

## Assessing molecular and agronomic diversity in flax genotypes based on SCoT marker profiling, fibre yield contributing characters, and seed yield-related traits

Gehan M. ANWAR<sup>1</sup>, Saber H. MOSTAFA<sup>2</sup>, Ehab M. ZAYED<sup>3</sup>,  
Sahar A.M. SHAMSELDIN<sup>4</sup>, Abdelrahman E.A. OMAR<sup>5</sup>,  
Fatmah A. SAFHI<sup>6\*</sup>, Nora M. Al ABOUD<sup>7</sup>, Diaan ABD EL-MONEIM<sup>8</sup>,  
Elsayed MANSOUR<sup>5\*</sup>

<sup>1</sup>Minia University, Faculty of Agriculture, Genetics Department, Minia 61519, Egypt; [gehan\\_ma@mu.edu.eg](mailto:gehan_ma@mu.edu.eg)

<sup>2</sup>Agricultural Research Center, Field Crops Research Institute, Fibre Crops Research Department, Giza 12619, Egypt; [sabermostafa@gmail.com](mailto:sabermostafa@gmail.com)

<sup>3</sup>Agricultural Research Center, Field Crops Research Institute, Cell Study Research Department, Giza 12619, Egypt; [eabzayed977@gmail.com](mailto:eabzayed977@gmail.com)

<sup>4</sup>Ain Shams University, Women College for Arts, Science and Education, Botany Department, Cairo 11566, Egypt; [shams.sahar@women.asu.edu.eg](mailto:shams.sahar@women.asu.edu.eg)

<sup>5</sup>Zagazig University, Faculty of Agriculture, Department of Crop Science, Zagazig 44519, Egypt; [omaromar1971@yahoo.com](mailto:omaromar1971@yahoo.com); [sayed\\_mansour\\_84@yahoo.es](mailto:sayed_mansour_84@yahoo.es) (\*corresponding author)

<sup>6</sup>Princess Nourah bint Abdulrahman University, College of Science, Department of Biology, Riyadh 11671, Saudi Arabia; [faalsafhi@pnu.edu.sa](mailto:faalsafhi@pnu.edu.sa) (\*corresponding author)

<sup>7</sup>Umm Al-Qura University, Faculty of Science, Department of Biology, Makkah, Saudi Arabia; [nmaboud@uqu.edu.sa](mailto:nmaboud@uqu.edu.sa)

<sup>8</sup>Arish University, Faculty of Environmental and Agricultural Sciences, Department of Plant Production (Genetic Branch), El-Arish 45511, Egypt; [dabdelmoniem@aru.edu.eg](mailto:dabdelmoniem@aru.edu.eg)

### Abstract

Exploring genetic diversity is crucial for effective breeding strategies to enhance crop performance under diverse environmental conditions and accelerate selection with improved yield and adaptability to climate change. This study aimed to explore the genetic diversity of eleven flax genotypes (*Linum usitatissimum* L.) using Start Codon Targeted (SCoT) markers and evaluate their performance in fibre yield contributing characters and seed yield-related traits in two field trials during 2021-2022 and 2022-2023 seasons. SCoT markers revealed varying degrees of polymorphism, with primers SCoT-1, SCoT-24, and SCoT-27 displaying 100% polymorphism, indicating high genetic diversity among the genotypes. Genetic similarity analysis categorized the genotypes into four diverse groups. The agronomic performance indicated that 'S.620', 'Sakha 3', and 'S.651' excelled in straw yield and associated traits such as technical length, plant height, fibre percentage, total fibre yield, fibre length, and straw yield, while 'Sakha 5' and 'S.945' showed the lowest. On the other hand, 'Sakha 5' followed by 'S.933/15/2' and 'Sakha 6' were the top performers for seed yield and related characteristics, including fruiting zone length, number of capsules per plant, seed index, seed yield, oil percentage, and seed and oil yields, while 'IV2' exhibited the lowest values. Hierarchical clustering divided the

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genotypes into distinct clusters based on straw and seed performance. Moreover, the principal component analysis highlighted a clear separation between genotypes excelling in straw and seed traits, with strong associations among related characters. These findings provide valuable insights for breeding programs to improve flax fibre and seed production.

**Keywords:** agronomic performance; crop breeding; environmental adaptability; fibre yield; genetic analysis; oil percentage; SCoT markers; yield traits

## Introduction

Flax (*Linum usitatissimum* L.) is a multipurpose crop recognized for its utility in producing fibre and oil (Kauser *et al.*, 2024). In present agriculture, flax holds economic and ecological importance due to its role in sustainable farming practices and its health benefits as a source of omega-3 fatty acids and dietary fibre (Stavropoulos *et al.*, 2023). It is cultivated primarily in three types: fibre flax, linseed for oil production, and dual-purpose varieties (Husain *et al.*, 2009). In Egypt, flax is the leading fibre crop after cotton, significantly impacting the cultivated area and the national economy. Its importance extends to export markets and local industries, emphasizing the need for robust flax breeding programs (El-Borhamy *et al.*, 2017). These programs focus on developing new dual-purpose or fibre specific varieties to meet the rising demand for high-quality fibres and seeds (El-Borhamy *et al.*, 2017).

Exploring genetic diversity using marker profiling enables more targeted and efficient breeding strategies, accelerating the development of flax cultivars that contribute to sustainable agricultural production and economic growth (Mansour *et al.*, 2018; Hoque *et al.*, 2024). Various molecular markers have recently been utilized for genetic mapping and genetic diversity assessments (Ponce-Molina *et al.*, 2012; Dossa *et al.*, 2024). Start Codon Targeted (SCoT) markers are recognized for their efficiency, informativeness, and cost-effectiveness in genetic studies (Mondal and Bandyopadhyay, 2024). SCoT utilizes single 18-mer primers designed to be complementary to conserved regions flanking the ATG start codon, making them highly effective for various applications, including phylogenetic hypotheses, cultivar identification, and genetic variation studies (Yeken *et al.*, 2022). SCoT marker technique offers robust molecular analysis by targeting short, conserved regions around the ATG start codon in plant genes (Altaf *et al.*, 2024). It is particularly valuable for genetic studies, bulk segregation analysis, and quantitative trait loci (QTL) mapping (Yan *et al.*, 2016). It has been effectively utilized for various plant species for genetic diversity analysis and elucidating genetic relationships (Luo *et al.*, 2011; Feng *et al.*, 2015; Majeed *et al.*, 2024).

Exploring genetic diversity within flax genotypes provides a substantial variability that could be exploited to develop superior cultivars with enhanced fibre productivity, increased oil content, and improved resilience to environmental stresses (Kaur *et al.*, 2023). Breeders can select and combine desirable traits by employing this diversity, creating varieties that meet specific industry requirements and adapt to varying climatic conditions (Habibullah *et al.*, 2021; ElShamey *et al.*, 2022; Kaur *et al.*, 2024). In principle, genotypic diversity extends the genetic pool available for breeding and offers solutions to the challenges of modern flax cultivation, ensuring its continued relevance and profitability in the agricultural sector (Sertse *et al.*, 2019). Consequently, this study aimed to assess the agronomic performance of eleven flax genotypes, comprising commercial varieties and potential new genotypes under field conditions, focusing on key parameters such as fibre yield, seed yield, plant height, and oil content. Also, the genetic diversity among the flax genotypes was investigated using SCoT markers to identify genetic variations and relationships. This could assist in identifying genotypes with superior agronomic performance and desirable traits that could be exploited to develop potential candidates for breeding programs and commercial cultivation.

## Materials and Methods

### *Plant material and experimental design*

Eleven flax (*Linum usitatissimum* L.) genotypes were evaluated, including six local varieties, four promising strains, and one imported variety, are presented in Table 1. The field experiment was performed at Agricultural Research Station, Giza, Egypt (30° 34' N and 29° 55' E) during the winter growing seasons of 2021/2022 and 2022/2023. The experimental site was characterized by clay soil with the following composition: 57.4% clay, 30.9% silt, and 11.7% sand. Sowing was conducted in the first week of November for two seasons. The experiments employed a completely randomized block design with four replicates, and each plot measured six m<sup>2</sup>. Each plot comprised ten rows, each 3 meters in length, with a spacing of 20 cm between rows.

**Table 1.** Pedigree and plant type of the eleven evaluated flax genotypes

Genotype	Pedigree	Type*
'Giza 12'	S.148/6/1 × S.2419	Dual.
'Sakha 1'	I.Bombay × I. 1485	Dual.
'Sakha 3'	Blenika (2E) × I.2096	Fib.
'Sakha 2'	I.2348 × I.Hira	Dual.
'Sakha 5'	I.2561 × I.370	Dual.
'Sakha 6'	Giza 8 × S. 2419	Dual.
'I.V2'	Imported from Holland	Fib.
'S.620'	Giza-7 × S.422	Fib.
'S.945'	I.1563 × Romania-14	Dual.
'S.651'	I.1563 × (S.402/1 × I.Iriana)	Fib.
'S.933/15/2'	S.2419 × I.Herms	Dual.

\*Dual.: dual type for both fibre and oil, and Fib.: only fibre type

### *DNA Extraction and PCR conditions for SCoT analysis*

Genomic DNA was isolated from freshly harvested leaves of 15-day-old seedlings. The leaves were ground to a fine powder, and DNA was extracted utilizing 700 µl of Cornell extraction buffer (100 mM Tris HCl; 500 mM NaCl; 50 mM EDTA; 0.84% SDS equilibrated to 65 °C, mixed with 0.38 g sodium bisulfite per 100 ml buffer). The pH of the extraction buffer was altered to 7.8-8.0 with NaOH. DNA purity and concentrations were measured using a spectrophotometer. SCoT analysis was performed using 10 SCoT primers (Table 2).

**Table 2.** Base sequences of used ten SCoT primers

No.	Primers	Sequence
1.	SCoT-1	5'-CAACAATGGCTACCACCA-3'
2.	SCoT-2	5'-CAACAATGGCTACCACCC-3'
3.	SCoT-3	5'-CAACAATGGCTACCACCG-3'
4.	SCoT-4	5'-CAACAATGGCTACCACCT-3'
5.	SCoT-5	5'-CAACAATGGCTACCACGA-3'
6.	SCoT-13	5'-ACGACATGGCGACCATCG-3'
7.	SCoT-22	5'-AACCATGGCTACCACCAC-3'
8.	SCoT-24	5'-CACCATGGCTACCACCAT-3'
9.	SCoT-27	5'-ACCATGGCTACCACCGTG-3'
10.	SCoT-35	5'-CATGGCTACCACCGGCC-3'

The PCR program consisted of initial denaturation at 94 °C for 5 minutes, 40 cycles of denaturation at 94 °C for 40 seconds, annealing at 50 °C for 50 seconds, extension at 72 °C for 1 minute, and the final elongation step was performed at 72 °C for 7 minutes. The amplified products were confirmed by electrophoresis on 2% agarose gels stained with ethidium bromide, and their sizes were estimated using a 100 bp DNA ladder. DNA fragment sizes were analysed using GelAnalser 3.

#### *Data collection*

Upon reaching full maturity, ten randomly harvested individual plants per plot were assessed for following characteristics: Total length (cm), stem diameter (mm), technical length (cm), fruiting zone length (cm), number of capsules per plant, seed index (g), straw yield (ton ha<sup>-1</sup>), fibre percentage (%), total fibre yield (kg ha<sup>-1</sup>), seed yield (kg ha<sup>-1</sup>), oil yield (kg ha<sup>-1</sup>) and oil percentage (%).

#### *Statistical analysis*

R software (version 4.1.2) was employed for statistical analyses. Differences among the evaluated genotypes were identified using LSD at a significance level of  $p \leq 0.05$ . The cluster, principal component, and heatmap analyses were applied using packages of FactoMiner, gplots, and pheatmap.

## **Results**

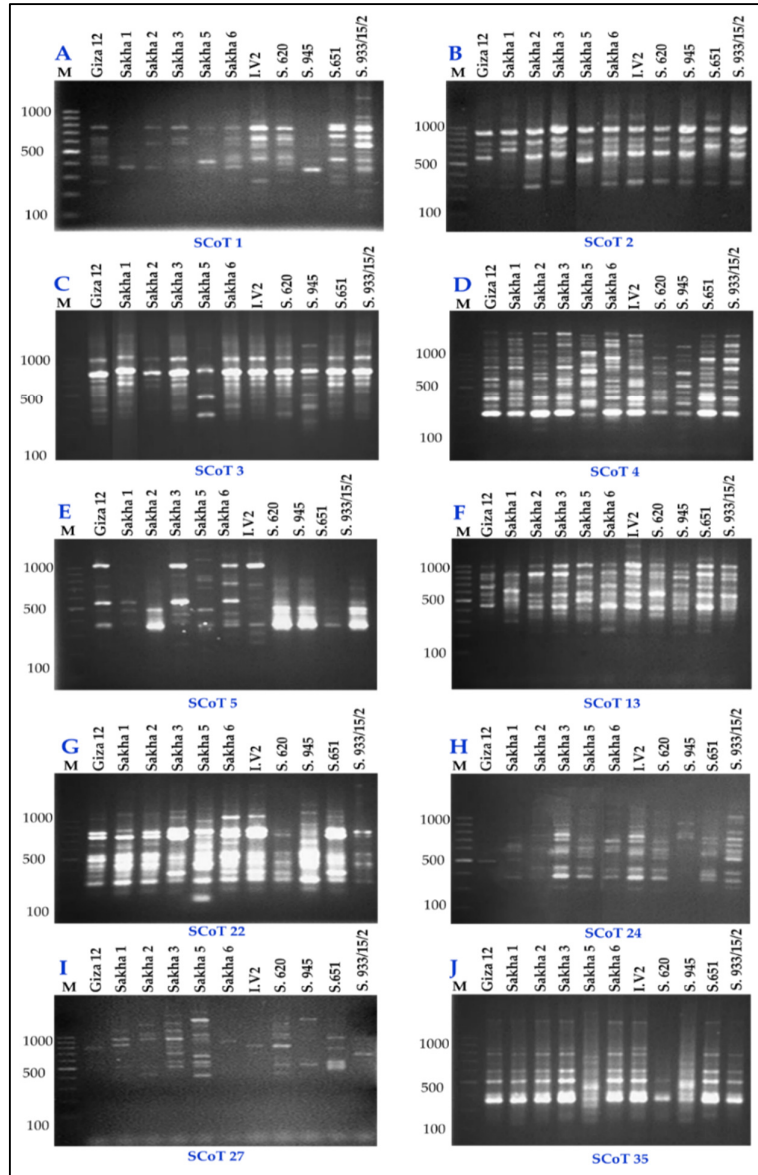
#### *Genetic diversity and molecular markers*

Ten SCoT molecular markers were employed to assess the genetic diversity of flax genotypes (Figure 1). The presence of monomorphic bands for specific primers, with the number of monomorphic bands per primer ranging from 0 to 4, is presented in Table 3. The level of genetic variation detected by each primer among the eleven flax genotypes is indicated in Table 3.

**Table 3.** Number of polymorphic, monomorphic, and unique PCR fragments and percentage of polymorphism obtained utilizing ten SCoT primers for the assessed flax genotypes

Primers	Monomorphic bands	Unique bands	Polymorphic bands		Total number of bands	Polymorphism (%)
			without unique	with unique		
SCoT-1	0	5	6	11	11	100.0
SCoT-2	2	1	4	5	7	71.00
SCoT-3	1	5	6	11	12	91.70
SCoT-4	1	4	14	18	19	94.70
SCoT-5	1	5	10	15	16	93.75
SCoT-13	4	10	1	11	15	73.33
SCoT-22	4	9	4	13	17	76.47
SCoT-24	0	7	8	15	15	100.0
SCoT-27	0	8	9	17	17	100.0
SCoT-35	1	2	7	9	10	90.00

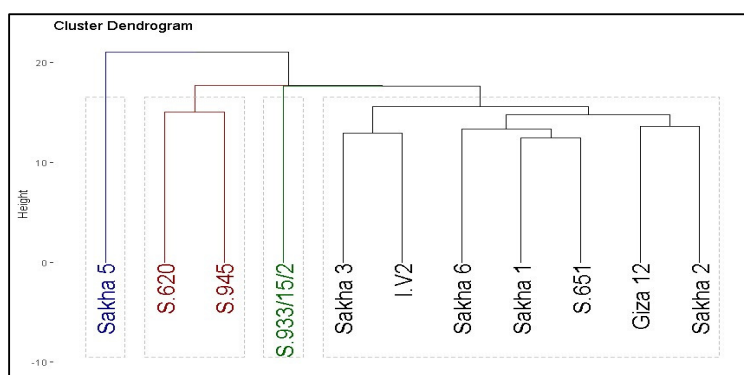
Primers SCoT-1, SCoT-24, and SCoT-27 showed 100% polymorphism, while primers SCoT-2, SCoT-3, SCoT-4, SCoT-5, SCoT-13, SCoT-22, and SCoT-35 exhibited polymorphism percentages ranging from 71.00% to 94.70%. The high polymorphism percentages detected by multiple primers highlight genetic diversity within the analyzed flax genotypes. This genetic variation can be further explored to identify markers associated with desirable traits and develop breeding strategies to improve crop performance and resilience. Table 4 details the unique bands and their distribution among different genotypes for each primer. The unique bands ranged from one to ten, produced by primers SCoT-2 and SCoT-13, respectively. Based on genetic similarities among the studied flax cultivars, the dendrogram revealed four groups (Figure 2): Group 1, represented by ‘Sakha 5’; Group 2 encompassed ‘S.620’ and ‘S.945’; Group 3 included ‘S.933/15/2’; and Group 4 contained the remaining seven flax genotypes.



**Figure 1.** SCoT-PCR amplification patterns of ten flax genotypes using ten SCoT primers (A: SCoT-1, B: SCoT-2, C: SCoT-3, D: SCoT-4, E: SCoT-5, F: SCoT-13, G: SCoT-22, H: SCoT-24, I: SCoT-27, and J: SCoT-35. A-J). M = 100 bp DNA ladder

**Table 4.** Number of unique PCR fragments obtained utilizing ten SCoT primers for assessed flax genotypes

Primers	Number of unique bands/Genotypes
SCoT-1	4('S. 933/15/2'), 1('S.651')
SCoT-2	1('Sakha 5')
SCoT-3	2('S.945'), 1('S.620'), 1('Sakha 5'), 1('Giza 12')
SCoT-4	1('Giza 12'), 1('Sakha 5'), 1('S.945'), 1('S. 933/15/2')
SCoT-5	1('Sakha-3'), 1('Sakha 5'), 1('Sakha 6'), 1('I.V2'), 1('S. 933/15/2')
SCoT-13	1('Giza 12'), 1('Sakha 2'), 2('Sakha1'), 1('Sakha 5'), 1('Sakha 6'), 2('I.V2'), 1('S.620'), 1('S.651')
SCoT-22	1('Giza 12'), 1('Sakha2'), 1('Sakha-1'), 1('Sakha 3'), 2('Sakha 5'), 2('S.945'), 1('S.651'),
SCoT-24	3('S. 933/15/2'), 1('Sakha 1'), 1('Giza 12'), 1('Sakha2'), 1('S.945')
SCoT-27	2('Sakha 5'), 1('Sakha 2'), 2('Sakha 3'), 1('S. 933/15/2'), 1('Sakha 6'), 1('Giza 12')
SCoT-35	2('Sakha 5')

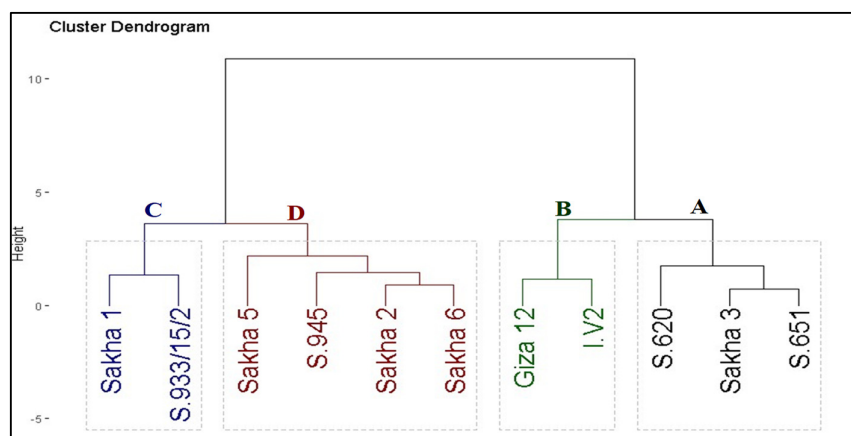
**Figure 2.** Cluster analysis of the eleven studied flax genotypes based on the multiple alignments of their sequences generated by 10 SCoT markers

#### *Straw yield and related characters*

The performance of straw yield and its contributing traits for eleven flax genotypes is illustrated in Table 5. The promising strain 'S.620' recorded higher values for most straw yield traits, except for stem diameter assigned for 'I.V2'. Besides, 'S.651' and 'Sakha 3' followed 'S.620' and displayed high values of technical length, plant height, stem diameter, straw yield, total fibre yield, fibre percentage, and fibre length. Otherwise, 'Sakha 5' and 'S.945' recorded the lowest values of the abovementioned characters. Total plant length ranged from 99.0 cm in 'Sakha 5' to 117.5 cm in 'S.620', with an average of 108.5 cm. The technical length varied from 79.0 cm in 'Sakha 5' to 105.0 cm in 'S.620', averaging 93.7 cm. The stem diameter extended from 1.19 mm in 'S.945' to 1.85 mm in 'I.V2', averaging 1.54 mm. Straw yield ranged from 9548 kg ha<sup>-1</sup> in 'Sakha 5' to 10953 kg ha<sup>-1</sup> in 'S.620', averaging 10256 kg ha<sup>-1</sup>. Total fibre yield contrasted from 1575 kg ha<sup>-1</sup> in 'Sakha 5' to 2223 kg ha<sup>-1</sup> in 'S.620', averaging 1813 kg ha<sup>-1</sup>. The fibre percentage changed from 16.0% in 'Sakha 1' and 'Sakha 2' to 20.3% in 'S.620', averaging 17.7%. Fibre length differed from 77.1 cm in 'Sakha 5' to 103.0 cm in 'S.620', averaging 92.1 cm. The eleven assessed flax genotypes were clustered based on straw yield and its related characters into four groups, as shown in Figure 3. Group A, which included three genotypes ('S.620', 'Sakha 3', and 'S.651'), recorded the highest straw yield and related characters. Following group B, which consisted of two genotypes ('Giza 12' and 'I.V2'), it was also characterized by high performance. Group C contained two genotypes ('Sakha 1' and 'S.933/15/2') that showed moderate performance. Conversely, Group D included four genotypes ('Sakha 5', 'S.945', 'Sakha 2', and 'Sakha 6'), encompassing genotypes with the lowest performance.

**Table 5.** Performance of straw yield and its contributed traits over 2021-2022 and 2022-2023 growing seasons

Genotype	Plant height (cm)	Technical length (cm)	Stem diameter (mm)	Straw yield (kg ha <sup>-1</sup> )	Total fibre yield (kg ha <sup>-1</sup> )	Fibre percentage (%)	Fibre length (cm)
'Giza 12'	110.5 c	96.50 c	1.829 ab	10238 d	1843 bcd	18.00 c	96.70 d
'Sakha 1'	105.0 d	92.00 de	1.285 e	10500 c	1680 cde	16.00 f	89.70 ef
'Sakha 2'	105.5 d	89.50 e	1.333 cd	9810 e	1676 cde	16.00 f	87.60 f
'Sakha 3'	113.5 b	103.00 ab	1.800 b	10762 b	2045 ab	19.00 b	100.40 ab
'Sakha 5'	99.00 f	79.00 g	1.321 d	9548 f	1575 e	16.50 ef	77.10 f
'Sakha 6'	103.5 de	85.50 f	1.356 c	9881 e	1631 de	16.50 ef	83.50 e
'I.V2'	112.0 bc	101.5 b	1.851 a	10476 c	1868 bc	19.04 b	99.00 cd
'S.620'	117.5 a	105.0 a	1.814 b	10953 a	2223 a	20.30 a	103.0 a
'S.945'	101.5 e	85.00 f	1.190 f	9572 f	1646 de	17.20 d	86.00 ge
'S.651'	115.5 ab	102.0 ab	1.853 a	10810 ab	2000 b	19.60 b	100.2 c
'S.933/15/2'	110.5 c	92.00 de	1.350 cd	10262 d	1757 cde	17.00 de	90.20 e

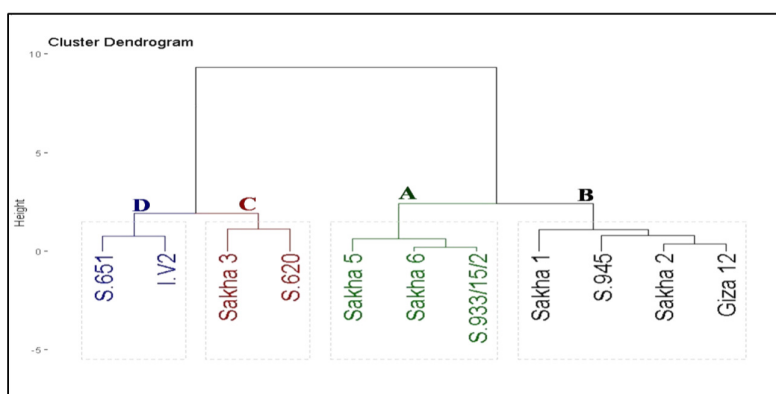
**Figure 3.** Dendrogram illustrating phenotypic distances among eleven flax genotypes based on straw yield and its contributing traits

#### *Seed yield and related traits*

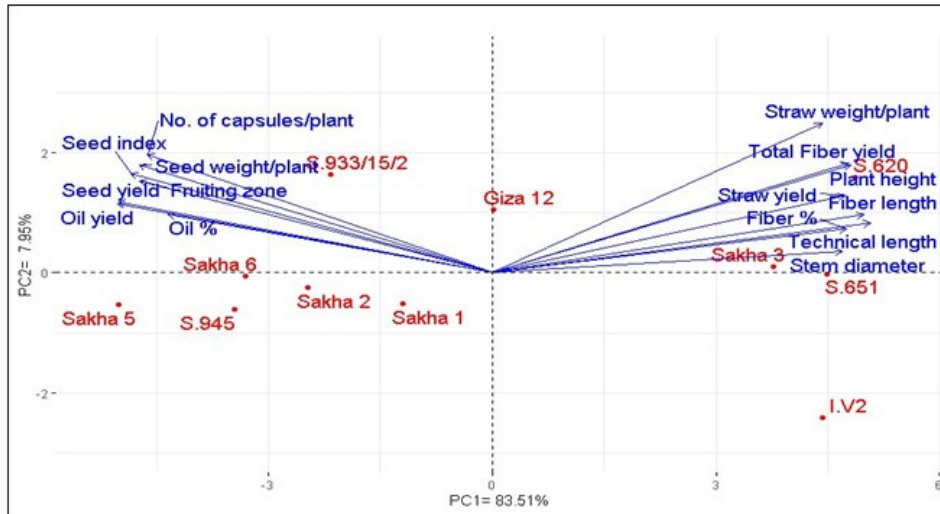
The performance of seed yield and contributing traits for the eleven flax genotypes is presented in Table 6. The newly developed flax genotype 'Sakha 5' ranked first. It exhibited the highest values of the fruiting zone, number of capsules per plant, seed index, seed yield, oil percentage, and oil yield, followed by the promising strain 'S.933/15/2' and 'Sakha 6'. In contrast, the introduced genotype IV2 recorded the lowest values. The fruiting zone varied from 9.5 cm in 'I.V2' to 20.0 cm in 'Sakha 5', averaging 14.6 cm. The number of capsules per plant ranged from 8.0 in 'I.V2' to 23.0 in 'Sakha 5', averaging 16.2. Seed index contrasted from 4 g in 'I.V2' to 9.9 g in 'Sakha 5', averaging 7.8 g. Seed yield fluctuated from 726.2 kg ha<sup>-1</sup> in 'I.V2' to 1714 kg ha<sup>-1</sup> in 'Sakha 5', averaging 1339 kg ha<sup>-1</sup>. Oil percentage differed from 35.6% in 'I.V2' to 41.6 % in 'Sakha 5', averaging 39.6%. Oil yield differed from 258.1 kg ha<sup>-1</sup> in 'I.V2' to 538.6 kg/ ha<sup>-1</sup> in 'Sakha 5', averaging 713.2 kg ha<sup>-1</sup>. The assessed flax genotypes were clustered based on seed yield and related traits into four groups, as shown in Figure 4. Group A, which included three genotypes ('Sakha 5', 'Sakha 6', and 'S.933/15/2'), recorded the highest seed yield and related traits. Following group B, which consisted of four genotypes ('Sakha 1', 'S.945', 'Sakha 2', and 'Giza 12'), it was also characterized by high performance. Group C contained two genotypes ('Sakha 3' and 'S.620') that showed moderate performance. On the contrary, Group D included two genotypes ('S.651' and 'I.V2') containing genotypes with the lowest seed yield and related traits.

**Table 6.** Performance of seed yield and its contributing traits over 2021-2022 and 2022-2023 growing seasons

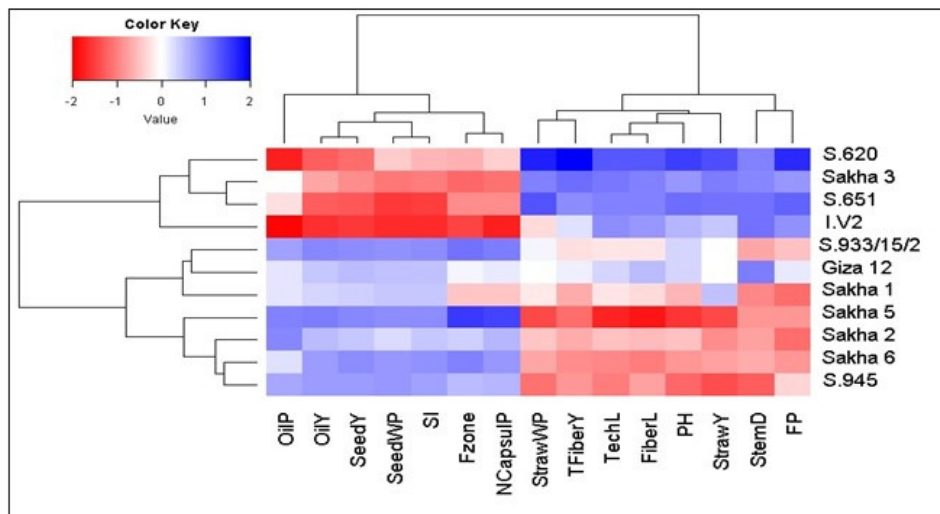
Genotype	Fruiting zone (cm)	No. capsules/plant	Seed index (g)	Seed yield (kg ha <sup>-1</sup> )	Oil percentage (%)	Oil yield (kg ha <sup>-1</sup> )
'Giza 12'	15.00 d	17.00 bc	8.890 d	1548 c	40.00 abc	619.1 de
'Sakha 1'	13.00 e	14.00 c	8.845 e	1488 d	40.00 abc	595.3 e
'Sakha 2'	16.00 d	19.00 ab	8.862 de	1521 cd	41.50 a	631.4 d
'Sakha 3'	10.50 fg	11.00 cd	5.420 g	976.2 e	39.50 bc	413.8 f
'Sakha 5'	20.00 a	23.00 a	9.850 a	1714 a	41.60 a	713.1 a
'Sakha 6'	18.00 bc	20.00 ab	9.765 b	1695 a	40.08 abc	677.6 bc
'I.V2'	9.50 g	8.00 d	3.950 i	726.2 h	35.55 d	258.1 h
'S.620'	12.50 e	14.50 c	6.400 f	892.9 f	36.00 d	321.4 g
'S.945'	16.50 cd	19.00 ab	9.450 c	1645 b	41.00 ab	674.5 c
'S.651'	11.50 ef	12.00 cd	4.253 h	821.4 g	39.00 c	320.5 g
'S.933/15/2'	18.50 ab	21.00 ab	9.830 a	1703 a	41.10 ab	699.8 ab

**Figure 4.** Dendrogram illustrating phenotypic distances among eleven flax genotypes based on seed yield and its contributed traits*Interrelationship among assessed fax genotypes and studied straw and seed traits*

The association between assessed fax genotypes and studied straw and seed traits was explored utilizing principal component (PC) analysis. The first two PCs depicted a substantial portion of the variance (83.51% by PC1 and 7.95% by PC2) and were shown in the PC biplot (Figure 5). PC1 exhibited higher variation and effectively separated the flax genotypes into those with negative and positive values along this axis. The genotypes on the positive side of PC1 were linked to higher straw yield and related characters, notably 'S.620', 'Sakha 3', 'S.651', and 'I.V2'. On the other hand, the genotypes on the negative side, such as 'Sakha 5', 'S.945', 'Sakha 6', 'S.933/15/2', and 'Sakha 2', demonstrated higher performance in seed yield and contributing characters. The middle genotypes, such as 'Giza 12', exhibited intermediate performance in both trait groups, straw and seed characters. The closeness of vectors demonstrated a robust positive association among the characters. Specifically, straw yield was positively associated with technical length, plant height, stem diameter, total fibre yield, fibre percentage, and fibre length. Conversely, seed yield is positively associated with the fruiting zone, number of capsules per plant, oil percentage, seed index, and oil yield. Notably, the two groups exhibited a negative correlation with each other. A heatmap and hierarchical clustering based on the studied straw and seed traits separated the flax genotypes into two diverse clusters (Figure 6). The genotypes 'S.620', 'Sakha 3', 'S.651', and 'I.V2' exhibited the highest straw characters (represented in blue), whereas the lowest seed traits (characterized in red). On the other hand, 'S.933/15/2', 'Sakha 5', and 'Sakha 6' exhibited the highest seed traits (described in blue), whereas the lowest straw character (depicted in red).



**Figure 5.** PC-biplot displays the relationship between assessed flax genotypes, evaluated straw and seed yields, and their contributing traits



**Figure 6.** Heatmap and hierarchical clustering analysis were employed to explore the association between flax genotypes and evaluated straw and seed yields, and their contributing traits

TechL: Technical length, PH: Plant height, StemD: Stem diameter, StrawWP: Straw weight/plant, StrawY: Straw yield, TFiberY: Total fibre yield, FP: Fibre %, FibreL: Fibre length, Fzone: Fruiting zone, NCapsulP: No. of capsules/plant, SeedWP: Seed weight/plant, SI: Seed index, SeedY: Seed yield, OilP: Oil %, and OilY: Oil yield

## Discussion

Genetic diversity enables the development of flax genotypes with improved straw and seed yield, which are essential for meeting the growing global demand for flax products. As climate change continues to pose challenges to agriculture, exploring genetic diversity becomes even more critical in breeding programs to ensure food security and economic stability for farmers (Mansour *et al.*, 2018; Selem *et al.*, 2022; Ogunbode *et al.*, 2025). The present study assessed the genetic diversity of eleven flax genotypes using ten (SCoT) markers. It

was evaluated in two field trials to examine the differences among the studied flax genotypes. The results of SCoT markers provided significant insights into the genetic variation among these genotypes. The presence of both monomorphic and polymorphic bands across the primers highlighted varying degrees of genetic uniformity and diversity. Specifically, detecting monomorphic bands for primers such as SCoT-2, SCoT-3, SCoT-4, SCoT-5, SCoT-13, SCoT-22, and SCoT-35 indicates a certain level of genetic uniformity among the genotypes for these specific sequences. However, the discovery of 100% polymorphism in primers SCoT-1, SCoT-24, and SCoT-27 pointed out significant genetic variation, demonstrating the effectiveness of these markers in distinguishing between the genotypes. The unique bands identified by different primers further highlight specific genetic variations that can be associated with particular traits of interest. The high polymorphism percentages observed across multiple primers indicate substantial genetic diversity within the flax genotypes, which is crucial for breeding programs. This diversity can be leveraged to identify molecular markers linked to desirable traits, ultimately aiding in developing superior flax varieties. The dendrogram analysis, which clustered the genotypes into major groups, revealed distinct genetic relationships, with 'Sakha 5' forming a separate cluster, indicating its genetic divergence from the other genotypes. This suggests that 'Sakha 5' may possess distinctive genetic traits valuable for breeding. Expanding the analysis to include a broader range of primers and molecular markers could provide a more comprehensive understanding of the genetic relationships among these genotypes, enhancing their classification and exploring their phylogenetic relationships. The results align with previous research, reinforcing the value of SCoT markers in evaluating genetic diversity across various plant species. In this context, Bhattacharyya *et al.* (2013) demonstrated the effectiveness of SCoT markers in endangered plant species like *Dendrobium nobile*, confirming that these markers reveal diversity and contribute to conservation efforts. Cabo *et al.* (2014) demonstrated the effectiveness of SCoT markers for assessing genetic diversity in Tritordeums. Moreover, Mahmoud *et al.* (2022) highlighted a polymorphism for SCoT markers, underscoring the importance of these markers in evaluating genetic diversity. The study revealed high reproducibility of the markers, making them suitable for evaluating genetic diversity in various contexts. The insights gained from these studies emphasized the importance of incorporating SCoT markers into breeding programs to improve genotype resilience and crop performance.

The evaluation of straw yield and its contributing traits across the eleven flax genotypes revealed significant differences in performance. Genotype 'S.620' is considered the most promising strain, displaying superior values for all crucial attributes of straw yield, except for stem diameter, where 'I.V2' exhibited the highest value. The strong performance of 'S.620', along with 'S.651' and 'Sakha 3', in traits such as plant height, technical length, stem diameter, straw yield, total fibre yield, fibre percentage, and fibre length indicated their potential as high-yielding flax genotypes for straw production. The observed differences in straw yield among the genotypes can likely be attributed to variations in their genetic makeup. Similar results have been reported by El-Shimy *et al.* (2016); El-Borhamy *et al.* (2017); El Sayed *et al.* (2018); Emam (2020); Praczyk and Wielgusz (2021); Abdelmasieh *et al.* (2024), who also noted the influence of genetic factors on flax straw yield. The clustering of genotypes based on straw yield and associated traits into four distinct groups further highlights the genetic diversity in straw yield potential among these flax genotypes. Group A, which includes 'S.620', 'S.651', and 'Sakha 3', demonstrated the highest performance, making them prime candidates for breeding programs focused on improving straw yield. Conversely, Group D, including 'Sakha 5', 'S.945', 'Sakha 2', and 'Sakha 6', recorded the lowest performance, indicating their limited suitability for high straw yield production. These genotypes may require targeted improvement or be utilised for different breeding objectives. The significant variation in traits such as total plant length, technical length, stem diameter, and fibre yield among the genotypes highlights the potential for selecting and breeding flax varieties with enhanced straw characteristics. In terms of seed yield and contributing characteristics, 'Sakha 5' was identified as the top-performing genotype, exhibiting the highest values for crucial characteristics such as the fruiting zone, seed index, number of capsules per plant, seed yield, oil percentage, and oil yield. The superior performance of 'Sakha 5', followed by 'S.933/15/2' and 'Sakha 6', suggests that these genotypes possess desirable seed yield

improvement and oil production traits. The wide variation in seed yield-related traits among the genotypes highlights the genetic diversity and the opportunity for selective breeding to enhance flax seed yield and oil content. The clustering of genotypes based on seed yield and related traits into four distinct groups reflects this genetic diversity in seed yield potential. Group A, comprising the top-performing genotypes, offers ideal candidates for breeding programs to enhance seed and oil yield. In contrast, Group D, which included genotypes with the lowest seed yield and related traits, may require targeted breeding strategies to improve their performance or could be used for other breeding objectives. These findings are consistent with the research conducted by Rahimi *et al.* (2011); Gul *et al.* (2016); Silska and Bocianowski (2018); Goreeva *et al.* (2020); Mahmoud *et al.* (2022); Kaur *et al.* (2024) who stated significant role of genetic diversity in determining the performance of flax genotypes regarding seed yield and oil production.

Principal component analysis provided beneficial insights into the interrelationships among the genotypes and the evaluated traits (Salem *et al.*, 2020; Swailam *et al.*, 2021; Yadav *et al.*, 2024). The high percentage of variance captured by PC1 suggests its effectiveness in differentiating the genotypes based on their performance in straw and seed traits. The PC biplot clearly separated the genotypes into those associated with higher straw yield and related characteristics on the positive side of PC1 and those linked to higher seed yield and associated traits on the negative side of PC1. This separation indicates a negative correlation between straw yield and seed yield traits, implying that genotypes excelling in straw yield tend to have lower seed yield and vice versa. This finding is further supported by the heatmap and hierarchical clustering analysis, which grouped the genotypes into two distinct clusters based on their performance in straw and seed traits. Strong positive correlations were observed within the straw yield-contributing characters, e.g., technical length, plant height, stem diameter, and total fibre yield, and within seed yield-related traits, e.g., number of capsules per plant, fruiting zone, seed index, and oil percentage, suggesting that these traits are closely linked and can be targeted simultaneously in breeding programs. However, the negative correlation between straw yield and seed yield characters presents a challenge for breeders, as improving one trait group may reduce the other. Therefore, breeding strategies should carefully consider these trade-offs to develop balanced flax varieties that meet specific production goals.

## Conclusions

This study explored molecular diversity, straw yield, seed yield, and their interrelationships among eleven flax genotypes, providing crucial insights for flax breeding programs focused on improving both straw and seed yield. Significant genetic variation was identified using ten SCoT molecular markers, with primers SCoT 1, SCoT 24, and SCoT 27 demonstrating 100% polymorphism, making them highly effective in distinguishing between genotypes. Performance analysis identified 'S.620' as the top genotype for straw yield traits, followed by 'Sakha 3' and 'S.651', while 'Sakha 5' excelled in seed yield and oil production, followed by 'Sakha 6' and 'S.933/15/2'. The findings emphasized the potential of these genotypes for targeted breeding strategies to optimize both straw and seed productivity, ultimately supporting the development of balanced flax varieties designed for specific agricultural goals. The principal component analysis further elucidated the relationships between straw and seed traits, with genotypes clustering into groups that either excelled in straw yield or seed traits, underscoring the inherent trade-offs between these characteristics.

## Authors' Contributions

Conceptualization: GMA, SHM, EMZ, SAMS; Data curation: GMA, SHM, EMZ, SAMS, AEAO; Formal analysis: AEAO, FAS, NMA, DE, EM; Funding acquisition: FAS, NMA, DE, EM; Investigation: GMA, SHM, EMZ, SAMS; Methodology: AEAO, FAS, NMA, DE, EM; Resources: FAS, NMA, DE, EM;

Software: FAS, NMA, DE, EMZ, EM; Supervision: SHM, EMZ, SAMS; Validation: GMA, FAS, NMA, D.E., EM; Visualization: GMA, SHM, EMZ, EM; Writing - original draft: GMA, SHM, EMZ, SAMS; Writing - review and editing: AEAO, FAS, NMA, DE, EM. All authors read and approved the final manuscript.

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### **Availability of Data and Materials**

The data are available from the corresponding author upon reasonable request.

### **Conflicts of Interest:**

The authors declare no conflicts of interest to report regarding the present study.

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