716	0.295	70.302	1.304	0.649	0.229	0.166	ND
White seeds (Control)	14.441	0.137	60.503	1.375	0.579	0.257	0.166	ND
EW12	13.390	0.340	8.785	1.431	0.622	0.307	0.216	ND
HNCW8	13.025	0.277	5.531	1.329	0.642	0.322	0.226	0.043
Shandaweel3 (Control)	14.378	0.416	5.3667	1.459	0.750	0.296	0.209	0.053
VESh1	13.833	0.329	5.897	1.239	0.610	0.279	0.146	ND

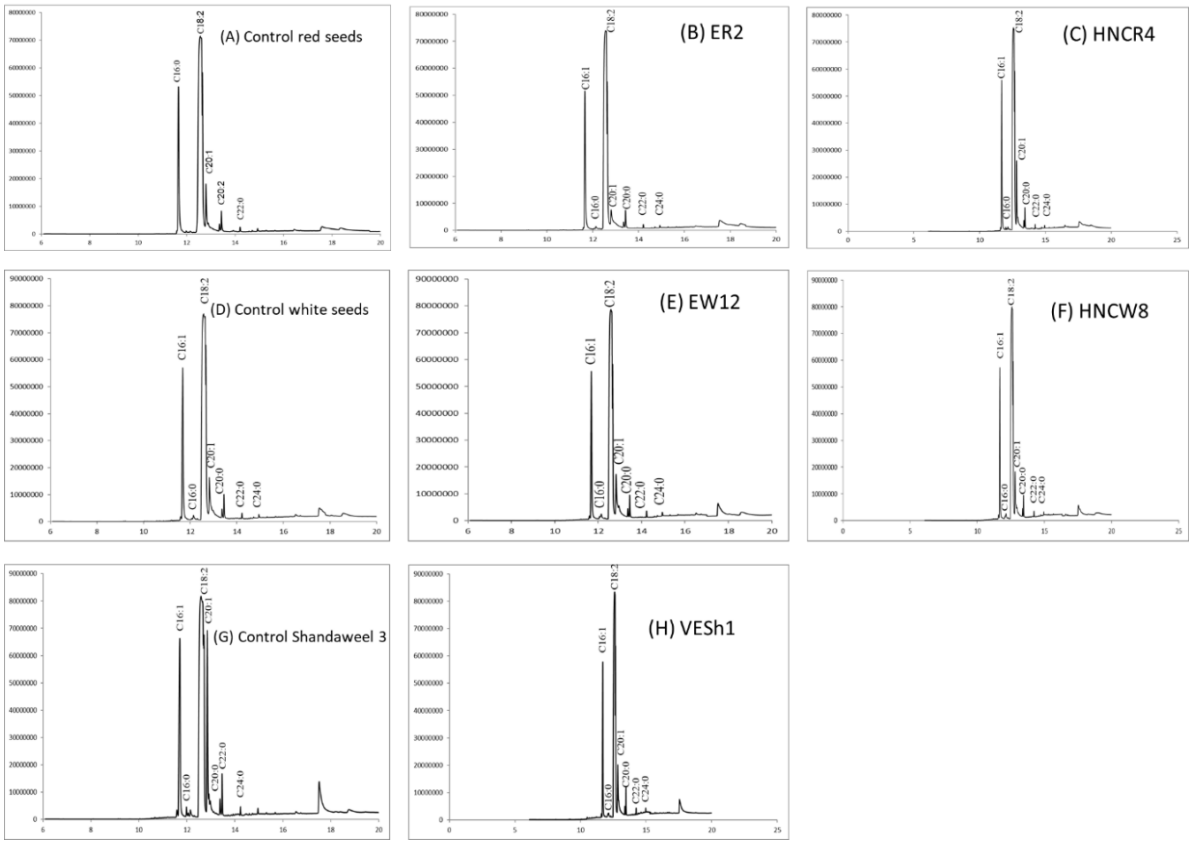

Figure 8. Gas chromatograms illustrating the fatty acid profiles of three sesame genotypes (Red seeds, White seeds, and 'Shandaweel3') and their respective mutants (ER2, HNCR4, EW12, HNCW8, and VESh1). The labeled peaks indicate the retention times of different fatty acids present in the seed oil.

The Red seeds genotype had high linoleic acid content (60.27%) followed by palmitic acid (15.06%). Arachidic, gondoic, and behenic acids were presented in smaller amounts (1.43%, 0.59%, and 0.29%, respectively), while palmitoleic, lignoceric, and cerotic acids were not detected. In contrast, ER2 mutant showed significantly lower linoleic acid content (5.34%) and palmitic acid (14.14%). This mutant uniquely contained palmitoleic acid (0.37%) and lignoceric acid (0.18%), which were absent in the parent Red seeds genotype. Arachidic (1.45%), gondoic (0.65%), and behenic (0.28%) acids were also present in the ER2 mutant. HNCR4 genotype demonstrated the highest linoleic acid content among all genotypes with 70.30% palmitic acid at 14.72%. Similar to ER2 mutant, HNCR4 also contained palmitoleic acid (0.30%) and lignoceric acid (0.17%), along with arachidic (1.30%), gondoic (0.65%), and behenic (0.23%) acids. Cerotic acid was consistently absent in the Red seeds genotype and its respective mutants.

The White seeds genotype compared its mutants exhibited a high content of linoleic acid at 60.5029%, followed by palmitic acid at 14.4409%. Other fatty acids present included arachidic at 1.3754%, gondoic at 0.5794%, behenic at 0.2573%, palmitoleic at 0.1372%, and lignoceric at 0.1657%. Cerotic acid was not detected in the control. In contrast, the EW12 genotype showed a significantly lower linoleic acid content of 8.7852% and palmitic acid at 13.3902%. This genotype also contained palmitoleic acid (0.3402%), arachidic acid (1.4308%), gondoic acid (0.6216%), behenic acid (0.3071%), and lignoceric acid (0.2157%), but like the control, lacked cerotic acid. The HNCW8 genotype presented the lowest linoleic acid content at 5.5306%, with palmitic acid at 13.0252%. This genotype, however, uniquely contained cerotic acid (0.0426%), which was absent in the other two genotypes. Palmitoleic acid (0.2765%), arachidic acid (1.3299%), gondoic acid (0.6423%), behenic acid (0.3216%), and lignoceric acid (0.226%) were also observed in HNCW8.

The analysis of fatty acid profiles for the 'Shandaweel3' and its mutant VESh1, revealed distinct compositions. 'Shandaweel3' exhibited a palmitic acid content of 14.3778%, palmitoleic acid at 0.4157%, and linoleic acid at 5.3667%. It also contained arachidic at 1.4591%, gondoic at 0.7503%, behenic at 0.2959%, lignoceric at 0.2095%, and cerotic acid at 0.0526%. In contrast, the VESh1 mutant showed slightly lower levels for most detected fatty acids compared to its parent: Palmitic acid was 13.8328%, palmitoleic acid 0.3294%, and linoleic acid 5.8971%. Arachidic acid was present at 1.2385%, gondoic acid at 0.6104%, behenic acid at 0.2786%, and lignoceric acid at 0.1459%. notably, cerotic acid was not detected in the VESh1 genotype.

DISCUSSION

Mutation breeding is a powerful tool for generating novel genetic variation, essential for overcoming productivity constraints in sesame cultivation. Enhancing genetic variability through induced mutations facilitates the development of superior cultivars with improved adaptability and yield stability across diverse environmental conditions. This study demonstrated significant genetic diversity among the evaluated sesame genotypes induced by gamma irradiation and sodium azide, indicating the efficacy of these mutagenic treatments in generating beneficial variability for breeding objectives. This genetic variability is essential for selecting and enhancing desirable yield and quality traits in sesame, which aligns with previous research revealing mutation breeding as an effective method to increase genetic diversity in crops (Al Mamun et al., 2025; Shahnaz et al., 2025).

The observed variability in days to maturity provides valuable insights for breeding programs targeting diverse agroecological niches. Early-maturing mutants such as VER1, EW9, EW12, and EW3 exhibited potential for utilization in regions with short growing seasons or limited water availability. Conversely, late-maturing genotypes, including EW11, VESh1, and ESh3, could exploit extended growth periods, potentially enhancing biomass and yield under optimal environmental conditions. These findings are consistent with studies of Kettani et al. (2024) and Sanni et al. (2025), who elucidated the importance of selecting genotypes adapted to specific environmental contexts. Significant variations in plant height highlight the potential for different management practices, particularly mechanized harvesting and high-density planting systems. Short-statured genotypes White-Seeds, EW11, and HNCW8 exhibited suitability for mechanization and intensive cultivation, which aligns with modern agricultural practices aiming for labor reduction and yield optimization (Sivakumar et al., 2024). On the other hand, taller genotypes, including ER2, VESh1, Red-Seeds, HNCR4, ESh3, and EW9, demonstrated the potential for higher yield through increased biomass and capsule-bearing nodes, corroborating earlier findings that plant height directly influences yield capacity in sesame (Raut et al., 2020).

The number of capsules per plant is a critical yield determinant, and genotypes such as VESh1, ER2, ESh4, and HNCR4 showed superior performance. These genotypes could serve as vital resources for breeding high-yielding sesame cultivars. Previous studies have similarly reported a strong correlation between capsule number and seed yield, representing the importance of capsule formation efficiency in sesame breeding (Wang et al., 2024). Variations observed in seeds per capsule and seed weight traits reveal their significance in enhancing sesame productivity. Mutants ESh4, VESh1, and ER2 consistently displayed high seed set and seed index, indicative of their potential for high-yielding and oil-rich sesame production. These traits align with previous findings that seed size and seed number per capsule are critical yield components in sesame breeding strategies (Sravanthi et al., 2021). The superior performance of mutants VESh1, ESh4, ER2, and HNCR4 in seed yield demonstrated their suitability for yield-focused breeding programs. These genotypes exhibited enhanced seed-filling capacity and significantly greater yield potential compared to the other mutants. Such outcomes confirm earlier studies revealing the importance of identifying high-yield mutants through mutation breeding for crop improvement (Francis and Selvaraj, 2024).

The evaluation of oil and protein content among the 17 sesame genotypes indicated significant genetic variability, which is essential for breeding programs targeting seed quality improvement. The observed range of oil content (21.7% to 47.5%) and protein content (6.9% to 32.6%) revealed the impact of induced mutagenesis in generating novel allelic combinations that influence seed composition traits. The results are in agreement with earlier findings that highlight the importance of exploiting induced mutations to enhance seed quality traits in sesame (Wang et al., 2024). The highest oil content was recorded in the mutant genotype EW12 (47.5%), which significantly surpassed all other genotypes, while the lowest was observed in VESh5 (21.67%).

This range is consistent with previous reports that highlight the effectiveness of mutation breeding in expanding the variations of oil content in sesame (Jayaramachandran et al., 2020). The superior oil yield of EW12 suggests its potential as a donor parent in breeding programs focused on enhancing oil productivity. Protein content also exhibited considerable variation, with VESh5 (32.6%) and HNCR4 (26.0%) as the top performers. The inverse relationship observed between oil and protein content in certain genotypes, such as EW12 (high oil, low protein) and VESh5 (low oil, high protein), aligns with the negative correlation between these two traits in oilseed crops (Hossain et al., 2019; Kambhampati et al., 2019). This trade-off is attributed to the competition for C and N resources during seed development, which is regulated by both genetic and environmental factors. The identification of genotypes with both moderate oil and protein content, such as EW11, EW9, and ER2, is particularly valuable. These genotypes offer a balanced nutritional profile and may serve as stable sources for developing cultivars with improved seed quality and adaptability.

Hierarchical cluster and heatmap visualization separated the genotypes into distinct groups based on their agronomic and seed quality performance. Distinct clustering patterns reflect the genetic variability induced by gamma irradiation and sodium azide treatments. The mutants VESh1, ER2, ESh4, and HNCR4 were characterized by consistently high values for most productivity-related traits, marking these genotypes as elite candidates for yield improvement. The genotypes EW12, EW11, 'Shandaweel3', White-Seeds, HNCW11 displayed moderate trait values, suggesting these lines possess balanced agronomic profiles suitable for broader adaptation. In contrast, VESh5, EW3, HNCW8, ER10, EW9, VER1, ESh3, Red-Seeds were associated with lower to moderate performance, indicating potential utility in stress environments or as sources of specific traits for future breeding efforts. The PCA biplot further elucidated the structure of genetic diversity and trait associations among the genotypes. The first two principal components (PC1 and PC2) accounted for 58.25% of the total phenotypic variance, underscoring the effectiveness of these axes in summarizing the major sources of variation within the population. High-yielding genotypes such as VESh1, ER2, ESh4, and HNCR4 were distinctly separated along the positive axis of PC1, in close association with vectors representing seed weight per plant, 1000-Seed weight, number of capsules per plant, and oil content. This distribution indicates that these traits are primary drivers of genotypic differentiation and superior agronomic and seed quality performance. Conversely, genotypes such as VESh5, EW9, VER1, and Red-Seeds, positioned in the lower left quadrant, were characterized by generally lower performance across most productive traits, suggesting limited yield potential but possible suitability for specific environments. Using multivariate such as hierarchical clustering, heatmap visualization, and principal component analysis are consistent with studies of Gaballah et al. (2024), Dhillon et al. (2025), and Galal et al. (2025) elucidated the power of multivariate approaches in dissecting genetic diversity and facilitating targeted selection in crop improvement programs.

The evaluation of fatty acid compositions in three sesame genotypes (Red seeds, White seeds, and 'Shandaweel3') and their five respective mutants (ER2, HNCR4, EW12, HNCW8, and VESh1) revealed significant variations in the profiles of eight key fatty acids: Palmitic, palmitoleic, linoleic, arachidic, gondoic, behenic, lignoceric, and cerotic acids. These findings highlight the impact of induced mutations on lipid biosynthesis pathways in sesame.

For the Red seeds genotype and its mutants (ER2, HNCR4), the parent Red seeds exhibited a high content of linoleic acid (60.27%) and palmitic acid (15.06%), with minor amounts of arachidic, gondoic, and behenic acids. Notably, palmitoleic, lignoceric, and cerotic acids were absent. The ER2 mutant; however, demonstrated a drastic reduction in linoleic acid (5.34%) and a slight decrease in palmitic acid (14.14%). This mutant uniquely synthesized palmitoleic (0.37%) and lignoceric (0.18%) acids, indicating a successful alteration in fatty acid desaturation and elongation pathways. Conversely, the HNCR4 mutant showed the highest linoleic acid content among all genotypes (70.30%) while maintaining a similar palmitic acid level (14.72%). Similar to ER2, HNCR4 also acquired the ability to produce palmitoleic (0.30%) and lignoceric (0.17%) acids. The consistent absence of cerotic acid in the Red seeds genotype and its mutants suggests a conserved genetic block in the very-long-chain fatty acid synthesis beyond C24. These results suggest that mutations can significantly modulate the expression of genes involved in fatty acid biosynthesis, leading to both quantitative shifts in major fatty acids and the qualitative appearance of new fatty acids (Ju et al., 2021).

In the case of the White seeds genotype and its mutants (EW12, HNCW8), the parent White seeds displayed a high linoleic acid content (60.50%) comparable to Red seeds, along with palmitic acid (14.44%) and detectable levels of palmitoleic, arachidic, gondoic, behenic, and lignoceric acids. Cerotic acid was absent. The EW12 mutant exhibited a substantial reduction in linoleic acid (8.79%) and a decrease in palmitic acid (13.39%), similar

to the trend observed with ER2. However, it maintained the presence of other fatty acids found in the parent, with slight variations in their proportions. The HNCW8 mutant presented the most significant reduction in linoleic acid (5.53%) and palmitic acid (13.03%) among the White seed-derived lines. Intriguingly, HNCW8 uniquely developed the capacity to synthesize cerotic acid (0.04%), a fatty acid absent in its parent and the EW12 mutant. This highlights the potential of induced mutagenesis to activate or de-repress pathways for very-long-chain fatty acid synthesis (Nath and Chan, 2016).

Finally, the comparison between the 'Shandaweel3' genotype and its mutant VESh1 also revealed noteworthy changes. 'Shandaweel3' exhibited a relatively lower linoleic acid content (5.37%) compared to the Red and White seed controls, with palmitic acid at 14.38%. It also contained palmitoleic, arachidic, gondoic, behenic, lignoceric, and cerotic acids (0.05%). The VESh1 mutant generally showed slightly reduced levels for most detected fatty acids compared to its parent, including palmitic (13.83%), palmitoleic (0.33%), and linoleic acids (5.90%). However, a key observation was the complete absence of cerotic acid in the VESh1 mutant, indicating a loss or suppression of the genetic mechanism responsible for its synthesis, a stark contrast to its presence in the 'Shandaweel3' parent. This suggests that induced mutations can lead to either the gain or loss of specific fatty acid production, underscoring the unpredictable yet impactful nature of mutagenesis in altering metabolic pathways (Wang et al., 2023).

CONCLUSIONS

This study demonstrated that gamma irradiation and sodium azide treatments are effective techniques for inducing valuable genetic variability in sesame. Significant variations were observed among the evaluated genotypes in agronomic traits, revealing the potential of selected mutants for targeted breeding. Genotypes such as VESh1, ER2, ESh4, and HNCR4 could be considered as superior candidates due to their enhanced agronomic performance. The substantial variability in oil and protein content among the evaluated sesame genotypes demonstrates the potential of mutation breeding for improving seed quality. Moreover, the mutants EW12, VESh5, and HNCR4 represent promising genetic resources for the development of high-value sesame cultivars to meet specific nutritional and industrial needs. Mutants such as EW12 (high oil) and VESh5 (high protein) demonstrate the potential for targeted breeding, while those with balanced profiles (EW11, EW9, ER2) are promising candidates for developing cultivars with improved nutritional quality and adaptability. Fatty acid composition analysis demonstrate that induced mutagenesis in sesame is an effective tool for modifying fatty acid profiles, leading to significant changes in lipid composition. These changes include substantial alterations in the proportions of major fatty acids like linoleic and palmitic acids, as well as the appearance or disappearance of minor fatty acids such as palmitoleic, lignoceric, and cerotic acids. Such modifications have significant implications for the nutritional and industrial applications of sesame oil, offering avenues for developing new cultivars with tailored fatty acid compositions.

Authors contribution

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Acknowledgements

The authors extend their appreciation for the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number RGP2/279/46. Also, the authors extend their appreciation for the Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2026R366), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia, and the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Grant No. KFU254094).

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