



Research article

Treatment of seeds with sodium azide for quantitative and qualitative capsule traits at M2 generation of Fourteen Ethiopian sesame (*Sesamum indicum* L.) genotypes

Micheale Yifter Weldemichael^{a,*}, Yemane Tsehaye Baryatsion^b,
Desta Berhe Sbhatu^c, Girmay Gebresamuel Abraha^d, Hagos Mohammedseid Juhar^a,
Abraha Birhan Kassa^e, Fiseha Baraki Sibhatu^f, Hailay Mehari Gebremedhn^a,
Mohammed Mebrahtu Mossa^b, Mullubrhan Mekonen Gebru^a,
Birhanu Kahsay Meresa^a, Medhin Teklay^g, Birhanu Debesay Berhe^g,
Haftay Abadi Gebru^g

^a Department of Biotechnology, CDANR, Mekelle University, P.O. Box 231, Mekelle, Tigray, Ethiopia

^b Department of DCHS, CDANR, Mekelle University, P.O. Box 231, Mekelle, Tigray, Ethiopia

^c Department of BCEN, MIT, Mekelle University, P.O. Box 231, Mekelle, Tigray, Ethiopia

^d Department of LaRMEP, CDANR, Mekelle University, P.O. Box 231, Mekelle, Tigray, Ethiopia

^e Department of Chemistry, CNCS, Mekelle University, P.O. Box 231, Mekelle, Tigray, Ethiopia

^f Tigray Agricultural Research Institute, HuARC, P.O. Box 510, Humera, Tigray, Ethiopia

^g Tigray Biotechnology Center Pvt. Ltd. Co., P.O. Box 223, Mekelle, Tigray, Ethiopia



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ABSTRACT

This study aimed at investigating the effects of sodium azide (NaN_3) on quantitative and qualitative capsule traits in M2 generation of 14 Ethiopian sesame genotypes collected from Humera Agricultural Research Center (HuARC), Tigray. Both the treatment and control seeds were sown in well-prepared beds in greenhouse to develop M2 plants. Data on quantitative and qualitative traits were collected and analyzed using GenStat 16 software. Results showed significant differences among the M2 seeds treated with 0.75% NaN_3 . The highest mean number of capsules per plant was recorded in ACC44 and Baha Necho genotypes, while the lowest was recorded in Gumero, Setit 2, Hirhir, ADI, Bounji and Aberghele. The highest mean number of seeds per capsule was recorded in Humera 1, Baha Necho, Zeri Tesfay, and Gondar 1 genotypes and the lowest was recorded in Setit 1, Setit 2 and ADI. The highest mean capsule length was observed in Zeri Tesfay while the lowest was recorded in Aberghele. The qualitative data reported that Hirhir, Setit 1 and Setit 2 were changed from completely shattering to partially shattering, Gumero and Bounji were changed from completely shattering to non-shattering, and Zeri Tesfay was changed from partially shattering to non-shattering. The 14 genotypes were clustered into four distinct groups including cluster I containing six genotypes, cluster II and III containing two genotypes each and cluster IV containing four genotypes. The mutants developed from Zeri Tesfay, ACC44 and Baha Necho genotypes are considered as potential candidate mutants for further utilization in sesame improvement.

* Corresponding author.

E-mail addresses: y.mickye@gmail.com, micheale.yifter1@mu.edu.et (M.Y. Weldemichael).

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1. Introduction

Sesame (*Sesamum indicum* L.; $2n = 2 \times = 26$) is an oilseed crop belonging to the Pedaliaceae family that is famous for high-quality oil [1,2]. Sesame grain is traditionally used as main ingredients in preparing food, pharmaceutical, cosmetic and personal care products such as antibacterial mouthwash and perfumes [3]. The seeds are rich in both protein and oil, where the oil has a high demand in the global confectionary industry. Besides, the sesamin and sesaminol lignans of sesame fat contribute to the oxidative stability and antioxidative activities of sesame oil [4]. In addition, it has crucial benefit to human health [5] as its oil is characterized by a low level of saturated fatty acids (less than 15%). It may also provide relief from hypertension, oxidative stress and neurodegenerative diseases [6].

Sesame remained to be one of the principal economic and livelihood crops in Ethiopia since long ago. Despite its nutritional, pharmaceutical, industrial and economical importance to Ethiopia, sesame is challenged with many critical problems [7-10]. It remains to be an orphan crop receiving very little support from research and development institutes, industry and policy-makers as genetic improvement is concerned [11]. The huge fluctuations in its production and productivity are due to various biotic and abiotic factors that discourage large and small-scale producers and investors [12]. Despite many sesame collections including local landraces and wild relatives, improved breeding programs using conventional breeding are limited and did not bring about notable progress in solving the critical biological and agronomic problems of the crop.

Being one of the most significant concepts of modern biology, mutation deals with the alteration of genetic characters of any organism. Mutation breeding has, hence, become more popular and an effective tool for the production of improved and superior varieties of crops with better yield, resistance to diseases and pests, tolerance to abiotic stresses, grain quality and nutritional composition [13]. It is a handy strategy proved to achieve rapid genetic changes that bring about additional variability in the qualitative and quantitative traits and supplement existing germplasm for cultivar improvement in breeding programs [14-17]. It was successfully employed for creating additional heritable variability in many crops including maize [18], rice [15], sesame [14,19], coffee [20], tomato [21] and brassica [22].

Since its establishment, mutation breeding is more preferred because it shortens the time for the development of new cultivars via induced mutations as compared to hybridizations [23]. However, the crop has a lot of problems when it is subjected for breeding. Its germplasm is not as large as other crops [24]. The crop's morphology is poorly adapted to modern farming systems because of its indeterminate growth habit, sensitivity to wilting under intensive management, and seed shattering at maturity [25-27]. Moreover, the yield of the crop depends on several traits such as number of seeds per capsule, number of capsules per plant, seed weight, capsule dimensions, height of the first capsule axis, branching type, capsule shattering and plant growth habit as well as management practices and biotic and abiotic factors [28-30]. Although many mutations breeding studies have been conducted using radiation and chemical mutagens to improve yield and many other qualitative and quantitative traits [26,31-35], studies on growth habits, maturity, capsule number, number of seeds per capsule, capsule length, coat color and shattering habits are still limited. Hence, there was an urgent need to address these problems by using NaN_3 .

Sodium azide (NaN_3) has been found to be one of the most powerful chemical mutagens, the mutagenicity of which is mediated through the production of an organic metabolite of azide compound that enters into the nucleus, interacts with DNA and creates a point mutation in the genome [36]. It affects the different parts of the plant and their development phenomena by distributing the metabolic activity. Therefore, the present study was initiated to investigate the effect of NaN_3 on quantitative and qualitative capsule traits in M2 generation of 14 Ethiopian Sesame genotypes.

2. Materials and methods

2.1. Collection of seeds and pre-treatment handling

The study was carried out at Tigray Biotechnology Center, Pvt. Ltd. Co., which is located in Mekelle City, northern Ethiopia (Lat.: $13^\circ 30' 0''$ N; Long.: $39^\circ 28' 11''$ E; Alt. 2,080 masl). The entire duration of the experiment was from February 2019 to July 2019 starting from seed collection, chemical purchasing, laboratory work up to harvesting. However, the time taken from sowing to harvesting of the M1 seeds was four months. Once, the M1 seeds were obtained, the seeds were further advanced to obtain the M2 seeds, which took similar duration (four months) and all the genotypes had the same duration. Seeds of 14 sesame genotypes (45 seeds each), namely Aberghele, ACC44, ADI, Baha Necho, Borkena, Baha Zeyit, Bounji, Gondar 1, Gumero, Hirhir, Humera 1, Setit 1, Setit 2 and Zeri Tesfay were used in this study. These seeds were collected from HuARC, Western Tigray (Ethiopia). The seeds were disease-free, normal shaped, dry and quiescent. The protocols used by Weldemichael et al. [19], Mensah et al. [32]; Herwibawa et al. [37]; Smith et al. [38] were used for seed sterilization. Following this, the seeds were soaked in sterile water for 16 h.

2.2. Chemical treatment

Different concentrations of sodium azide (0.25%, 0.5%, 0.75% and 1%) were used to identify the optimum concentration of sodium azide (unpublished data) where 0.75% was found to be the optimum concentration. The procedures used in the work of Weldemichael et al. [19] were applied in this study for treatment of the seeds with NaN_3 . Then, the seeds were further advanced into M2 lines by collecting healthy, clean and good-looking seeds from the M1 plants. The M1 seeds of each line were planted on a plot size of $(2 \times 2) \text{ m}^2$ in greenhouse condition with a row and plant spacing of 40 cm and 10 cm, respectively, to generate the M2 plants. Planting was carried

Table 1
Morphological traits and their Codes used to Study Qualitative Traits of the 14 Sesame Genotypes.

SN	State of traits	Code	Scoring
1	Capsule Arrangement	CA	1 = Monocapsular; 2 = Multicapsular
2	Capsule Hairiness	CH	0 = Glabrous (hair absent); 3 = Weak or Sparse; 5 = Medium; 7 = Strong or Profuse
3	Color of Dry Capsule	CDC	1 = Green; 2 = Straw/Yellow; 3 = Brown/Tan; 4 = Purple
4	Capsule Dehiscence at Ripening	CDR	1 = Non-Shattering; 2 = Partial Shattering; 3 Complete Shattering
5	Seed Coat Color	SCC	1 = White; 2 = Cream; 3 = Beige; 4 = Light Brown; 5 = Medium Brown; 6 = Dark Brown; 7 = Brick Red; 8 = Tan; 9 = Olive; 10 = Grey; 11 = Dull Black; 12 = Bright Black; 99 = Other

Source [39]:

out in a factorial arrangement with completely randomized design (CRD) with three replications. All the necessary agronomic practices were carried out during the growth period of the plants. The plants were moderately watered every other day. Weeding and other cultural practices were also carried out as required.

All the required quantitative and qualitative data were collected at maturity stage. Recorded quantitative data include: (a) mean capsule length (MCL), (b) mean capsule width (MCW), (c) number of capsules per plant (NCP), (d) number of seeds per capsule (NSC) and (e) thickness of capsule monocarp (TCM). Likewise, the recorded qualitative data were (a) capsule hairiness (CH), (b) color of dry capsule (CDC), (c) capsule dehiscence at ripening (CDR) and (d) seed coat color (SCC) (Table 1).

2.3. Data analyses

All collected data were subjected to analysis of variance (ANOVA) using GenStat 16 software [40]. ANOVA comparisons of means were carried out using Duncan's Multiple Range Test (DMRT) at a fixed significance level of $p \leq 0.01$ [41].

3. Results and discussion

3.1. Effects NaN_3 on quantitative traits of M2 plants

The ANOVA of M2 capsule traits showed the existence of significant variation among the NaN_3 tested genotypes. The interaction effects of NaN_3 (0 and 0.75%) and genotypes (14 genotypes) were statistically significant on the quantitative data for NCP, NSC, MCW, TCM and MCL (Table 2).

3.1.1. Number of capsules per plant (NCP)

Mutation studies are important instruments in generating cultivars with the highest capsule number. In the present study, significant variations existed among the M2 lines for NCP (Table 3). The highest mean NCP was recorded in ACC44 (78.50) and Baha Necho (74.75) genotypes, while the lowest was recorded in Gumero (31.50), Setit 2 (33.00), Hirhir (37.62), ADI (38.25), Bounji (42.87) and Aberghele (42.37). The genotypes with higher NCP have better yield as compared to those genotypes with lower NCP. Similarly, the work of Parimala et al. [42] reported that the best NCP was found to be the most important character for seed yield. Besides, various findings revealed NCP to be the principal determinant for high grain yield in the crop [28,30,43–45]. Furthermore, NCP and other yield related traits were reported to be highly affected by the environment, rhythm of growth and development, and gene-gene interaction [46–48]. In fact, the inflorescences for most sesame varieties are indeterminate and hence continue growing if the environmental condition is suitable. In a study by Zhang et al. [49], the capsule numbers varied from 9.2 to 96.2. This variation in capsule number was significantly influenced by gene interaction, and its effects on capsule number varied across crosses (between mutant X non mutant) and environments [46]. It was established that the number of capsules on main stem and branches have a high positive direct effect on seed yield in sesame [50,51].

Recent functional genomics studies identified candidate genes such as *SiACS8* that control traits of capsule number [52]. In our

Table 2
Analysis of variance for various characters in M2 generation of *Sesamum indicum* L.

Source of variation	d.f.	Traits				
		NSC	MCW	MCL	NCP	TCM
Variety	13	699.6**	0.13604**	1.2057**	1638.2 ^{ns}	0.041346**
Treatment	1	2491.74**	0.15429**	5.2500**	36105**	0.014405 ^{ns}
Linear	1	2491.74**	0.15429**	5.2500**	36105**	0.014405 ^{ns}
Variety X Treatment	13	144.81*	0.07210**	0.1575 ^{ns}	898.1 ^{ns}	0.011826 ^{ns}
Variety X Linear	13	144.81*	0.07210**	0.1575 ^{ns}	898.1 ^{ns}	0.011826 ^{ns}
Residual	84	75.17	0.01141	0.1829	926.0	0.007684

MCL = mean capsule length; NCP = number of capsules per plant; NSC = number of seed per capsule; TCM = thickness of capsule monocarp; MCW = mean capsule width. **: $p \leq 0.01$; *: $p \leq 0.05$; ns: non-significant.

Table 3
Main effects of sodium azide supplements and genotypes on different quantitative traits.

Genotypes	Traits				
	NCP	MCL	MCW	TCM	NSC
ACC44	78.50 ^a	2.912 ^{cddefg}	0.7000 ^d	0.5000 ^{cdef}	62.75 ^{cde}
Baha Necho	74.75 ^{ab}	2.937 ^{cde}	1.0125 ^a	0.6000 ^{bcd}	78.38 ^a
Zeri Tesfay	58.62 ^{abc}	3.950 ^a	0.5250 ^e	0.4625 ^f	74.12 ^{ab}
Baha Zeyit	57.62 ^{abc}	2.850 ^{defg}	0.7000 ^d	0.5000 ^{cdef}	68.25 ^{bc}
Humera 1	55.75 ^{abc}	3.137 ^{bcd}	0.8500 ^{bc}	0.6375 ^b	81.25 ^a
Borkena	52.75 ^{abc}	3.250 ^{bcd}	0.7875 ^{cd}	0.5000 ^{cdef}	64.88 ^{bcd}
Gondar 1	50.06 ^{abc}	2.937 ^{cdef}	0.7375 ^{cd}	0.5000 ^{cdef}	73.75 ^{ab}
Setit 1	44.25 ^{abc}	2.612 ^{efg}	0.7812 ^{cd}	0.5750 ^{bcd}	54.62 ^{ef}
Bounji	42.87 ^{bc}	3.437 ^b	0.7625 ^{cd}	0.5625 ^{bcd}	61.13 ^{cdef}
Aberghele	42.37 ^{bc}	2.450 ^{fg}	0.6750 ^d	0.4875 ^{ef}	61.875 ^{cde}
ADI	38.25 ^c	2.850 ^{defg}	0.8500 ^{bc}	0.5375 ^{cdef}	54.50 ^{ef}
Hirhir	37.62 ^c	3.125 ^{bcd}	0.9500 ^{ab}	0.6000 ^{bc}	56.00 ^{def}
Setit 2	33.00 ^c	3.375 ^{bc}	0.6875 ^d	0.5125 ^{cdef}	51.63 ^f
Gumero	31.50 ^c	3.487 ^b	0.9500 ^{ab}	0.7250 ^a	60.69 ^{cdef}
LSD	30.28	0.4256	.1063	0.08722	8.627
Treatment					
Control	29.488 ^b	2.793 ^b	0.721 ^b	0.526 ^a	56.94 ^b
Treated	62.071 ^a	3.274 ^a	0.821 ^a	0.564 ^a	69.1286 ^a
LSD	13.21	0.1857	0.0464	0.03807	3.765
CV	61	13.8	13.6	15.9	13.5

NCP = number of capsules per plant; MCL = mean capsule length; MCW = mean capsule width; NSC=Number of seeds per capsule; TCM = thickness of capsule monocarp. Means followed by a different letter indicate significant differences at $P \leq 0.01$, i.e., Means with different letters in the column are significant, while means with the same letter(s) in the column are non-significant.

study, we have found 78.50 capsules per plant from the genotype ACC44, which is almost closer to the maximum capsule number reported in China [49] and, hence, this is a promising genotype for further breeding strategies. The mutants with higher NCP appear to be superior to those with lower NCP for some agronomically important characters that facilitate mechanized harvesting such as synchronous flowering, uniformity and early maturity. The observations from this study showed that the mutants were earlier at flowering and maturation stages. The mutants with better NCP could, therefore, play a vital role in developing new genotypes. Several authors have studied different traits that contribute to the seed yield formation in sesame. Distinctly, the capsule number per plant was reported to be a primary determinant for high seed yield in sesame [28,43,45,53]. Our results match well with those in the literature, as we found capsule number and seed size-related traits strongly correlated with yield indexes.

3.1.2. Number of seed per capsule (NSC)

Holding other yield components positive, it is apparent that higher NSC leads to higher yield. In this regard, the present study looked into the effects of 0.75% NaN_3 mutagen on NSC at M2 lines of 14 Ethiopian sesame genotypes (Table 3). Accordingly, the highest mean NSC was recorded from Humera 1 (81.25), Baha Necho (78.38), Zeri Tesfay (74.12), and Gondar 1 (73.75) genotypes, while the lowest mean NSC was from Setit 1 (54.62), Setit 2 (51.63), and ADI (54.50). This was in agreement with another finding where the NSC varied from 17.4 to 117.7, of which 450 (59.0%) accessions had 50–70 seeds per capsule [49]. In fact, in many studies, NSC is most often reported as an important contributor to sesame grain yield [28,54,55]. Thus, NSC is one of the quantitative traits targeted as a selection index for sesame improvement [56]. Our observations clearly indicated that the higher NSC (81.25) was observed in Humera 1 which is better than the NSC [50–70] reported from accessions in China [49]. This result would, hence, be used as the best option for improving the yield of sesame in Ethiopia. Besides, identifications of mutant genotypes with the best desirable traits are the key findings for further molecular breeding research in the country.

3.1.3. Mean capsule length (MCL)

Capsule length (CL) is another important quantitative trait that affects grain yield in this oilseed crop. CL thus becomes a focus of research in the crop's improvement. In the present study, 0.75% NaN_3 mutagen resulted in a significantly highest MCL (3.90 cm) in one of the farmers' landraces (i.e., Zeri Tesfay). MCL for Gumero (3.487 cm) and Bounji (3.437 cm) were also significantly higher than that of other genotypes (Table 3). Interestingly, the mutagen resulted in the lowest MCL in the other farmers' landrace called Aberghele (2.450 cm). Longer CL yielded more seeds than the genotypes with shorter CL. Similar results were observed in another study where the tip zone lengths of 763 accessions studied in China ranged from 0.0 cm to 26.5 cm [49]. Molecular biology studies showed that sesame transcriptome, hormone, and genome have incorrect splicing mutation of *SiCRC* in the auxin signal transduction pathway, which is simultaneously responsible for two important yield contributing traits, namely NSC and CL [57]. Likewise, a recent functional genomics study identified a novel candidate gene called *SILPT3* that is believed to be involved affecting sesame grain yield through controlling CL [52]. Therefore, the maximum CL was found in the study, one of the most important traits for yield improvement.

3.1.4. Mean capsule width (MCW)

Capsule width (CW), another important yield component of sesame, was significantly affected by NaN_3 treatment. NaN_3 resulted

Table 4
Effect of NaN₃ on different qualitative traits of sesame genotypes at M2 stage (%).

Capsule morphology	Treatment	Name of genotypes													
		Aberghela	ACC44	ADI	Baha Necho	Baha Zeyit	Borkena	Bounji	Gondar 1	Gumero	Hirhir	Humera 1	Setit 1	Setit 2	Zeri Tesfay
Capsule hairiness															
Glabrous	Control	37.5				100.0		37.5		37.5					37.5
	Treated	62.5	100.0	100.0				62.5		62.5					62.5
Weak/sparse	Control								100.0		37.5				
	Treated						100.0				62.5	100.0	100.0		
Medium	Control													100.0	
	Treated														100.0
Strong of profuse	Control		100.0	100.0	37.5		100.0				100.0	100.0	100.0	100.0	
	Treated				62.5	100.0			100.0						
Color of dry capsule															
Brown	Control	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5
	Treated	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
Capsule dehiscence at ripening															
non-shattering	Control														
	Treated							100.0		100.0					100.0
partially shattering	Control				37.5	37.5	37.5					37.5			100.0
	Treated				62.5	62.5	62.5				100.0	62.5	100.0	100.0	
Completely shattering	Control	37.5	37.5	37.5				100.0	37.5	100.0	100.0		100.0	100.0	
	Treated	62.5	62.5	62.5					62.5						
Seed coat color															
White	Control	100.0	100.0					100.0			100.0	100.0			100.0
	Treated			100.0											
Cream	control				100.0	100.0			100.0						
	Treated	100.0	100.0					100.0			100.0	100.0	100.0	100.0	100.0
Light brown	control									37.5				100.0	
	Treated				100.0					62.5	100.0				
Dark brown	control						100.0								
	Treated							100.0							
Grey	control			100.0									100.0		
	Treated					100.0	100.0		100.0						

N.B. In CDC no significant change has been observed in all the genotypes in CDC (37.5% are control and 62.5% are treated), in SCC all genotypes changed except Gumero (both treated and control have light brown), in CDR eight genotypes have been observed with no change when treated with NaN₃; while the remaining six genotypes have changed from completely shattering to partial shattering and others changed from completely shattering to non-shattering due to NaN₃.

the highest MCW in Baha Necho (1.013 cm), Hirhir (0.950 cm) and Gumero (0.950 cm) and the lowest MCW in Zeri Tesfay (0.525 cm) and Abergehele (0.675 cm) (Table 3). Similar findings have been reported by Ganesh and Sakila [49] for CW to be 0.61–2.20 cm. Mutation breeding, hence, is important for the enhancement of genetic variation through the influence of different mutagens. This involves procedures that increase genetic variation, select desirable genotypes, evaluate selected genotypes and finally multiply and release new cultivars.

3.1.5. Thickness of capsule monocarp (TCM)

Capsule monocarp is also an important part of capsule which is paramount important for sesame yield improvement. The M2 lines of the 14 Ethiopian sesame genotypes with 0.75% NaN_3 treatment resulted varied changes in capsule traits. The mutagen resulted the highest mean TCM in Gumero (0.725 mm) followed by Humera 1 (0.638 mm) and the lowest mean TCM in Zeri Tesfay (0.463 mm) and Abergehele (0.488 mm) (Table 3). A great number of sesame mutants, including TCM, small capsule, small seed size, determinate flowering habit, short internode, branch density, short stalk, short flowering period, resistance to diseases, male sterility and other agronomic traits have been discovered [58–60].

3.1.6. Effects NaN_3 on qualitative traits of M2 plants

This study also investigated the effects of 0.75% NaN_3 on four qualitative sesame capsule traits at M2 stage. The qualitative capsule traits were CDR, SCC, CDC and CH (Table 4). The responses of each trait to 0.75% NaN_3 treatment are provided below.

3.1.7. Capsule dehiscence at ripening (CDR)

Sesame inflorescence is indeterminate and the flowering stages of some varieties can last for more than one month. Therefore, shattering becomes the principal factor in affecting seed harvesting time, grain yield, and grain quality. Non-dehiscent varieties with high shattering resistance can reduce harvest loss of mature seeds even with mechanized harvest. Unfortunately, capsules of most sesame genotypes worldwide are dehiscent with low shattering resistance. Nearly all shattering genotypes are characterized by 60–70% yield loss under dry weather conditions [61]. Moreover, since 99% of sesame is harvested manually [62], the loss due to shattering can be even higher. And yet, past efforts have been focusing on improving the content of the crop's grain oil rather than on developing non-shattering varieties [63–65].

Genetic improvement programs aiming at developing non-dehiscent (non-shattering) sesame varieties are very critical for Ethiopian sesame producers. All the 14 genotypes considered in the present study are shattering because almost all Ethiopian sesame genotypes are shattering type. NaN_3 treatment changed 100% in capsule dehiscence for Hirhir, Setit 1 and Setit 2 genotypes from completely shattering to partially shattering; Gumero and Bounji were changed from completely shattering to non-shattering; and Zeri Tesfay was changed from partially shattering to non-shattering (Table 4). Therefore, these mutants have better resistance to shattering and less yield loss. Similarly, findings reported on capsule shattering significantly affected sesame yield loss [29]. Besides, Çağırğan et al. [66] stated that screening for closed capsules is advisable to arrange M2 populations in the form of M1 plant progenies instead of bulk, although the *cc-1* is selected in a bulk. Furthermore, other studies developed non-dehiscent sesame varieties that succeeded in producing and releasing dozens of high shattering resistance varieties [67,68]. Moreover, Couch et al. [62] developed improved non-dehiscent sesame variety (named S29) with shattering resistance. In addition, Zhang et al. [69] reported that *SiCL1* gene and *cl-1* mutant supply the chance to discover advanced regulation of leaf and capsule, which would improve resistance to shattering and facilitate adoption of mechanized harvesting for sesame.

The desirable lines developed in the present study would, therefore, be very critical for developing sesame varieties with more yield and better shattering resistance when crossed with elite cultivars. This finding would also open new opportunities towards understanding the genes responsible for shattering and investigate how the mutants will behave under various agronomic practices and environmental conditions.

Table 5
Change in seed color due to NaN_3 treatment.

SN	Genotypes	Color Change by Treatment		Desirability of Change by Treatment		Gain or Loss
		Before	After	Before	After	
1	Abergehele	Cream	Cream	Desired	Desired	No Change
2	ACC44	White	Cream	Desired	Desired	No Change
3	ADI	Grey	White	Undesired	Desired	Gain
4	Baha Necho	Cream	Light Brown	Desired	Undesired	Loss
5	Baha Zeyit	Cream	Grey	Desired	Undesired	Loss
6	Borkena	Dark Brown	Grey	Undesired	Undesired	No Change
7	Bounji	White	Cream	Desired	Desired	No Change
8	Gondar 1	Cream	Grey	Desired	Undesired	Loss
9	Gumero	Light Brown	Light Brown	Undesired	Undesired	No Change
10	Hirhir	White	Light Brown	Desired	Undesired	Loss
11	Humera 1	White	Cream	Desired	Desired	No Change
12	Setit 1	Grey	Cream	Undesired	Desired	Gain
13	Setit 2	Light Brown	Cream	Undesired	Desired	Gain
14	Zeri Tesfay	White	Cream	Desired	Desired	No Change

3.1.8. Seed coat color (SCC)

Ethiopia is a home to many creamy and white-colored sesame genotypes. The seed colors of the 14 varieties considered in this study were white (5 genotypes), cream (4 genotypes), light brown (2 genotypes), grey (2 genotypes) and dark brown (1 genotype) (Table 5). This implies that sesame improvement programs targeting seed color have to be considered. The NaN_3 treatment in this study, hence, brought changes in SCC in many of the genotypes. It changed the SCC of ACC44, Bounji, Zeri Tesfay and Humera 1 genotypes from white to cream, Setit 2 genotype from light brown to cream, Hirhir genotype from white to light brown, Baha Necho genotype from cream to light brown, Baha Zeyit and Gondar 1 genotypes from cream to grey, Borkena genotype from dark brown to grey, Setit 1 genotype from grey to cream and ADI genotype from grey to white. In line to this, the SCCs of sesame were categorized into white, cream, beige, light brown, medium brown, dark brown, brick red, tan, olive, grey, dull black and bright black [39].

Seed coat color is highly polymorphic ranging from white to black through all intermediate colors [70,71]. It is an important agronomic trait in sesame and was found to be associated with biochemical functions involved in protein and oil metabolism, antioxidant activity, and disease resistance [71-73]. Recently, this trait was reported to be a more suitable trait for estimating sesame evolution than geographic origin [74] since the evolution was from wild species to black cultivars [74,75]. Besides, seed coat color is an important agronomic trait in sesame, which varies from white to black. As compared to the white sesame seeds, black sesame seeds usually have higher ash and carbohydrate contents, but lower protein content, oil content, and moisture ratios [76]. On the other hand, white sesame seeds typically have higher oil, sesamin or sesamol content [77]. Color changes from white or cream to any other colors are, therefore, regarded as undesirable, while changes from other colors to white or cream are considered as desirable changes. In this

Table 6

Mean of the quantitative traits for each cluster of sesame genotypes.

Clusters	Mean of quantitative traits				
	Number of capsules per plant	Mean capsule length	Mean capsule width	Thickness of capsule monocarp	Number of seed per capsule
I	49.30	3.117	0.8117	0.5300	63.57
II	46.90	3.460	1.0400	0.7300	59.60
III	81.8	3.300	0.9200	0.6300	87.10
IV	79.0	3.405	0.6750	0.5000	73.25

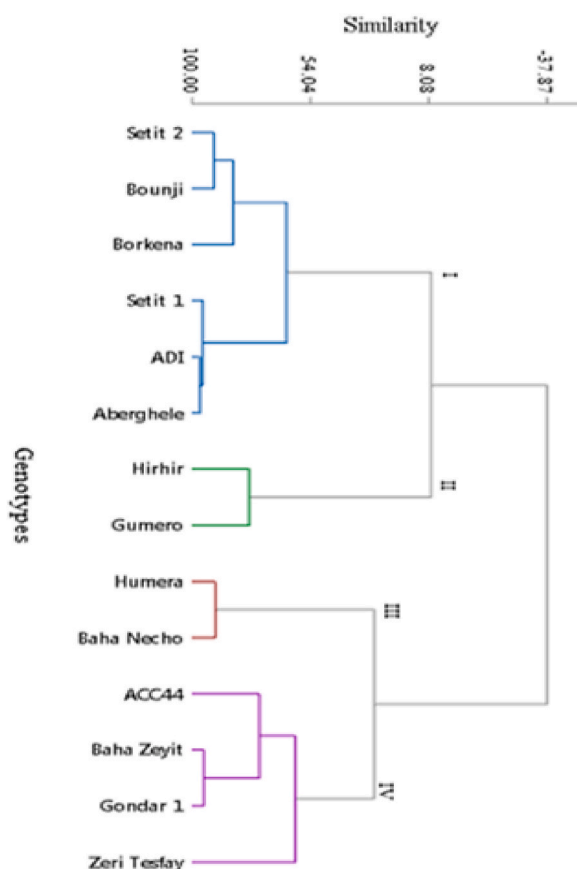


Fig. 1. Dendrogram using Ward's method based on dissimilarity matrix of 14 sesame (*S. indicum* L.) genotypes.

Table 7
Mahalanobis distance between clusters.

Clusters	I	II	III	IV
I	–	93.17	38.802	38.300
II		–	139.395	232.533
III			–	40.215
IV				–

regard, the treatments in this study resulted three desirable changes (gain) and four undesirable changes (loss).

3.1.9. Capsule hairiness (CH)

The chemical treatment resulted changes of CH in some of the M2 lines. Treatments with NaN_3 changed the CH of Hirhir and Humera 1 genotypes from glabrous to weak or sparse. It resulted 100% change in CH from strong profuse to weak or sparse in the Borkena, Humera 1 and Setit 1 genotypes and from strong or profuse to glabrous in the ACC44 and ADI genotypes. It also changed the CH of Setit 2 from strong to medium and that of Gondar 1 from weak or sparse to strong or profuse (Table 4). Hairiness may be related to drought tolerance, where the target environment is frequently subjected to drought. Developing drought tolerant lines could, therefore, be another key requirement for cultivation of sesame under rainfed conditions.

4. Grouping and calculating Mahalanobis distance

4.1. Grouping of fourteen sesame genotypes

Cluster mean values for each trait are given in Table 6. The 14 genotypes were clustered into four distinct groups (Fig. 1). Cluster I contains six genotypes, of which four genotypes including ADI, Borkena, Setit 1 and Setit 2 are research improved genotypes with the exception of Abergehele and Bounji, which are local landraces. They are characterized by relatively moderate mean NCP (49.30), lower MCL (3.117), relatively moderate MCW (0.8117), TCM (0.5300) and NSC (63.57). Cluster II, on the other hand, includes two genotypes (i.e., Gumero and Hirhir). These genotypes had the highest MCL (3.460), MCW (1.0400) and TCM (0.7300). However, their performance in terms of mean NCP (46.90) and NSC (59.60) were below average. Cluster III has two genotypes, namely, Humera 1 and Baha Necho, which are characterized by the highest NCP (81.8), MCW (0.9200) and NSC (87.10), as well as relatively moderate MCL (3.300) and TCM (0.6300). Finally, cluster IV includes four genotypes, namely, ACC44, Baha Zeyit, Gondar 1 and Zeri Tesfay, which had relatively higher mean NCP (79.0), MCL (3.405) and NSC (73.25), but lower MCW (0.6750) and TCM (0.5000).

4.2. Mahalanobis distance between clusters of fourteen sesame genotypes

The genotype grouping observed from the cluster analysis was further confirmed by the Mahalanobis distance analysis among clusters (Table 7). The distance values ranged from 93.17 (between clusters I and II) to 139.395 (between clusters II and III) and all the distance values were significantly different from each other ($P \leq 0.01$). The significant difference among clusters as depicted by the Mahalanobis distance would have a breeding implication in sesame improvement programs in Tigray, Ethiopia.

5. Conclusion

NaN_3 has been found to bring about a mutagenic effect on both qualitative and quantitative traits of sesame. Significant differences were observed in almost all of the morphological features that were studied in the mutant seeds of each treatment and the control. NaN_3 at the identified concentration of 0.75% played the most important role in most traits of sesame. This study believed that there is a significant improvement on the main components of sesame seed yield per plant i.e. NSC and NCP. The mutants developed from ACC44, Baha Necho, and Zeri Tesafy were the best and promising candidate mutants. Zeri Tesafy was developed as a promising candidate mutant for the production of non-shattering genotype. It can, therefore, be recommended that sesame seeds treated with NaN_3 should be used to create beneficial mutants of other sesame varieties. Besides, additional research should be conducted on M3 and beyond generations to confirm the isolating effect of any particular mutant. Further research should also be done on identification of different molecular markers linked to the closed capsule mutant trait.

Declarations

Author contribution statement

Micheale Yifter Weldemichael: Conceived and designed the experiments; Performed the experiment; Contributed reagents materials, Analysis tools or data; Analyzed and interpreted the data; Wrote the paper.

Yemane Tsehaye Baryatsion, Desta Berhe Sbhatu, Girmay Gebresamuel Abraha, Hailay Mehari Gebremedhn, Tesfakiros Semere Gebrelibanos, Abraha Birhan Kassa: Performed the experiment; Wrote the paper.

Mohammed Mebrahtu Mossa, Birhanu Kabsay Berhe, Mullubrhan Mekonen Gebru: Analyzed and interpreted the data; Wrote the

paper.

Hagos Mohammedseid Juhar, Haftay Abadi Gebru, Birhanu Debesay Berhe, Medhin Teklay: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

The data that has been used is confidential.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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