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MORPHOLOGICAL, BIOCHEMICAL, AND MOLECULAR ANALYSES TO ASSESS THE FLAX (*LINUM USITATISSIMUM* L.) GENOTYPES UNDER SANDY SOIL CONDITIONS

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SUMMARY

Flax (*Linum usitatissimum* L.) is a significant marketable crop for seed, oil, fiber, and pharmaceutical products. The latest study transpired to assess the morphological, biochemical, and yield traits with inter-simple sequence repeat (ISSR) markers to determine the genetic diversity among flaxseeds of Sakha-1, Sakha-2, Sakha-3, Sakha-5, Sakha-6, Giza-12, and Amon genotypes under sandy soil conditions. Results indicated that all flax varieties varied significantly ($P > 0.05$) in most studied characteristics. Sakha-6 exceeded all genotypes in seed yield (0.888 t ha^{-1}), straw yield (6.093 t ha^{-1}), oil yield (0.302 t ha^{-1}), and biological yield (6.981 t ha^{-1}). These increases were due to the rise in different biochemical contents of carotenoids (0.601 mg), proline (34.81 mg), free amino acids (396.85 mg), oil (35.46%), phenolic (189.61 mg), and total carbohydrates (32.07%), which reflected on seed yield plant⁻¹ (0.509 g). Flax genotypes showed high genetic variations; eight of the 15 ISSR primers employed resulted in 74 bands, with 35 as polymorphic. The average percentage polymorphism of the amplified loci ranged from 16.67% to 50%, the average number of polymorphic bands per primer was 4.38, and the average number of amplified bands per primer was 9.25. The marker index for ISSR values ranged from 0.08 to 2.40 for UBC-846 and UBC-825, respectively. The similarity between genotypes ranged from 0.58 to 0.90. Three markers showed significant regression association with the six traits.

Keywords: Flax (*L. usitatissimum* L.), genotypes, genetic variability, biochemical, physiological, and yield traits, ISSR markers, cluster analysis

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Key findings: Seven flax (*L. usitatissimum* L.) genotypes incurred morphological, biochemical, and ISSR markers evaluation. The flax genotypes varied significantly in all studied traits. They showed a high level of genetic variations, with the genotypes divided into two main clusters and a dendrogram created using the similarity matrix produced by UPGMA.

INTRODUCTION

The flax (*Linum usitatissimum* L.) plant is one of the family Linaceae plants; it has multiple uses such as food, textile fiber, and medicine. It is one of the essential fiber crops, extracting fibers from the stem of flax plants by retting operations for manufacturing textiles. In addition, it is a good source of oilseed. Recently, the need for flax fibers and seeds has increased globally because flax fibers served to make excellent paper, tent cloth, twine, and long, fine-fiber linen cloth. Moreover, flaxseed cakes are applicable as dairy animal feed and linseed oil for medicine; both can be edible oils for human use.

Additionally, oil seed is beneficial to create printing ink, paint, and varnish. It has become a medium for producing novel bioproducts due to its adaptability and product diversity. Research into using flax crops for bioproducts is progressive in North America, Australia, Asia, and Europe (Amit and Hall, 2010). According to numerous studies, it has medical uses in lowering hyperglycemia and hypercholesterolemia in humans, and linseed cake is a good protein supplement for poultry and ruminants (Morris, 2007). An agronomic scan showed the performance of flax genotypes differs in various environmental conditions (Nofal *et al.*, 2011; Al-Doori, 2012; Bakry *et al.*, 2012a, 2012b).

Molecular markers are effective methods for assessing genetic diversity in plant populations (Powell *et al.* 1995), particularly the highly polymorphic ISSR markers. Wiesner and Wiesnerová (2003) evaluated whether increased PCR productivity would produce additional bands in ISSR patterns by adding an extra PCR re-amplification round to the standard ISSR-PCR process in fingerprinting 53 different flax cultivars with nine anchored ISSR primers. Wiesner and Wiesnerová (2004) investigated the genetic diversity assessment within flax germplasm collections. According to

Gui *et al.* (2007), ISSR applications may help examine phylogenetic breeding characteristics and their genetic structure and diversity, which produce greater levels of DNA polymorphism.

Past studies used molecular (90 SSR and 10 ISSR markers) and morphological markers for genetic diversity analysis to demonstrate the genetic diversity of 48 flax genotypes (Pali and Mehta 2016). Therefore, this work aimed to study the physiological, morphological, seed, and oil yields and ISSR molecular markers analysis to screen seven flax genotypes grown under sandy soil conditions. Additionally, probing the population structure and genetic diversity of flax germplasm will help future programs improve this crop by identifying the degree of genetic relatedness and diversity in various cultivars.

MATERIALS AND METHODS

During the winters of 2019–2020 and 2020–2021, field studies on flax (*Linum usitatissimum* L.) started at the National Research Centre, District Al-Nubaria, and the Biotechnological Laboratory, Sohag University, Egypt. The soil of the experimental site is sandy (92.2% sand, 4.5% silt, and 3.3% clay). In a randomized complete block design (RCBD) experiment, seeds of flax cultivars Sakha-1, Sakha-2, Sakha-3, Sakha-5, Sakha-6, and Giza-12, sown on 15 November in both seasons, had rows 3.5 m long. The distance between rows was 20 cm, while the plot area was 10.5 m² (3.0 m in width and 3.5 m in length). Two thousand seeds m⁻² from each flax variety continued to sow under sandy soil conditions. Applying 63 kg ha⁻¹ of calcium superphosphate with 15.5% P₂O₅ preceded sowing. After emergence, nitrogen treatment as ammonium nitrate (33.5%) had a rate of 31.5 kg ha⁻¹, divided into five equal doses. Two equal amounts of 21 kg ha⁻¹ of potassium sulfate (48.52% K₂O) application followed. The

new method of sprinkler irrigation system provided irrigation every five days.

Plant samples collected 75 days after sowing underwent measurements of flax growth characteristics comprising plant height (cm), shoot fresh and dry weights (g) per plant, root length (cm), and root fresh and dry weights (g). Drying the samples took 48 h at 70 °C in an electric oven with a drift fan to achieve a constant dry weight. After 75 days from sowing, plant samples continued for chemical analysis. Harvesting flax plants occurred when marks of full maturity were visible, cutting them down and placed on the ground to dry out, gently removing the capsules. The following parameters' estimation at harvest used random samples of 10 guarded flax plants from each plot: number of fruiting branches plant⁻¹, technical stem length (cm), fruiting zone length (cm), total plant height (cm), no. of capsules plant⁻¹, no. of seeds per plant, 1000-seed weight (g), biological yield plant⁻¹ (g), seed yield plant⁻¹ (g), straw yield plant⁻¹ (g), biological yield (t ha⁻¹), and seed yield (kg ha⁻¹).

Biochemical analysis

Employing Lichtenthaler and Buschmann's (2001) methods aided the estimation of the total chlorophyll a, chlorophyll b, and carotenoid contents from fresh leaves. Larsen *et al.* (1962) methods helped extract and analyze indole acetic acid (IAA). After IAA extraction, determining the extract's total phenol content engaged the Danil and George (1972) approach. Estimating total soluble sugars applied the technique by Homme *et al.* (1992), and analysis was according to Yemm and Cocking (1955). Free amino acids and proline extractions followed the process by Vartainan *et al.* (1992). Obtaining the free amino acid utilized the ninhydrin reagent of Yemm and Willis (1954), with proline estimated by the Bates *et al.* (1973) method.

The antioxidant enzyme superoxide dismutase (SOD, EC 1.12.1.1) activity assayed spectrophotometrically at 560 nm comprised the nitro-blue-tetrazolium (NBT) reduction method by Chen and Wang (2006). Peroxidase (POX, EC 1.11.1.7) activity's

spectrophotometrical assay employed the procedure of Kumar and Khan (1982). Polyphenol oxidase (PPO, EC 1.10.3.1) activity determination used a spectrophotometric method based on an initial rate of increase in absorbance at 410 nm (Soliva *et al.*, 2001). Garnering the antioxidant activity (DPPH radical scavenging) engaged the method of Liyana-Pathiranan and Shahidi (2005).

DNA extraction and ISSR assay

Total genomic DNA extraction from young leaf pieces (approximately 1 cm²) used the CTAB protocol described by Poresbski *et al.* (1997). The PCR reactions ran in a 25 µl volume containing Taq polymerase (1 unit), 3 µl genomic DNA (50 ng), 2 µl of primer 10 pmol, 2 µl dNTPs (1 Mm), 2 µl MgCl₂, 3 µl 5× reaction buffer, and added to 25 µl of free nuclease water. PCR amplification ensued with a preliminary cycle of 5 min at 94 °C, followed by 35 cycles at 35 s at 94 °C, 50 s at 40 °C to 58 °C, 90 s at 72 °C, and a final extension of 7 min at 72 °C. Resolving the amplification products in 1.5% agarose gel followed the method of Sambrook *et al.* (1989).

Statistical analysis

The results reached statistical analysis using a randomized complete block design according to Snedecor and Cochran (1980). After testing the homogeneity of the error according to Bartlett's test, a combined analysis continued at 5% probability levels, using the least significant difference (LSD) test to compare means.

The bands' size measured received binary coding as one or zero for their presence or absence in each genotype. The data analysis employed the software package NTYSYS-pc (Ver.2.20), Rohlf (2000). Polymorphism information content (PIC) for the ISSR set attained determination according to the formula of Ghislain *et al.* (1999). Marker index (MI) calculation utilized the formula by Prevost and Wilkinson (1999). Regression between markers and morphological traits reached estimation according to the Mantel test (Mantel, 1967).

RESULTS

Growth characteristics

Growth traits (shoot and root length [cm], fresh and dry weight [g]) of seven flax genotypes (Sakha-1, Sakha-2, Sakha-3, Sakha-5, Sakha-6, Giza-12, and Amon) are available in Figure 1. Data showed significant variations ($P > 0.05$) in all recorded growth traits of the seven flax genotypes. Results revealed that the Amon variety exceeded the other varieties for length (53.72 and 12.83 cm), fresh weight (2.71 and 0.337 g), and dry weight (0.749 and 0.043 g) of plant shoot and root, respectively.

Photosynthetic pigments

Data in Figure 2 showed the effect of flax varieties on photosynthetic pigment constituents (chlorophyll a and b, carotenoids, and total pigments). Results provided significant differences ($P > 0.05$) between the seven tested flax varieties in photosynthetic pigment constituents, except Sakha-6, Giza-11, and Amon varieties, which were superior to the other varieties for different photosynthetic constituent amounts. The Amon variety was superior to other varieties for chlorophyll a, chlorophyll b, and total pigment contents, which gave 1.765, 0.893, and 3.22 mg g⁻¹ fresh wt., respectively. The variations were

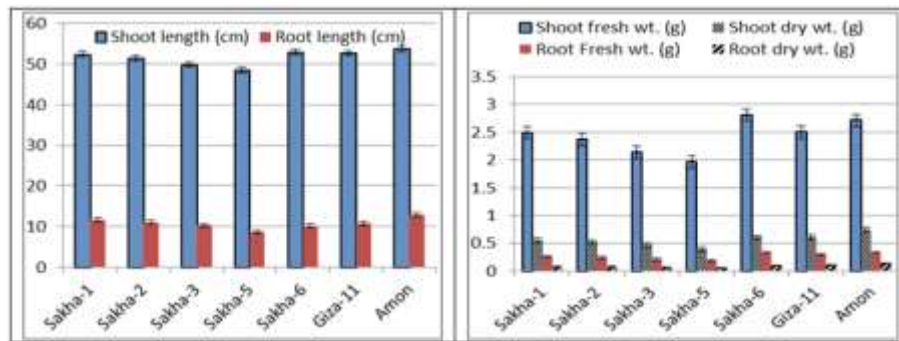


Figure 1. Effect of varietal differences on shoot and root length, fresh and dry weight of seven flax genotypes. LSD at a 5% significance level: shoot length - 1.4 cm; root length - 0.89 cm - shoot fresh wt. - 0.18 g; shoot dry wt. - 0.049 g; root fresh wt. - 0.033 g; root dry wt. - 0.043 g.

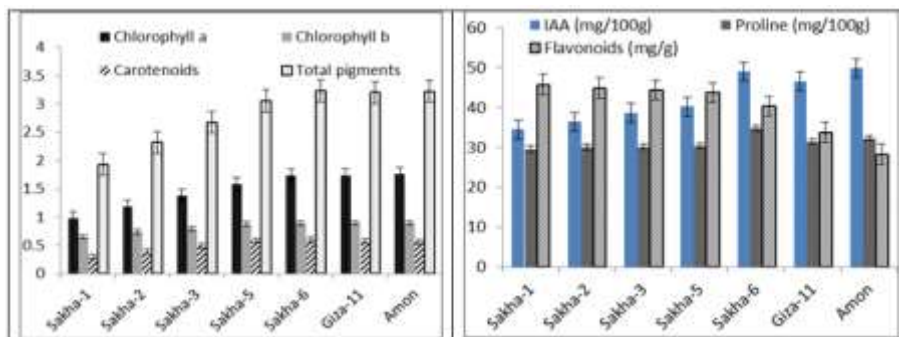


Figure 2. Effect of varietal differences on photosynthetic pigments (mg g⁻¹ fresh wt.) and IAA, proline, and flavonoid constituents of seven flax genotypes. LSD at a 5% significance level: chlorophyll a - 0.006 mg g⁻¹; chlorophyll b - 0.02 mg g⁻¹; carotenoids - 0.002 mg g⁻¹, total pigments - 0.02 mg g⁻¹; IAA - 0.45 mg 100 g⁻¹; proline - 0.4 mg 100 g⁻¹; flavonoids - 2.94 mg 100 g⁻¹

alike in chlorophyll and total pigment content among Amon, Sakha-6, and Giza-11 genotypes. In addition, the Sakha-6 variety gave the highest value of carotenoid contents (0.601 mg g^{-1} fresh wt.).

Chemical analysis

Data in Figures 2 and 3 signified differences among flax genotypes on some chemical constituents (IAA, proline, free amino acids, oil%, DPPH%, flavonoids, and total carbohydrates). Results indicated significant variations ($P > 0.05$) among the seven tested flax varieties. The Sakha-1 variety gave the foremost values of DPPH content (57.55%) and flavonoids (45.75 mg g^{-1} dry wt.). Likewise, the Sakha-6 variety was superior over all other flax varieties in the contents of proline ($34.81 \text{ mg } 100\text{g}^{-1}$), free amino acids ($396.85 \text{ mg } 100\text{g}^{-1}$), oil (35.46%), phenolics ($189.61 \text{ mg } 100\text{g}^{-1}$), and total carbohydrates (32.07%).

Yield and yield components

Outcomes in Figure 4 showed the effect of flax varieties on yield and its components (plant height, fruiting zone length, technical stem length, no. of fruiting branches, no. of capsules plant⁻¹, seed yield plant⁻¹, biological yield plant⁻¹, straw yield plant⁻¹, seed yield ha⁻¹, biological yield ha⁻¹, straw yield ha⁻¹, and oil yield ha⁻¹). Results expressed significant differences ($P >$

0.05) among the seven tested flax varieties. Sakha-1 variety gave the highest values for no. of fruiting branches and no. of capsules plant⁻¹ (4.5 and 14.14) and biological and straw yield plant⁻¹ (1.423 and 1.021 g). Similarly, the Sakha-2 variety was superior regarding fruiting zone length and no. of fruiting branches plant⁻¹ (22.97 cm and 4.50). In addition, Sakha-6 was better than all varieties in seed yield plant⁻¹ (0.509 g), straw yield (6.093 t ha^{-1}), seed yield (0.888 t ha^{-1}), biological yield (6.981 t ha^{-1}), and oil yield (0.302 t ha^{-1}).

ISSR molecular markers

Eight of the 15 primers indicated various levels of polymorphism (% P) among varieties. The eight primers produced 74 loci, of which 35 bands were polymorphic with 44.34% polymorphism (Table 1 and Figures 5 and 6). The polymorphic loci ranged from one (UBC-849) to eight (UBC-825), averaging 4.38. The smallest size of loci was 190 bp, and the largest was 1544 bp, generated by UBC-846 and UBC-887 primers, respectively. The dinucleotide (AC), (AG), (TC), and trinucleotide (GAG) repeat primers showed the highest level of polymorphism. The UBC-825, UBC-834, UBC-884, and UBC-887 primers detected polymorphism levels of 50%, 55.56%, 63.64%, and 80%, respectively.

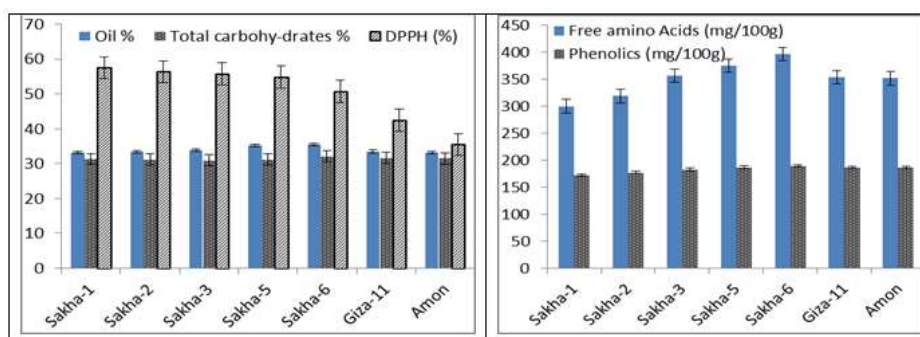


Figure 3. Effect of varietal differences on oil %, total carbohydrates %, DPPH %, free amino acid and phenolic constituents of seven flax varieties. LSD at a 5% significance level: oil % - 0.11; total carbohydrates % - 0.29; DPPH % - 0.51; free amino acids - $14.95 \text{ mg } 100 \text{ g}^{-1}$; phenolics - $14.4 \text{ mg } 100 \text{ g}^{-1}$.

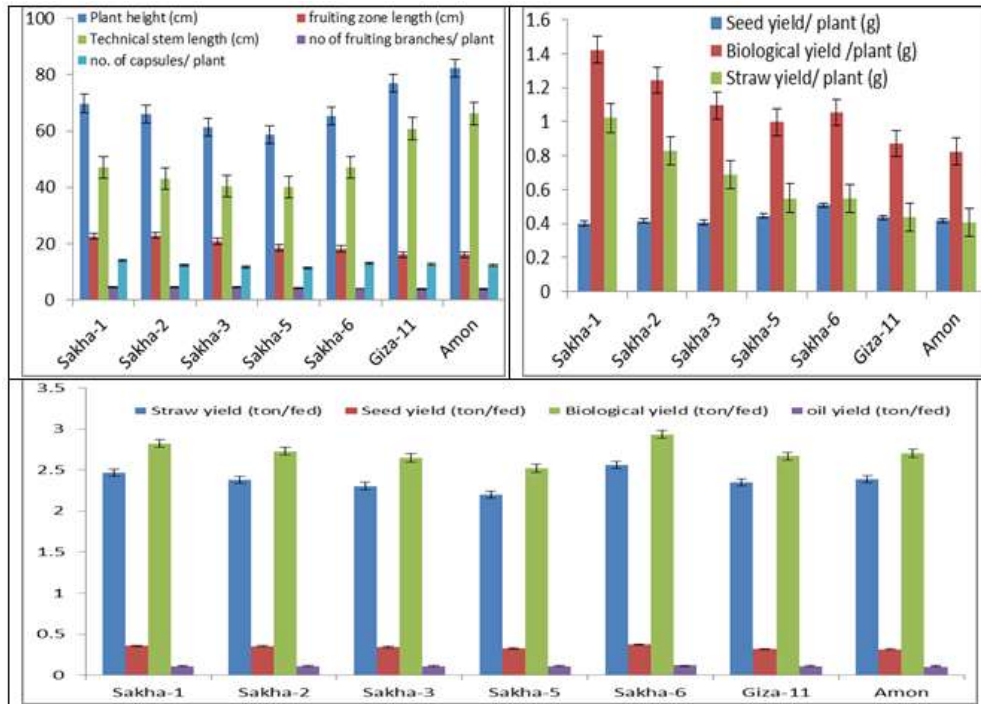


Figure 4. Effect of varietal differences on yield and yield components of seven flax varieties. LSD at a 5% significance level: plant height - 3.82 cm; fruiting zone length - 1.67 cm; technical stem length - 3.43 cm; no. of fruiting branches plant⁻¹ - 0.35; no. of capsules plant⁻¹ - 3.19; seed yield plant⁻¹ - 0.035 g; biological yield plant⁻¹ - 0.132 g; straw yield plant⁻¹ - 0.145 g; straw yield ha⁻¹ - 0.064 tons; seed yield ha⁻¹ - 0.059 tons; biological yield ha⁻¹ - 0.035 tons; oil yield ha⁻¹ - 0.021 tons.

Table 1. Different ISSR primers used for detecting polymorphism among seven flax genotypes.

Primer	Primer Sequence 5'.....3'	Amplified bands		% P	PIC	MI	RP	Fragments size (bp)	
		loci number	Polymorphic loci					Larger	Smallest
UBC 809	(AG) ₈ G	9	2	22.22	0.10	0.20	1.44	1507.00	204.00
UBC 825	(AC) ₈ T	10	8	80.00	0.30	2.40	4.58	1519.00	236.00
UBC834	(GA) ₈ TT	11	7	63.64	0.23	1.61	3.72	1431.00	204.00
UBC 846	(CA) ₈ AT	6	2	33.33	0.08	0.16	0.57	1028.00	190.00
UBC 847	(CA) ₈ AC	9	3	33.33	0.13	0.39	1.72	871.00	285.00
UBC 849	(GT) ₈ YA	6	1	16.67	0.08	0.08	0.86	1016.00	208.00
UBC 884	ACT AG) ₇	9	5	55.56	0.23	1.15	3.15	694.00	235.00
UBC 887	GCA(TC) ₇	14	7	50.00	0.14	0.98	2.57	1544.00	212.00
Means		9.25	4.38	44.34	0.16	0.87	2.33		
Total		74	35						

% P - percentage of polymorphism; PIC - polymorphic information content; MI - marker index; RP - resolving power.

Using dinucleotide repeat primers resulted in the maximum level of polymorphism. PIC values ranged from 0.08 (UBC-846) to 0.30 (UBC-825), with an average value of 0.16. Employing resolving power (RP) measured the

discriminatory power of molecular markers. In this work, ISSR primers showed RP that ranged from 0.57 (UBC-846) to 4.58 (UBC-825), with a mean of 0.66 (Table 1).

Single-marker analysis

DNA markers indicate superiority to morphological and biochemical marker systems because they analyze polymorphism at the DNA level and allow differentiating genotypes undistinguishable by other tests. This investigation involved seven flax genotypes that exhibited moderate to high genetic variability using a simple linear regression method. Analysis of variances for simple regressions (Table 2) showed significant regression of six traits with three ISSR markers

from 35 polymorphic ISSRs. The ISSR markers (UBC-825_{388bp}) are options as candidate markers linked to straw yield ($t\ ha^{-1}$) and biological yield ($t\ ha^{-1}$). Also, the ISSR markers UBC-834_{1019bp} and UBC-834_{204bp} are connected to one trait (IAA) and four traits (root fresh wt. [g], straw yield [$t\ ha^{-1}$], shoot fresh wt. [g], and shoot dry wt. [g]), respectively. Maximum regression explanation referred to the linked indicators at 53.40% to 73.33% for the biological yield ($t\ ha^{-1}$) and shoot fresh wt. (g), respectively.

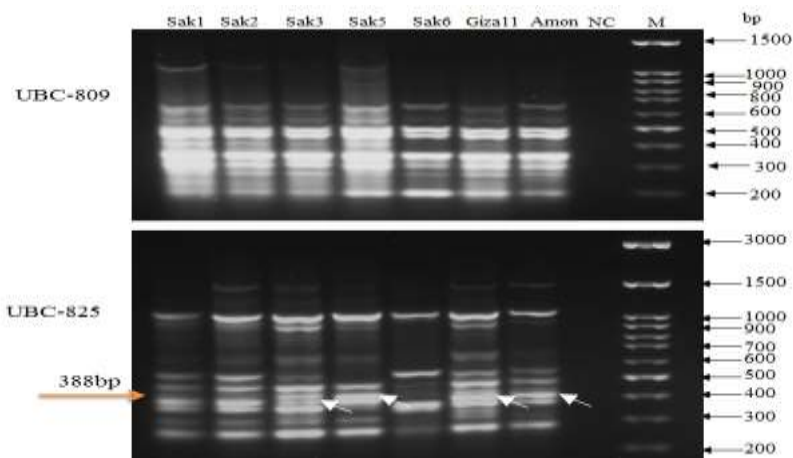


Figure 5. ISSR profiles for seven flax genotypes amplified with primers UBC-809 and UBC-825. M – 100 bp DNA ladder.

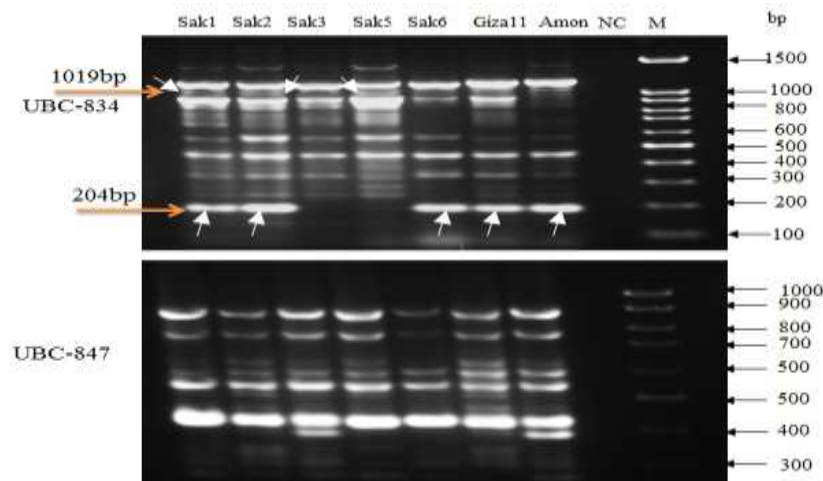


Figure 6. ISSR profiles for seven flax genotypes amplified with primers UBC-834 and UBC-847. M – 100 bp DNA ladder.

Table 2. ANOVA involving simple linear regression (R^2) for flax traits and ISSR primers.

Marker	Traits	S.V	d. f	SS	MS	R^2	P- value
UBC 825 _{388bp}	Straw yield (t/ha)	Genotypes	1	0.26	0.26*	57.78	0.04
		Error	5	0.19	0.04		
		Total	6	0.45			
	Biological yield (t/ha)	Genotypes	1	0.34	0.34*	57.63	0.05
		Error	5	0.25	0.05		
		Total	6	0.59			
UBC 834 _{1019bp}	IAA	Genotypes	1	137.09	137.09*	58.86	0.04
		Error	5	95.82	19.16		
		Total	6	232.91			
UBC 834 _{204bp}	Root fresh wt. (g)	Genotypes	1	0.0133	0.0133*	65.35	0.03
		Error	5	0.0068	0.0014		
		Total	6	0.0202			
	Straw yield (t/ha)	Genotypes	1	0.25	0.25*	55.56	0.05
		Error	5	0.20	0.04		
		Total	6	0.45			
	Shoot fresh wt. (g)	Genotypes	1	0.395	0.395**	73.33	0.01
		Error	5	0.144	0.029		
		Total	6	0.54			
	Shoot dry wt. (g)	Genotypes	1	0.0412	0.0412*	54.67	0.05
		Error	5	0.034	0.007		
		Total	6	0.075			

SV- source of variance; SS - sum of squares; DF - degrees of freedom; MS - mean square; R^2 - coefficient of determination (%).

Cluster analysis for ISSR markers

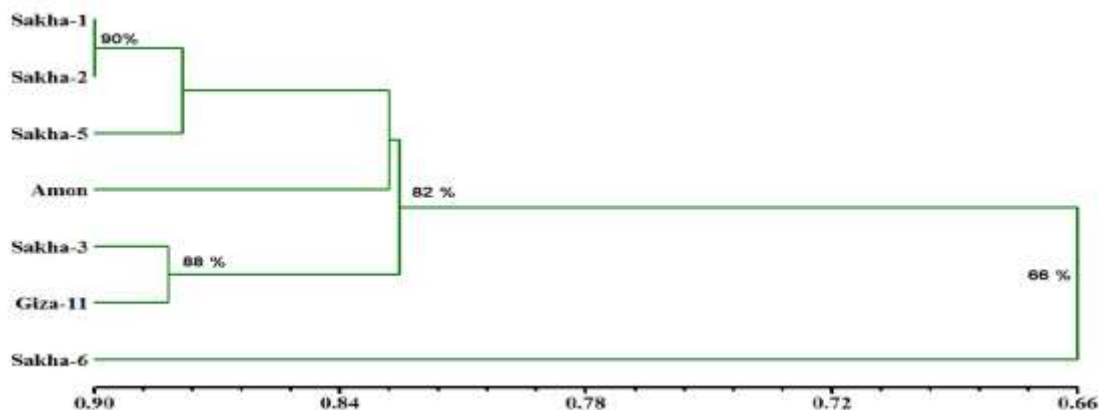
Cluster analyses divided the seven flax genotypes into two main groups based on Jaccard's similarity coefficient, where the average similarity index was 0.74 (Table 3). The similarity coefficient generated a dendrogram for flax genotypes based on the UPGMA analysis (Figure 7). The genetic tree divided the seven genotypes into two main groups. The first group formed three clusters containing six genotypes. The first cluster includes three genotypes (Sakha-1, Sakha-2, and Sakha-5) diverging at a 90% similarity percentage. The second cluster comprises one genotype, Amon, which diverged at 82%. The varieties Sakha-3 and Giza-11 diverged at 88% in the third cluster. The second group diverged at 66% with the variety Sakha-6. The correlation between the ISSR markers and morphological and physiological traits' estimation through the Mantel test revealed a negative and nonsignificant correlation ($r = -0.06, P < 0.05$).

DISCUSSION

The collected data confirmed the remarkable effect of genotypes, i.e., Sakha-1, Sakha-2, Sakha-3, Sakha-5, Sakha-6, Giza-11, and Amon on different growth characteristics (shoot and root length [cm], fresh and dry weight of shoot and root [g]) (Figures 1), photosynthetic pigments (chlorophyll a and b, carotenoids, and total pigments) (Figure 2), and phenolic, IAA, proline, and flavonoids free amino acids (Figure 2). Likewise, genotype effects emerged on seed yield, its components (Figure 4), nutritional contents (oil% and carbohydrate%), antioxidant compounds (flavonoids and phenolics), and antioxidant activity (DPPH %) of the seven flax varieties (Figure 3). These differences might be due to the variations between these genotypes in origin and growth habit, where growing these flax cultivars serve as double-purpose crops (oil and fibers) under the conditions of this experiment.

Table 3. Similarity matrix among the seven flax genotypes using ISSR markers.

Genotype	Sakha-1	Sakha-2	Sakha-3	Sakha-5	Sakha-6	Giza-11	Amon
Sakha-1	1.00						
Sakha-2	0.90	1.00					
Sakha-3	0.82	0.83	1.00				
Sakha-5	0.90	0.86	0.80	1.00			
Sakha-6	0.66	0.68	0.67	0.58	1.00		
Giza-11	0.81	0.88	0.88	0.80	0.67	1.00	
Amon	0.85	0.87	0.81	0.76	0.70	0.84	1.00

**Figure 7.** The constructed dendrogram by UPGMA and similarity matrix depending on the Jaccard similarity coefficient using ISSR markers.

Moreover, the study results agreed with those acquired by Bakry *et al.* (2019 and 2022) and Bakry *et al.* (2012a and 2012b) on different plant species in many regions worldwide. Furthermore, the superiority of Sakha-6 and Sakha-5 could refer to the increased rate of quenching of chlorophyll fluorescence, which markedly increased plant biomass. This study stated that they were better than the other cultivars. The superiority of these cultivars concerning the yield and its components might be due to the dominance in plant height, fruiting zone length, branches and capsules number plant⁻¹, and seed yield plant⁻¹ (Figure 4).

The superiority of the Sakha-6 genotype over other genotypes in seed and oil yield ha⁻¹ is due to its significant number of capsules, fruiting branches, seed yield plant⁻¹, oil %, IAA, proline, total carbohydrate %, free amino acids, and phenolics contents (Figures 3 and 4). Bakry *et al.* (2016) reported that Sakha-5 significantly surpassed the other

cultivars regarding biological and straw yields (t ha⁻¹), with this study also confirming the superiority of Sakha-5 cultivar regarding plant height, technical stem length, biological yield plant⁻¹, and straw yield plant⁻¹.

The study findings illustrated the ISSR data's applicability for examining genetic diversity in seven genotypes and accessions of flax (*Linum usitatissimum* L.). The findings indicated the markers were highly informative, as eight ISSR markers produced fragments with an average of 9.25. The PIC and marker index values measured high levels of phylogenetic relationship. The average PIC value is 0.16, while the average value for the marker index is 0.87 (Table 1). These results were similar to earlier research (Osman-Marwa *et al.*, 2021; Amangaliev *et al.*, 2023; El-Bassiouny *et al.*, 2024). In evaluating the phylogenetic tree among 12 flax genotypes, they observed that 13 ISSR primers effectively generated 177 reproducible bands, with a polymorphism rate of 64.40%.

The ISSR markers showed the maximum polymorphism, PIC, and MI. Rajawade *et al.* (2010) and Pali and Mehta (2016) stated that the similarity matrix by ISSR primers ranged from 0.60 to 0.97 among flax genotypes. The seven flax varieties underwent classification into two main clusters. Ahmed *et al.* (2019) studied the phylogenetic relationship between nine flax genotypes by 10 ISSR primers; the mean of polymorphism was 54.80%. The seven flax genotypes could become two main cluster groups using the UPGMA. The Jaccard similarity coefficient varied from 0.73 to 0.97, averaging 0.857. Previous research on generating molecular markers in flax has restrictions, focusing only on cultivar characterization. The data from morphological features and molecular markers aided in building two distance matrices, with the correlation between them evaluated by El-Sherbeny *et al.* (2018), who found a negative and statistically significant association ($r = -0.25$, $P \leq 0.05$).

CONCLUSIONS

The results of this study concluded the superiority of the Sakha-6 genotype over all other genotypes in seed and oil yield ha^{-1} , morphological, and most physiological traits. Moreover, the ISSR technique effectively distinguished the seven examined flax genotypes based on their genetic diversity. The study indicated the applicability of ISSR data to investigate genetic diversity in seven flax genotypes and accessions. It facilitates and boosts the recommended use of these flax genotypes to be promising in flax breeding and improvement programs. The research advises farmers to cultivate the Sakha-6 genotype under the same conditions as this trial.

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