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Evaluation and Identification of Flax (*Linum usitatissimum* L.) Genotypes Using Agro-Morphological Traits and SCOT Markers

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Abstract

A two-year study was conducted at Giza Research Station, Agricultural Research Center, Giza, Egypt during 2022/2023 and 2023/2024 seasons to evaluate the yield potential of twelve promising flax lines, identify seed morphological traits, yield components, technological traits and molecular markers comparing to commercial Giza 12 and Sakha 3 as check varieties. Results indicated that differentiation existed between all genotypes under investigation for seed morphological characters. Germination and seedling traits showed significant differences among genotypes. Yield and its components differed significantly among the flax genotypes for all the studied traits. S.3 and S.8 recorded the highest fiber yield per feddan. Meanwhile, S.6 and S.10 recorded the highest values seed yield per plant (g), seed yield per feddan (kg), and oil yield per feddan (kg). A total of 119 SCOT fragments were recorded, with 33 monomorphic and 86 polymorphic amplicons, resulting in 72% polymorphism. The polymorphism heterozygosity index values SCOT marker traits were ranged as H (0.44-–0.7), PIC (0.35–0.642), E (1–2.67) HAV (0.009–0.7), MI (0.025–0.7) and D (0.113–056). The proportion of observed polymorphism was 63%. SCOT-2 and SCOT-36 had the highest number of unique bands (4). There was a similarity between the PCA, heat map, and dendrogram distributions of S.8, S.9, S.3, S.7, Giza12 and Sakha3. All data revealed a significant differences, among the surveyed flax genotypes at the molecular level.

Keywords: Flax, Seed classification, Germination, Seed yield, Genetic diversity, SCOT.

1. Introduction

Flax (*linum usitatissimum* L.) consider as one of the important fiber crops in several countries of the world, as well as Egypt where flax is known in ancient history, It has grown since

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many years ago as a dual-purpose crop for its oil and fiber extracted from the seeds **[1]**. Flax, a product that can meet the increasing demand for agricultural production, plant of flax used in both oil and fiber production **[2]**. Flax is the source of three major classes of commercial products oil, fiber and bio-products **[3]**. Flax consumption of oil or flaxseed has significant beneficial impacts in prevention of some neurological and hormonal disorders, cardiovascular diseases, some cancers and inflammation **[4].**

Diversity analyses based only on morphological characters are. prone to environmental bias due to environmental influences. and complex genetic structure of different morphological .traits **[5-6]**. Genetic diversity in flax, based on morphological, biochemical, metabolic., and molecular markers, has been reported by many researchers. worldwide **[6,7, 8, 9,10].** However, the development of improved. plant varieties and cultivates are restricted mainly due to narrow genetic pool. which results into limited possibility to restructure the flax crop. Identification of genotypes based on morphological characteristics is according to International Union for Protection of New Plant Varieties (UPOV). For identification of varieties through morphological characters and conduct of Flax **[9,12, 13, 14, 15,16].**

Germination and seedling emergence are the most serious stages during life cycle of plants that determine the efficient use of the nutrients and water resources available to plants. The germination rate is one of the important features of the base of seed which grow rapidly will have less exposure to pest and disease attacks **[17]**. For example, the root length obtained during the germination period is an important indicator of the plants attachment to the soil and thus its survival in the face of stress conditions **[18]**. Using germination and seedling traits can be indicator to express of variation in genotypes during this stage.

Developing a breeding programs relies on understanding the crop's geographical distribution and its genetic variation in advance **[19]**. While, traditional plant breeding relied on visual traits to assess genetic diversity, modern techniques use molecular markers to analyze DNA variations and their impact on observable characteristics **[20]**. Genetic diversity analysis is an important task in plant breeding as diversity in plant genetic resources (PGR) provides an opportunity for plant breeders to develop new and improved cultivars with desirable characteristics **[21].** Discussed genetic diversity analysis in order to use the new methods and technology for better and rapid assessment, for utilization of germplasm from gene banks to their applied breeding programs. Molecular markers provide a direct measure of genetic diversity and

go beyond indirect diversity measures based on agronomic traits or geographic origin **[22]**. Molecular markers reflect the true expression of genotype, while morphological traits encompassed the expression of genotype, environment and their interaction **[23]**. Molecular characterization of flax germplasm has been made using various molecular techniques to assess genetic diversity of cultivated flax and polygenetic relationship. The success of any breeding programme mainly depends upon magnitude of genetic variability in hand and continuous supply of new germplasm for sustainable production **[24-25]**. SCOT technique was chosen for making fingerprint for newly cultivated and foreign flax genotypes to evaluate the genetic diversity among them, with the aim of providing facts and tools to increase the assortment for future flax genotypes propagation and assisting in developing and planning breeding strategies for crop improvement programs **[26]**. SCoT marker is a novel, simple, cheap and powerful tool to be used and effective for generating gene targeted in plants which was described and developed by **[27].** SCoT marker based on the short conserved region flanking the ATG start codon in plant genes. It is has been successfully applied in several sets of other plant species for genetic diversity analysis and relationship which included 35 chickpea genotypes and maize genotypes (*Zea mays* L.) **[27-28]**. In flax, various types of molecular markers such as RAPD, AFLP, ISSR and SSR have been used to estimate the genetic variation and relationships in different sets of germplasm **[29]**. Apart from molecular markers based diversity studies, limited reports are also available to describe the extent of genetic variability in flax based on morphological traits **[30]**. Start codon targeted (SCoT) marker is one of the reliable techniques as it is considered an efficient, informative and inexpensive tool. The knowledge regarding its nature and extent of genetic variability available in the germplasm and the correlation among various characters are important requirements for planning a successful breeding program for the linseed crop **[31]**. To evaluate the diversity in germplasm, various morphological, biochemical and molecular markers can be utilized.

The overall aims of the present study as follow: 1) classification of seed morphology and molecular markers, 2) evaluating germination and seedling establishment, 3) evaluating yield and its components for 12 promising flax lines and 2 check varieties grown under the prevailing Egyptian environmental conditions.

2. Materials and methods

2.1. Plant materials

New twelve promising flax strains recorded from Line S.1 to Line S.12 and were studied along with two commercial check varieties Giza 12 and Sakha 3. The genetic materials used in this study to comprised twelve flax lines introduced from Holland and two Egyptian commercial flax varieties which presented in Table 1.

Table 1: Sources of different flax genotypes of fiber types (F), dual purpose types (D) and oil types (O).

Genotypes	Pedigree	Classification
S.1	279 Ha	F
S.2	Hylbema V.2	D
S.3	259k Kindecodev bowk	F
S.4	2526 in Egyption	F
S.5	240 linede wermilio	F
S.6	258 lin dewnd gcrobyssine	O
S.7	Linolno.2	D
S.8	L. 26. Gosomer	D
S.9	266 OH 804D	O
S.10	305 OH 870B	O
S.11	160 Pale Blue	D
S.12	Percello	O
Giza 12	S.2419 × S.148/6/1	D
Sakha 3	I.2569 × Belinka	F

*S.: Strain

2.2. Field Experiments

Two field experiments were carried out in Giza Research Station, Agricultural Research Center, Egypt during the two successive seasons (2022/2023 and 2023/2024). Flax seeds of each genotype were sown during the second week of November for all trails in two seasons. The experimental design was a randomized complete block with three replications. Plot area was 6 m^2 (2× 3m). Each plot consisted of 10 rows with 3 meters' length and 20 cm apart. Flax seeds were sown at the rate of 2000 seeds/m^2. Normal cultural practices for flax production were done as recommended. At harvest, ten guarded plants were taken randomly from the central row to estimate the characters under study.

2.3. Seed Morphological Identification

The identification of the following seed morphological characters was conducted using the procedures of UPOV (The International Union for the Protection of New Varieties of Plant). Seed size, seed color, seed length, seed width and seed length to width ratio were classified at Laboratory of Seed Technology Department, Field Crops Research Institute, Agricultural Research Center. The decimal code for the growth stage of legume was also used to standardize **[32]**.

2.4. Laboratory Experiments

Laboratory experiments were conducted at Laboratory of Seed Technology Department, Field Crops Research Institute, Agricultural Research Center. Laboratory experiment laid out in completely randomized design (CRD) with four replications **[33]** .Germination test was performed according to ISTA rules, whereas 50 seeds of flax were sown in each sub-replication in sterilized Petri dishes covered at the bottom with two sheets of Whitman filter paper, then placed in an incubator at 20±2°C. Total numbers of seeds germinated were counted daily and germination percentage was calculated at 7^{th} day. Measurements were calculated as following: Seed germination (%) = (number of germinated seeds / total number of seeds) × 100. Germination rate (GR): It was calculated **[34]**. GR= a + (a +b) + (a + b + c) …….... (a + b + c + m) / n (a + b + c + m), where a, b, c is no. of seedlings in the first, second and third count, m is number of seedlings counted on successive days, n = total number of counts. Speed germination index SGI = (number of seeds germinated / days to first count) + ... + (number of seeds germinated / days to final count). Seeds were considered germinated when the radicle reached at least 2 mm in length **[35]**. On the 7^{th} day after planting, the root and shoot lengths (cm) of ten normal seedlings were measured. Seedling fresh weight was recorded by weighing the same ten seedlings. These seedlings were dried in a hot-air oven at 85°C for 12 hours to determine their dry weight (g). Seedling vigor index I (SVI) = germination (%) × seedling length (root + shoot). Seedling vigor index II (SVII) = germination (%) × seedling dry weight (root + shoot) **[36].**.

2.5. Yield and Yield Components:

Straw yield and related characters: Plant height (cm), technical length (cm), fruiting zone length (cm), straw yield/plant (g), straw yield/feddan (ton) and fiber yield/feddan (kg).

Seed yield and related characters: Number of capsules/plant, weight of capsules/plant (g), seed index (g), seed yield/plant (g), seed yield/feddan (kg) and oil yield/feddan (kg).

Technological characters: Fiber length (cm), Total fiber percentage (%) which calculated by (fiber yield/feddan) / (straw yield/feddan) × 100, Oil percentage (%) which was determined by Soxhlet extraction apparatus **[37]**.

2.6. Genomic DNA Extraction

Genomic DNA was extracted from young leaves of the flax according to https://primerdigital.com/dna.html using CTAB solution (2%CTAB, 1.5 M NaCl, 10 mM Na EDTA, 0.1 M HEPES-acid; 100 ml (2 g CTAB, 2.4 g HEPES-acid, 2 ml 0.5 M Na EDTA, 30 ml 5 M NaCl, Chloroform-isoamyl alcohol mix (24:1), 100% isopropanol (isopropyl alcohol, 2-propanol), 70% ethanol and 1xTE (10 mM Tris HCL, pH8.0; 1 m M EDTA).

PCR amplification of SCOT markers, thirteen primers SCOT was used in this study (Table 2). PCR were carried out in 20 µl for both markers containing 10 X PCR buffer, 25 mM MgCl, 10 mM dNTPs, 2 µM primer, 5 U Taq DNA polymerase and 100 ng templates DNA. All PCR reactions were carried out in an eppendorf Thermal Cycler. PCR programmed: 95ºC for 5 min; 35 cycles (95 ºC for 30 sec, 50 ºC for SCOT for 45 ºC, 72 ºC for 1:30 min) and 72 ºC for 5 min. The amplification products were resolved by electrophoresis in agarose gel containing ethidium bromide (0.5 µg/ml) in 1 X THE buffer at 80 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

Table 2. Name, Sequences and GC% for SCOT primer.

	SCOT primers	Sequences (5' - 3')	GC%
1	SCOT 1	CAACA ATGGCTACCACCA	50
2	SCOT 2	CAACA ATGGCTACCACCC	56
3	SCOT3	CAACA ATGGCTACCACCG	56
4	SCOT 5	CAACA ATGGCTACCACGA	50
5	SCOT 6	CAACA ATGGCTACCACGC	56
6	SCOT 7	CAACA ATGGCTACCACGG	56
7	SCOT 14	ACGAC ATGGCGACCACGC	67
8	SCOT 31	CC ATGGCTACCACCGCCT	67
9	SCOT 32	CC ATGGCTACCACCGCAC	67
10	SCOT 33	CC ATGGCTACCACCGCAG	67
11	SCOT 34	ACC ATGGCTACCACCGCA	61
12	SCOT 35	C ATGGCTACCACCGGCCC	72
13	SCOT 36	GCAACA ATGGCTACCACC	56

2.7. Statistical Analysis

Data was carried out according to use computer statistical program MSTAT-C v. 3.1. **[38-39]**. Least significant difference (LSD) was applied to compare mean values. Data of germination were statically analyzed by an analysis of variance (ANOVA) of Completely Randomized Design (CRD), while, yield and its components were statically analyzed by Randomized Block Design (RCBD). Data were analyzed using MEGA 5.10 (Molecular Evolutionary Genetics Analysis) version 7 (http: //www.megasoftware. net). The band profiles were scored as (1) for present bands and (0) for absent bands for SCOT marker analysis. The banding patterns generated by thirteen SCOT-PCR primers to determine the genetic relatedness of the samples under study. The genetic distances (GD) between two genotypes was estimated according to Dice coefficient **[40]**. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. Unweighted Pair Group Method using Arithmetic Average (UPGMA)

used for generate cluster analysis between genotypes **[41].** Dice formula: GSij = 2a/(2a+b+c) Where GSij is the measure of genetic similarity between individuals i and j, a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. At the first step, when each genotype represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters.

This method is called Unweighted Pair Group Method using Arithmetic Average (UPGMA) **[41]**. The basic information about molecular markers that determines their application in genetic mapping and genetic diversity was calculated for each marker using Polymorphism Information Content (PIC). PIC provides an estimate of the discriminatory power of a locus, or loci, by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles. PIC values were calculated by the algorithm: PIC = 1- $\sum_{i=1}^{n}$ fi2, Where fi2 is the frequency of the i th (presence of band) alleles in the set of all genotypes tested **[42]**. PIC values ranged from 0 (monomorphic) to 1 (very highly discriminative) with many alleles each in equal and low frequency. The analysis was conducted by using iMEC software programe online on an example data set taken for using SCOT primers with the fourteen flax genotypes (*Linum usitatissimum* L.) **[43]**.

The data set is available for download and the resulting calculations are summarized in Table 6. The D parameter (discriminating power of primer) was used evaluates the efficiency of the primers in identification of flax genotypes **[44]**. Marker Efficiency Calculator (iMEC software) is a simple computation of seven basic measures polymorphism indices for individual markers such as, iMEC calculates heterozygosity index (H), polymorphism information content (PIC), discriminating power (D), effective multiplex ratio (E), marker index (MI), arithmetic mean heterozygosity (Havp), and resolving power ® **[45]**. The source code used to develop iMEC is available on GitHub (https://github.com/ Limpfrog/iMEC). iMEC is available at *https:// irscope.shinyapps.io/iMEC/.* A principal component analysis (PCA) based on the SCoT data matrix was also produced using PAST software 4.02. Heat maps were created using ClustVis, an online application for visualizing the clustering of multivariate data **[46].**

3. Results and discussions

3.1. Seed Morphological Identification

Results in Fig. (1) Indicated that the seed morphological characters after harvest were different for all flax genotypes.

Seed size was low for genotypes S.2, S.5, S.11, S.12 and Sakha 3 and medium for lines S.4, S.6, S.7, S.8 and Giza 12 check variety, while was high for S.1 and S.9 and very high with lines S.3 and S.10. Seed coat color was light brown for genotypes S.1, S.3, S.6, S.9, S.10, Giza 12 and Sakha 3, while, was brown for genotypes S.2, S.4, S.5, S.7, S.8, S.11and S.12. Concerning seed length (Fig 1.), the data revealed that flax genotypes were short for lines S.2, S.5, S.7, and S.11, whereas, lines S.1, S.4, S.6, S.8 and Giza 12 were medium, and lines S.3, S.9, S.12 and sakha 3 were long, while, line S.10 recorded very long. On the other hand, Seed width of all genotypes was between narrow (lines S.8, S.11 and S.12), medium (genotypes S.4, S.6, S.10, Giza 12 and Sakha 3), broad (lines S.1, S.2, S.5, S.7 and S.9) and very broad (line S.3). In figure (1), all flax genotypes had moderately compressed for length to width ratio trait except for genotypes S. 1, S.2, S.4, S.9 and Giza 12 which were medium, whereas was moderately elongated for line S.12 and cultivar Sakha 3, and very elongated for line S.10.

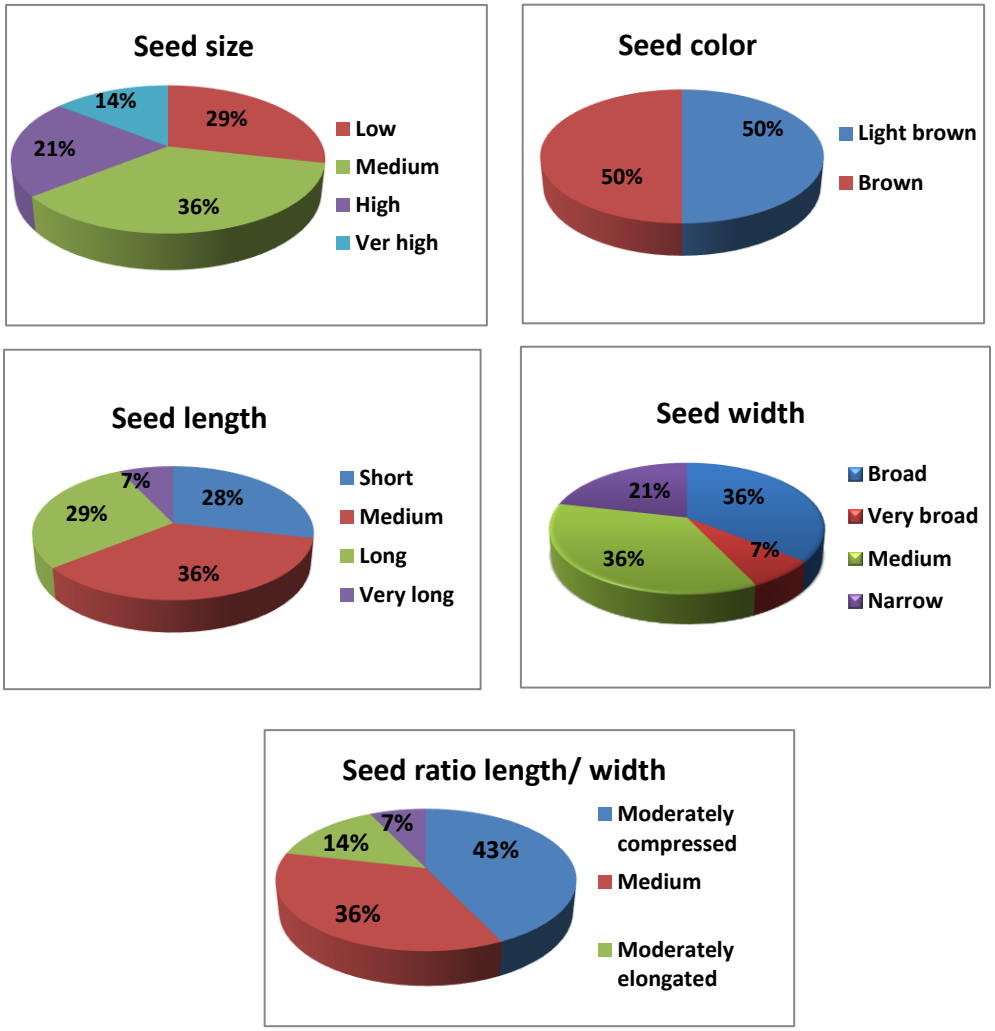

Fig. 1. Frequency distribution of seed flax morphological traits

3.2. Germination and Seedling Characters:

Germination characteristics

Means performance of the evaluated genotypes under laboratory conditions are presented in (Fig.2) clearly indicated significant differences at ($P < 0.05$) between lines and check varieties for all seed germination characters. The highest values of G % was observed on genotypes by line S.1 and Giza 12 which were 100% followed by the lines S.9, S.8 and S.2, respectively, not statistically significant. While, lines S.3, S.6 and S.12 recorded the lowest percentage compared with other genotypes. GR was highest in S.8 and S.5 (2.0 and 1.6, respectively), however, the lowest value was to S.9 (0.9) compared with other genotypes. Moreover, SGI is demonstrated in Figure (2), data indicated that line S.8 reached the maximum SGI which were (31.8), compared with other lines and check varieties, while the lowest Sakha 3 and line S.3 recorded the lowest

values which were 18.2 and 19.3, respectively. Meanwhile, Fig. (2) illustrates the MGT of the seed flax under the study, it indicates that highly significant differences were observed between different genotypes. Where, S.1 and S.8, and Giza 12 genotypes showed the supreme amount followed by S.5, S.2, S.4 and S.10 . Compared between genotypes, line S.9 was the lower value than other genotypes which was 5.6 (Fig.2).

Seedling parameters

Data presented in Figures (3, 4 and 5) show significant effect between different genotypes on shoot length (cm), root length (cm), seedling length (cm), seedling fresh weight (g), seedling dry weight(g), seedling vigor I (SVI) and seedling vigor II (SVII) of seed germinated under investigation. The date clear that some lines caused improvement of above mentioned parameters compared to check varieties under corresponding laboratory conditions.

The present study also indicates that lines significantly improved shoot, root and seedling length (cm) (Fig.3). However, lines S.1, S.2, S.5 and S.8 gave higher values (12.49 and 8.46 cm), (11.20 and 8.17 cm), (11.58 and 7.72 cm) and (11.07 and 7.49 cm) for seedling length and shoot length, respectively, and the lowest values were for S.9 and Sakha 3 (7.72 and 4.60 cm) and (7.54 and 3.38 cm), respectively. Whereas, it gave high values with S.4 and S.10 (4.63 and 4.79 cm, respectively) for root length and the lower value with S.2 (3.03 cm). On the other hand, seedling fresh weight and dry weight were significant increase with S.3, S8 and S.10 which were 0.71, 0.73 and 0.87g for seedling fresh weight and the lowest value for S.2 and S.6 (0.40 and 0.47g, respectively). For seedling dry weight, the values had increased to 0.07g (S.3), 0.05g (S.8) and 0.05g (S.10) followed by the rest of lines comparing with check varieties (Fig. 4). Maximum SVI in Fig.(5) was highly significant between different lines and check varieties, which had increased with S.3 (64.1),S.8 (48) and S.10 (40.1), while, it was decreased with S.2, S.5, S.7 and S.9 (12.6, 13.5, 7.5 and, 15.4 respectively). Data indicated that SVII was increased significant and gave the highest value for S.1 (1249.0) , S.2 (1083.6), S.5 (1118.3) and S.8 (1069.6). Whereas, it gave low values with S.9 which was 778.6 comparing with other genotypes (Fig. 5).

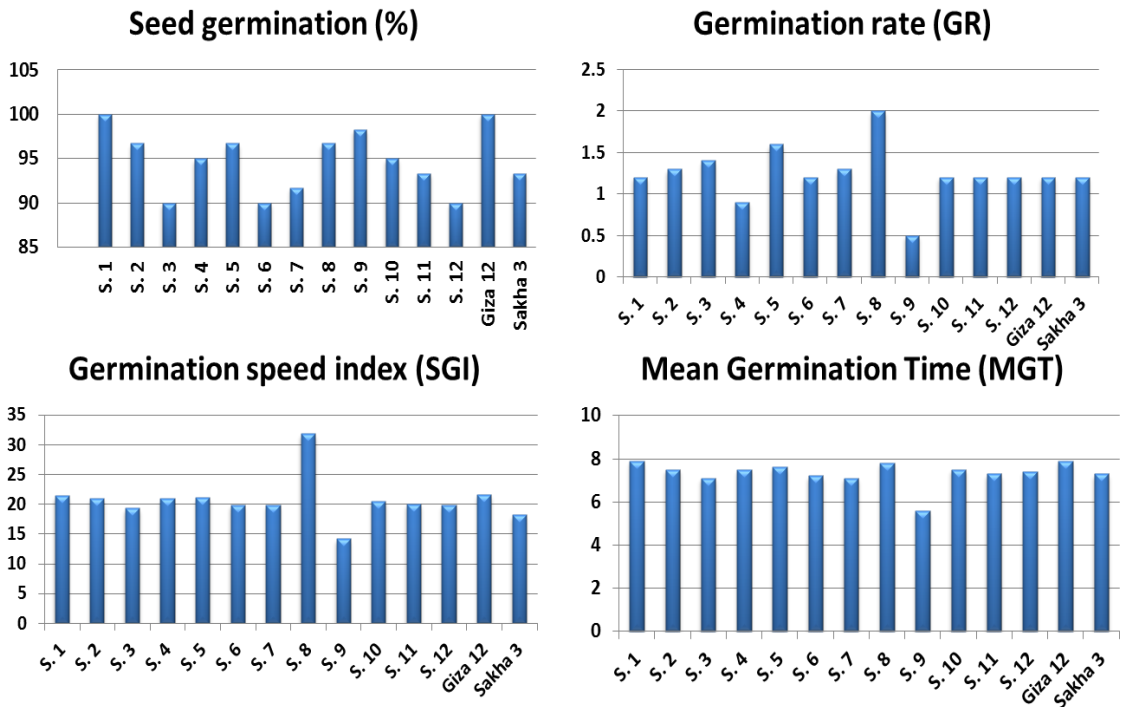

Fig. 2. Means of seed germination (%), germination rate, germination speed index (SGI), Mean germination time (MGT) of flax genotypes.

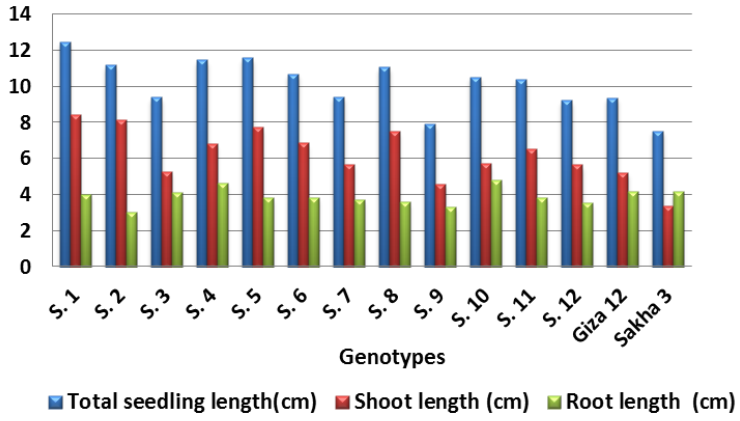

Fig. 3. Means of total seedling length (cm), shoot length (cm) and root length (cm), of flax genotypes.

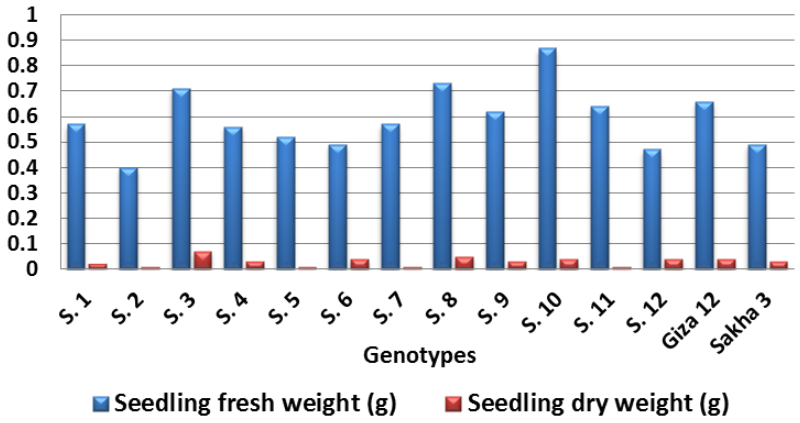

Fig. 4. Means of total seedling fresh weight (g) and seedling dry weight (g) of flax genotypes.

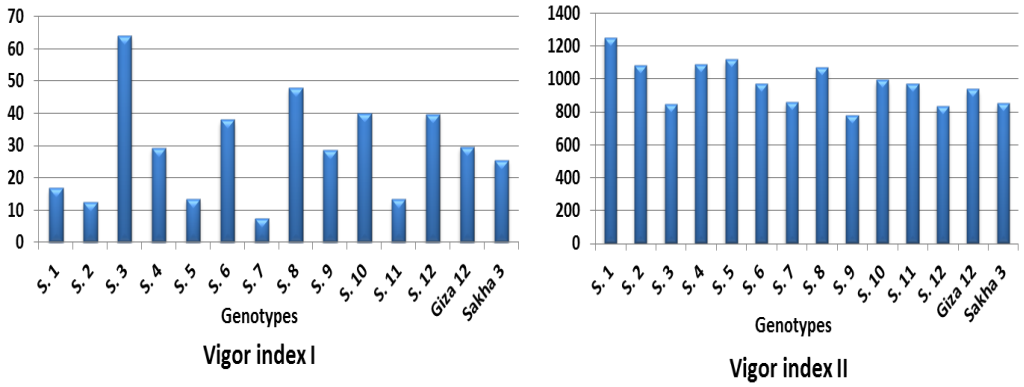

Fig. 5. Means of seedling vigor I and seedling II vigor of flax genotypes.

3.3. Yield and Yield Components:

Straw yield and related characters

The experiment results showed significant differences between evaluated genotypes in straw yield and related characters showed in Table 3. Data showed that commercial flax variety Sakha 3 ranked first in plant height and technical length (115.10 and 98.22 cm), respectively followed by Strain 3 (112.46 and 95.32 cm). While, Strain 12 and S.9 superiority in fruiting zone length (22.95 and 22.88 cm, respectively). The dual commercial variety Giza 12, S.3 and S.8 significantly surpassed other studied genotypes in straw yield / plant as well as per feddan (2.35, 2.35 and 2.36 g/plant) and (4.21, 3.82 and 4.19 ton/fed. , respectively). On the other hand, S.3 and S.8 recorded the highest values of fiber yield / feddan (820.15 and 812.87 kg). The superiority of S.3 and S.8 genotypes in fiber yield over than other studied genotypes might be attributed to genetic factors. The tested flax genotypes weren't similar in all straw yield and its traits these variations ought to the genetic factors.

Table 3: Combined main values of genotypes for straw yield and its attributes in seasons 2021/2022 and 2022/2023.

Genotypes	Plant height (cm)	Technical length (cm)	Fruiting zone length (cm)	Straw yield /plant (g)	Straw yield /fed. (ton)	Fiber yield /fed. (kg)
S. 1	104.64	83.53	21.11	1.90	3.65	697.51
S. 2	88.97	75.21	13.76	2.25	3.53	632.22
S. 3	112.46	95.32	17.14	3.35	3.82	820.15
S. 4	105.70	89.52	16.18	1.95	3.69	715.55
S. 5	108.64	92.95	15.69	1.98	3.74	716.21
S. 6	67.60	54.81	12.79	2.10	3.45	500.94
S. 7	87.53	70.68	16.85	2.28	3.50	654.50
S. 8	87.80	71.58	16.22	2.36	4.19	812.87
S. 9	65.65	42.88	22.88	2.12	3.40	511.02
S. 10	66.31	46.52	19.79	2.10	3.42	485.98
S. 11	91.78	75.48	16.30	2.04	3.55	657.46
S. 12	68.46	45.51	22.95	2.29	3.47	496.21
Giza 12	95.75	79.85	15.90	2.35	4.21	788.53
Sakha 3	115.10	98.22	16.88	1.83	3.97	796.04
LSD (0.05)	5.72	5.23	4.10	0.10	0.12	30.22

Seed yield and related characters

The results in Table 4 showed high variation among flax genotypes for seed yield and related characters. S.6 ranked first in number of capsules / plant and weight of capsules / plant (63.15 and 2.14 g) followed by S9 and S.10. While, Giza 12 exceeded the other genotypes in seed index (11.18g), while S.6 genotype (oil type) came in the second rank after commercial genotype Giza 12 in seed index (11.09 g). Seed yield, seed yield/fed and oil yield/fed had high variation between different genotypes . S.6 and S.10 recorded the highest values of these traits (2.40 and 2.33g seed yield/plant (g)), (680.22 and 667.40 seed yield/fed(kg)) (290.94 and 260.04 oil yield/fed(kg)) , respectively.

Table 4. Combined main values of genotypes for seed yield and its attributes in seasons 2021/2022 and 2022/2023.

Genotypes	No. of capsules /plant	Weight of capsules/ plant (g)	Seed index (g)	Seed yield/plant (g)	Seed yield/fed(kg)	Oil yield/ fed (kg)
S. 1	34.66	1.37	5.57	1.51	495.53	171.05
S. 2	40.24	1.12	7.89	1.86	515.60	189.01
S. 3	27.87	1.11	5.65	1.18	432.43	151.78
S. 4	31.31	0.91	5.53	1.25	440.55	152.43
S. 5	26.17	1.11	4.99	1.42	487.90	166.95
S. 6	63.15	2.14	11.09	2.40	680.22	290.94
S. 7	45.52	1.32	7.41	2.01	528.08	191.96
S. 8	49.88	1.27	7.48	1.93	520.91	186.48
S. 9	60.24	2.07	6.95	2.11	539.50	208.78
S. 10	59.82	1.97	9.10	2.33	667.40	260.04
S. 11	41.03	1.22	7.32	1.95	521.87	191.26
S. 12	58.32	1.49	7.01	2.23	550.92	233.84
Giza 12	47.85	1.47	11.18	2.31	606.50	240.39
Sakha 3	35.13	0.89	4.95	1.33	460.75	163.15
LSD (0.05)	11.12	0.39	0.17	0.29	9.43	10.22

Technological characters

The mean performance of the studied genotypes for technological characters was shown in Table 5 that indicated significant differences among flax genotypes in relation to the three technological characters i.e., fiber length, fiber percentage and oil percentage. S.3 genotype reported maximum value in fiber length (98.31 cm) and fiber percentage (21.53 %) followed by strain 8 (93.96 cm and 20.23%) and Sakha 3 (95.48 cm and 20.08%), respectively without significant differences. These results may be due to the variation in technical length of different flax genotypes (fiber, dual and oil types). While, the dual purpose genotype S.3 ranked first in oil percentage (44.59 %) followed by oil type Strain 10 (42.31 %), Giza 12 (41.74%) and Strain 12 (40.19 %) .

Table 5. Combined main values of genotypes for technological characters in seasons 2021/2022 and 2022/2023.

Genotypes	Fiber length (cm)	Fiber percentage (%)	Oil percentage (%)
S. 1	92.42	19.11	34.52
S. 2	73.88	17.91	36.66
S. 3	98.31	21.53	35.10
S. 4	92.15	19.39	34.60
S. 5	93.94	19.15	34.22
S. 6	49.38	14.52	44.59
S. 7	72.63	18.70	36.35
S. 8	93.96	20.23	35.80
S. 9	47.41	15.03	38.70
S. 10	47.15	14.21	42.31
S. 11	78.32	18.52	36.65
S. 12	50.85	14.30	40.19
Giza 12	79.51	18.73	41.74
Sakha 3	95.48	20.08	35.41
LSD (0.05)	10.27	1.39	0.50

3.4. Molecular Marker

SCoT analysis

As shown in Fig. 6; Tables 6 and 12 SCoT primers produced a total of 119 bands; 33 bands were monomorphic, and 86 bands were polymorphic, with 72% (polymorphism) including 19 unique bands (7 positive specific markers and 12 negative specific markers). The molecular size ranged from 100 to 2000 bp, and the number of bands was between 4 and 12. In addition, SCoT-35 and SCOT-32 produced 12 bands, followed by SCoT-7, which produced 11 bands; SCoT-2 and SCoT-3 produced 10 bands; and SCoT-14, SCoT-31, and SCoT-33 and SCOT- 34 produced 9 bands. SCOT-3, SCOT-6 and SCOT-36 produced 8 bands. The highest polymorphism percentage was 100% produced by SCOT-14. And the lowest polymorphism percentages were produced by SCOT-33 was 56%. The primers SCoT-2, SCoT-7 and SCoT-14 had the highest number of polymorphic bands (9 bands).

While SCOT-3 produced the lowest number of polymorphic bands (2 bands) the primers SCoT-2 and SCoT-36 primer generated the highest number of unique bands (4). In addition, primer SCoT-3, SCoT-6, SCoT-33 and SCoT-34 recorded the lowest number of unique bands (1 band). The molecular genetic distinctions between the fourteen flax genotypes were clarified by using the

data shown in Table 6 which also identified the unique markers for each genotype to serve as a basis for classification. In addition, these bands can be considered molecular genetic markers for each genotypes. The primer SCoT-2 exhibited four unique fragments (two positive unique bands marked (S.8) with 1000 and bp 1100 and two negative unique bands marked (S.2) with 700 and 800 bp. The primer SCOT-36 marked two genotypes, S.2 with two positive unique fragments 900 and 1000pb and two negative unique fragments marked genotype S.9 at 700 and 800pb. Three negative unique markers were generated by the primer SCoT-32, distinct genotype S.4 (400bp), S.8 (700pb) and S.12 (800pb). Two negative unique bands marked genotypes S.8 and S.9 with 500 and 600pb respectively. Two positive unique fragments distinct genotype S.8 with 1200 and 1500pb. Primer SCOT-3, SCOT-6 and SCOT-34 produced one negative unique fragments marked genotypes S.11, S.7 and S.8 with 800, 700 and 500 respectively. One positive unique bands marked genotype S.1 with 700pb.

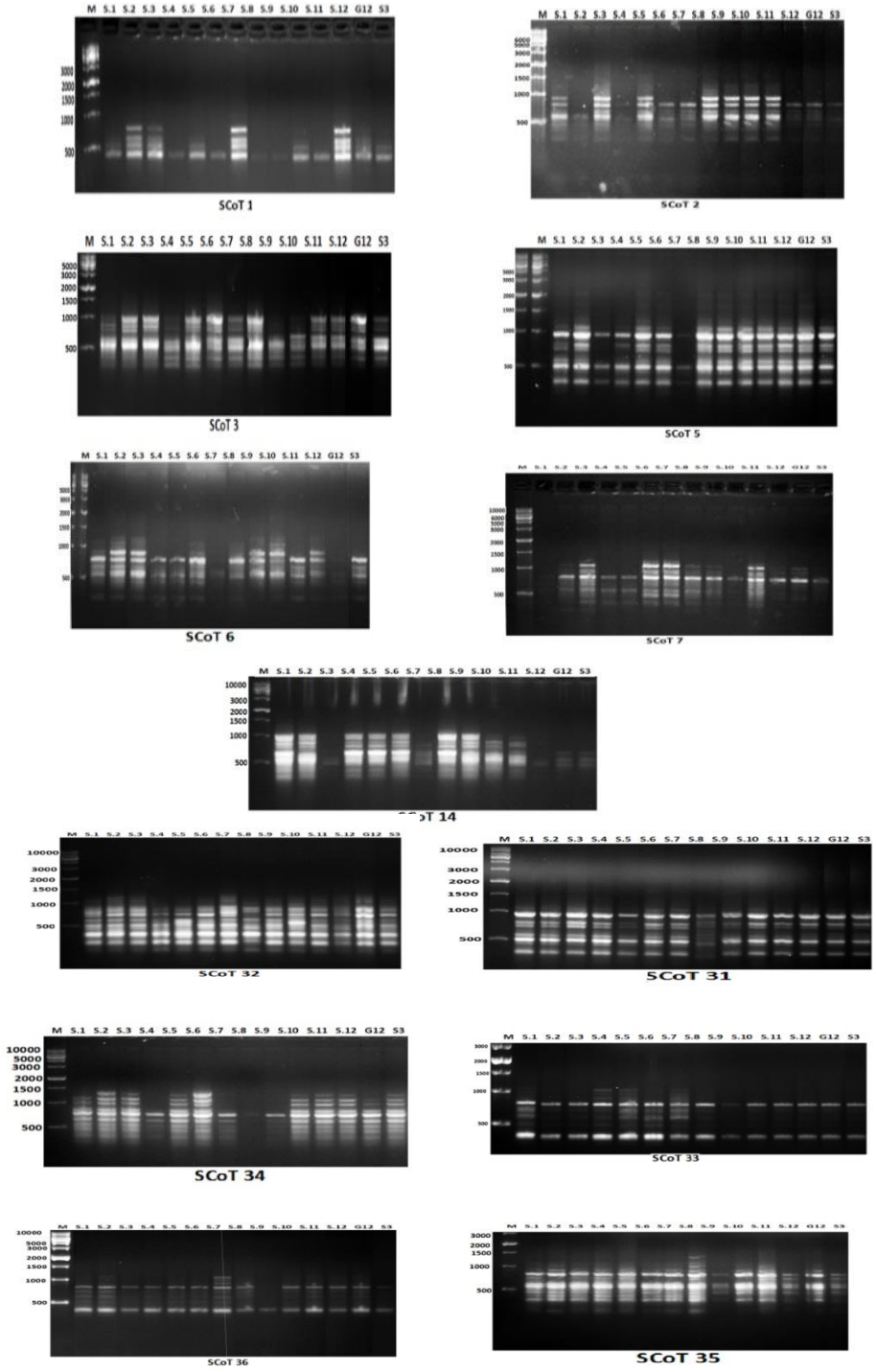

Fig. 6. SCoT fingerprints of the 14 flax genotypes tested using 13 SCOT primers. M (marker), Lanes 1–14 flax genotypes.

Table 6: SCOT amplification in 14 Flax genotypes. FR Fragment range, TB total bands, PB polymorphic bands, MB monomorphic band, , , P% polymorphism percentage, UB Unique bands, + ve UB positive unique bands, − ve UB negative unique bands, G Genotype, H heterozygocity index, PIC polymorphism information content, E efective multiplex ratio, H.av arithmetic mean of H, D Discriminating power, MI marker index, RP resolving power.

primers	FR	TB	PB	MB	P%	UB	ve+	band size	G	ve-	band size	G	H_0	PIC_0	E_0	H.av_0	MI_0	D_0	R_0
SCOT 1	400 -700	4	3	1	75	0	0			0	0	0	0.444	0.346	2.667	0.009	0.025	0.560	2.667
SCOT 2	300- 1100	10	9	1	90	4	2	(1000, 1100)	8	2	(700-800)	2	0.700	0.641	1.000	0.700	0.700	0.254	
SCOT 3	300 -1000	8	2	6	25	1	0			1	800	11	0.593	0.513	1.000	0.593	0.593	0.113	
SCOT 5	300 - 2000	10	7	3	70	0	0	0		0	0	0	0.606	0.552	1.000	0.606	0.606	0.254	
SCOT 6	300 -1100	8	7	1	88	1	0			1	700	7	0.699	0.642	1.000	0.699	0.699	0.276	
SCOT 7	300 - 1200	11	9	2	82	0	0	0		0	0	0	0.667	0.611	1.000	0.667	0.667	0.295	
SCOT 14	300 - 1000	9	9	0	100	0	0	0		0	0	0	0.517	0.470	1.000	0.517	0.517	0.183	
SCOT 31	300 -1100	9	5	4	56	2	0			2	500	8	0.620	0.548	1.000	0.620	0.620	0.152	
											600	9							
SCOT 32	100 - 1100	12	7	5	58	3	0			3	400	4	0.612	0.554	1.000	0.612	0.612	0.189	
											700	8							
											800	12							
SCOT 33	400 - 1000	9	5	4	56	1	1	700	1	0	0	0	0.674	0.615	1.000	0.674	0.674	0.154	
SCOT 34	300 - 1300	9	8	1	89	1	0			1	500	8	0.651	0.585	1.000	0.651	0.651	0.260	
SCOT 35	100 - 1500	12	8	4	67	2	2	(1200-1500)	8	0	0	0	0.679	0.620	1.000	0.679	0.679	0.214	
SCOT 36	400 - 1100	8	7	1	88	4	2	(900 - 1000)	7	2	(700-800)	9	0.663	0.605	1.000	0.663	0.663	0.168	
Total		119	86	33	72	19	7			12			0.625	0.562	1.128	0.592	0.593	0.236	

On the other hand, the lowest value for heterozygosity index (H) was 0.44 produced by SCOT-1 and the highest was 0.7 produced by SCOT-2. Polymorphism information content (PIC) was high by SCOT-6 (0.642) and the lowest value was obtained by SCOT-1 (0346), the arithmetic mean of H (H.av) and marker index (MI) for SCOT-2 was (0.7) and the lowest was (0.009) obtained by primer SCOT-1 and (0.025) for MI, Discriminating power (D) was high for primer SCOT-1 (0.560) and the lowest value was obtained by SCOT-3 (0.113), and resolving power (RP) was obtained with primer SCOT-1 (2.667).

Cluster analysis

The dendrogram of the 14 genotypes that were studied is displayed in Fig. 7 based on the SCOT marker. The genotypes fell into two major clusters, according to UPGMA cluster analysis. S.8 and S.9 were found in the same cluster. The remaining 13 genotypes are clustered into a subcluster within the second main cluster. Thus, this supported their high degree of commonality. S.7 separated from 12 genotypes in separated sub-cluster. Giza12 and Sakha3 found in the same subcluster with S.12. While S.1, S.5, S.10, S.2, S.11, S.3 grouped near to each other in one sub-cluster.

Genetic distances

Table 7 shows the levels of genetic distances among the 14 genotypes that were examined using the SCoT marker. Genetic distance between flax genotypes ranged between 0.560 and 0.061 . The Lowest genetic distance value was between genotypes S.12 and Giza12 (0.061). The highest genetic distance value was between genotypes S.4 and (S.12) 0.560.

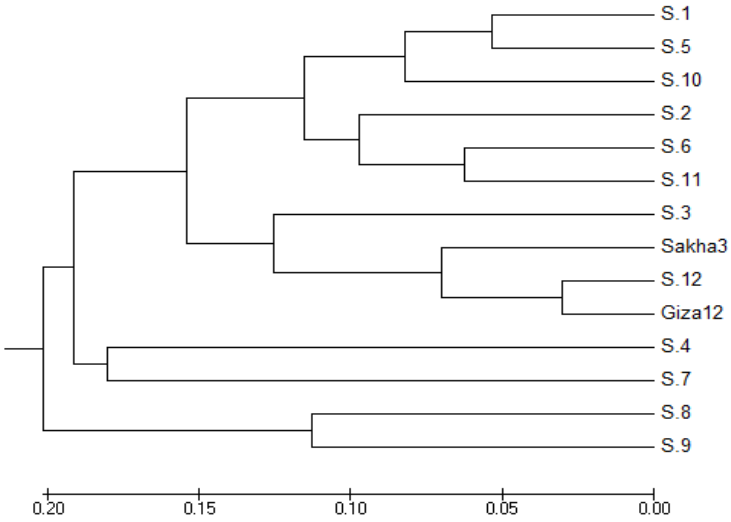

Fig.7. UPGMA cluster analysis based on genetic distances, showing the genetic relationships among the 14 Flax genotypes tested, obtained from SCoT markers.

Table 7: Genetic distances values generated using MEGA 5 software for the data produced from SCoT primers for the 14 flax genotypes.

	S.1	S.2	S.3	S.4	S.5	S.6	S.7	S.8	S.9	S.1	S.1	S.1	Giza	Sakh
S.1	-													
S.2	0.2	-												
S.3	0.3	0.2	-											
S.4	0.2	0.4	0.4	-										
S.5	0.1	0.2	0.3	0.1	-									
S.6	0.2	0.1	0.2	0.3	0.2	-								
S.7	0.4	0.4	0.2	0.3	0.4	0.3	-							
S.8	0.3	0.3	0.4	0.4	0.3	0.3	0.5	-						
S.9	0.3	0.3	0.4	0.4	0.3	0.3	0.5	0.2	-					
S.10	0.1	0.2	0.3	0.3	0.1	0.2	0.4	0.4	0.3	-				
S.11	0.2	0.2	0.2	0.3	0.2	0.1	0.4	0.3	0.2	0.2	-			
S.12	0.3	0.2	0.2	0.5	0.3	0.3	0.4	0.5	0.4	0.3	0.2	-		
Giza	0.4	0.3	0.2	0.4	0.3	0.3	0.3	0.5	0.3	0.3	0.2	0.0	-	
Sakh	0.3	0.2	0.2	0.4	0.2	0.2	0.3	0.4	0.3	0.2	0.2	0.1	0.10	-

Principle component Analysis

PCA showed clear differentiation between flax genotypes (Fig. 8), it separated flax to three groups genotype Sakha3, Giza12, S.3 and S.7 found in separated groupe from other genotypes. Genotype S.8 and S.9 found closed to each other in one group. While, S.1, S.2, S.4, S.5, S.6, S.10, S.11, S.12 found scattered in one group.

Heat map

Heat map divided flax genotypes to three clusters (Fig. 9), first cluster have genotypes S.8 and S.9 in one subcluster seprated from S.2, S.6 and S.11. Second cluster gave S.1, S.5, S.4 and S.10, third cluster have S.3, S.7, Giza12, Sakha3 and S.12.

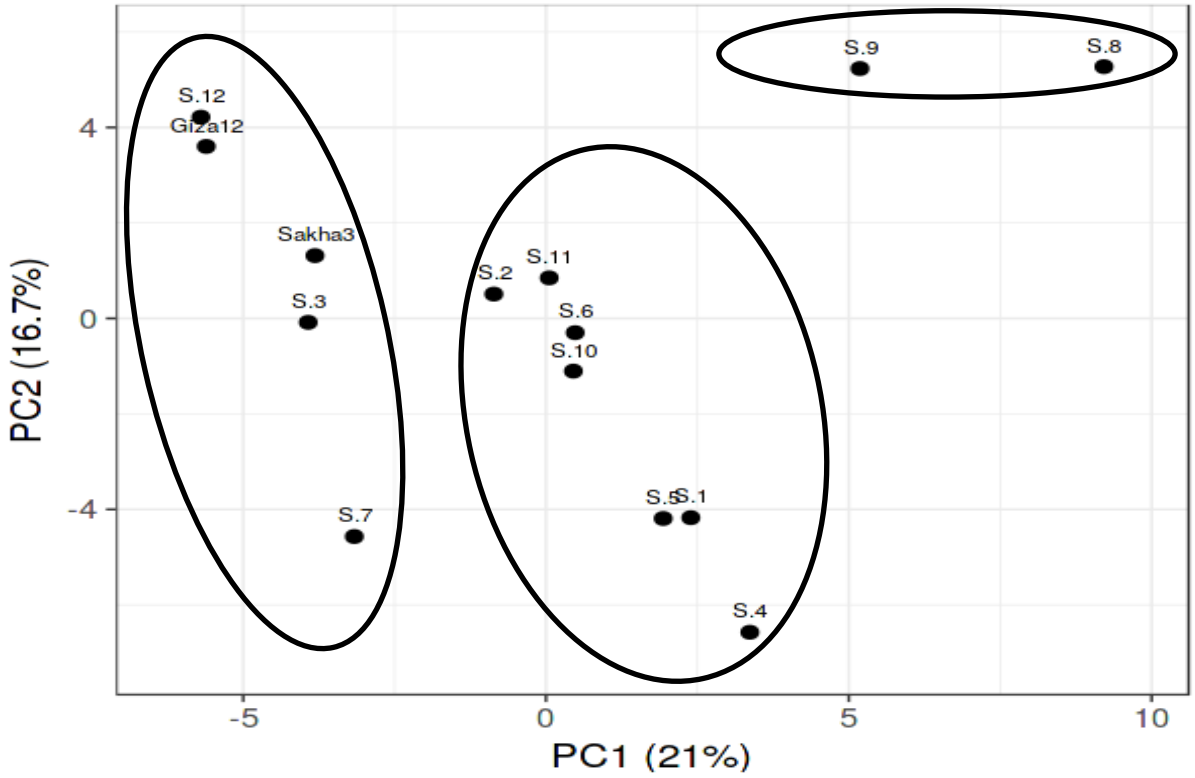

Fig. 8. Illustration of the genetic diversity expressed in 14 Flax genotypes, according to a principal component analysis (PCA) based on polymorphism of SCoT using PAST software.

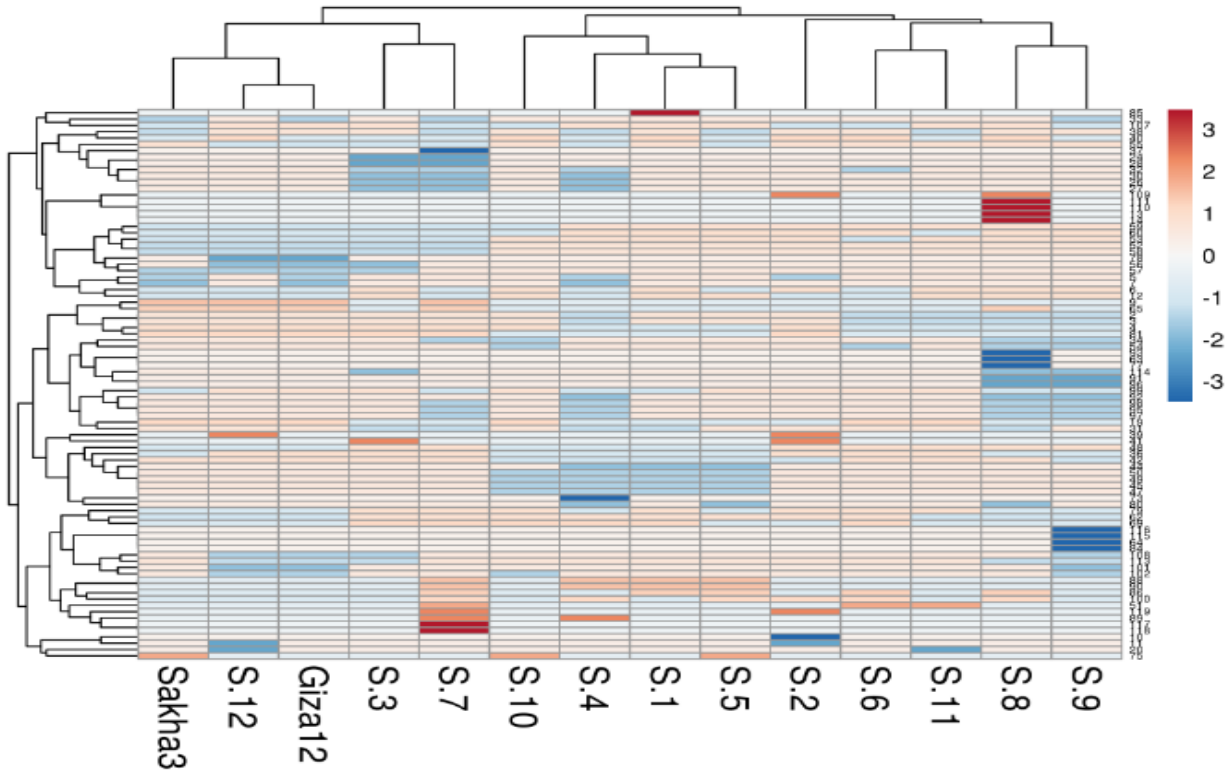

Fig. 9. Multivariate Heatmap illustrating the genetic diversity of 14 flax genotypes, based on 13 SCoT primers for using the module of a Heatmap of ClustVis—an online tool for clustering and visualizing of multivariate data.

4. DISCUSSION

Selection of diversified materials is crucial for widening the genetic base of breeding genotypes collection.The success of breeding program usually depends on the right choice of parental groups at the inception **[47]**. In our study, we had used a total of fourteen genotypes for the diversity evaluation of 12 lines from Holland and two Egyptian check varieties. It has evaluated seed germination traits, seed morphology, oil yield and its related traits, fiber and its related traits, and molecular marker under Egypt conditions and results showed that there was high variation between all genotypes under study. Genetic diversity has been crucial in the evolution of crop plants. For a breeding program effort targeted at crop development on good and rational foundation, knowledge of the current genetic diversity in the crop species is required. The choice of suitable diverse parents is crucial when breeding cultivars for diverse agro-climatic zones because hybridizing genetically diverse parents for aparticular character may be helpful in unleashing a wide spectrum of variability in segregating generations, adding new gene pools to the population and extending the range of variation **[48]**. It has been evaluation of the use of flax

seeds plants for feed , food, fibre and value-added product has been conducted **[49-50].** The seed characters in different crops with different genotypes had high differentiation in many traits of seeds. linseed is one of the richest plant-based sources of omega-3 fatty acids and contains about 55% ALA of the total fatty acids **[9,10,11,12,13,14]**. Additionally, Dut to that flax seeds high omega-3 fatty acid concentration, dietary fibre, high-quality protein and lignans , linseed has been known as a nutrient rich food **[51-5]**. Many studies revelaed that the germination stage is an important stage for all crops showed that the flax cultivar can be classified into two groups, Sevanade and Bavaria the best performing under stress conditions, the two cultivars having medium tolerance to water stress at germination stage and early seedling growth **[10,11,12,53]**. Increasing of seed yield was due to increase of dry matter accumulation in the later formed capsules may be attributed to high temperature and long photoperiod that exist during capsules development **[54].** The above cited results are due to the higher reproductive growth of strains. It has been high variation between the tested flax genotypes which were not similar in all straw and seed yield and its traits, and technological characters addition to morphological traits **[55-56**].

Here, we aimed to exploit SCOT markers to determine the levels and patterns of genetic diversity and polymorphism of flax genotypes. This work to identify genetic diversity for agronomic or morphological characters; provides data on linseed crop diversity; can help choose parents for crosses in breeding program; and suggests appropriate sites for increased activity in germplasm conservation or collection. The profile and distribution of the genetic relatives in the dendrogram showed that the Flax genotypes were distributed in two clusters (Fig.5). The first cluster contains the two genotypes closed to each other S.8 and S.9 they have similar traits in seed morphological traits and seed germination rate also they found in the same groupe in PCA and in the same cluster in Heat map. Using the same parameters to study the genetic diversity of 48 flax germplasm using 90 SSR and 10 ISSR markers and morphological markers for molecular diversity analysis **[57]**. Study of genetic diversity between 28 flax genotypes using RAPD and ISSR makers analysis and recording high genetic variation between oil and fiber flax, they found 9 exotic lines and showed high level of genetic variation with respect to oil and fiber contents **[58]** .These results are relatively consistent with our results which recorded that S.3, S.7, S.12, Giza12 and Sakha3 found in the same cluster in heat map and dendrogram and in the same group in PCA also have similar traits in seed morphological traits (seed size), germination rate, seed germination index, straw yield and seed yield. The other traits found scattered in one group in PCA and distriputed in dendrogram and heat map and they have different traits in all parameters. Four promising

Egyptian flax (*Linum usitatissimum* L.) genotypes identified as the most diverse genotype using 13 ISSR markers for the genetic examination yielding 119 loci, of which 86 were polymorphic **[59]**.

5. Conclusion

The current investigation was conducted to evaluate 12 promising flax lines comparing to flax commercial varieties, based on germination, seed Agro-morphological traits and molecular levels. By evaluating the novel flax lines, genotypes showed a wide variance concerning the different studied traits. Based on the resulted yield component for the studied flax genotypes, it was obvious that the some lines exceed in oil%, seed yield per plant (g), seed yield per feddan (kg) such as S.6 and S.10. meanwhile, S.3 and S.8 recorded the highest fiber yield per feddan. According to the findings, it was obvious that the promising lines could be considered as a rich source and valuable genetic resource; and introduced breeding material by direct and indirect selection for improving seed yield and fiber quality and quantity in the future breeding programs. Also, SCOT markers can used to assess the genetic diversity and study genetic relationships between flax genotypes.

6. Data availability statement.

Data that supporting the paper is available on request

7. Author contributions

Conceptualization and design of study: AAA, seed morphological and germination tests: AAA. Field experiment; procedure, sampling and data collection: AAA, RHHA. DNA and SCOT marker: MG. Data analysis: AAA, MG, RHHA. First manuscript draft: AAA, RHHA, MG. Reviewing and editing of manuscript: AAA. All authors commented on previous versions of manuscript. All authors read and approved final manuscript version.

8. Conflict of interest

The authors report there are no competing interests to declare.

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