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Full Length Research Paper

Genetic Diversity Assessment of some Flax Genotypes Using Morphological and Molecular Markers

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ABSTRACT: Twelve flax genotypes (10 promising strains and two control varieties) were evaluated for straw, seed, technological characteristics, and genetic diversity using morphological and molecular characters, which will assist breeders in evaluating genetic diversity and relationships among the different flax genotypes at Giza Exp. Station, Field crops Res. Inst., Agricultural Research Center (A. R. C.), Egypt during two consecutive seasons 2018/2019 and 2019/2020, The design of experimental was a randomized complete block arrangement with three replications. The results showed that the new promising Strain 620/3/5 had the highest estimates in total plant height, technical stem length, straw yield/plant as well as per hectare, fiber yield per hectare, and fiber length in both seasons, while S.651 had the highest total fiber percentage. S.889/50/5/9 obtained the highest values in terms of number of seeds per capsule, seed yield/plant as well as per hectare, oil yield per hectare, and oil percentage in both seasons, but only in terms of number of capsules / plant and seed index in the second season. For genetic diversity, sixteen molecular markers were used (8 ISSR and 8 SCOT primers). ISSR contains more polymorphic information content (PIC) than SCOT. The PIC for ISSR primers ranged from 0.37 to 0.57, with an average of 0.49, and the PIC for SCOT ranged from 0.19 to 0.52, with an average of 0.39. PIC, Marker index (MI), heterozygosity (H), effective multiplex ratio (E), discriminating power (D), resolving power (R), and havp (arithmetic mean heterozygosity) for ISSR higher than SCOT. Both markers showed promising sources of variation, assisting breeders in assessing the genetic diversity and interactions between the various flax genotypes. Total DNA fragments generated for both markers were 106, and an average of 7.0 bands per primer was found. While 58 of the fragments were monomorphic and 48 were polymorphic, ISSR had a greater polymorphism proportion than SCOT, at 59.4% against 39.3%. The dendrogram produced by the UPGMA algorithm effectively distinguished the two clusters of flax genotypes. The genotypes and genetic distances varied between 0.029 to 0.620. The data for straw and seed yield as well as technological characteristics are highlighted in PCA for morphological data.

Keywords: Flax, genetic diversity, straw, seed, molecular marker

INTRODUCTION

Flax is an ancient crop, the cultivation of flax dating back to 7,000 BC. Utilization of stem fiber and seed oil can be traced back to early societies in Egypt and the Middle East. Flax seed is indeed utilized for a variety of industrial, food and feed purposes (Dunford, 2015; Gudmewad *et al.,* 2016). Seed flax is mostly grown for oil which contains a lot of omega-3 fatty acid. Flaxseed becoming more popular in the human diet due to its high dietary fiber, omega-3 oils, and anti-carcinogenic lignans (Thompson *et al.,* 1991; Westcott and Muir 2003).

Flaxseed oil is also utilized in paints and varnishes for its unique drying properties (Przybylski 2005). The "Linen" obtained from flax fiber is one of the best raw materials for textile production. Flax contains 80-90% cellulose, it is considered to have superior in strength and durability to cotton and stronger than cotton (Upadhyay *et al.,* 2019).Variation in phenotypic characteristics and genotypic background leads to diversity in genetic materials. Initially, morphological parameters were used to estimate the diversity of flax germplasm (Diederichsen

and Raney 2006; Saeidi 2012). Developing the response of genotypes with high productivity is essential to improving the efficiency of flax production. The only way to increase production is to expand the area unit by enhancing and developing various production resources, which requires the development of high yield genotypes and advanced in quantity and quality of production and adapted to local conditions. Numerous researchers discovered variation among flax genotypes in yield and its components (Stafecka *et al.,* 2019, Soto-Cerda *et al.,* 2019, Hoque *et al.,* 2020 and Jassim and Aziz, 2022).

Flax is traditionally grown in no more than 20 countries worldwide. The largest growers of fiber flax are Russia, China, Belarus, France, Ukraine, Egypt, and Belgium, with other contributions from many European countries (Mackiewicz-Talarczyk, 2000). In traditional plant breeding, genetic diversity was usually diagnosed through observational selection. But now, with the development of molecular biology, this work is determined at molecular level based on DNA changes and their effects on the phenotype (Bernardo, 2008).

Breeding programs require prior knowledge of crop distribution and its genetic diversity (Hoque *et al.,* 2020). In general, genetic biodiversity techniques provide information about the useful genes in germplasm resources. Such genes can be transferred during breeding programs through marker-assisted selection by backcrossing (Rahman *et al.,* 2016). Markers are widely used to assess genetic variations and utilized to evaluate genetic differences among accessions as well as to investigate seed clarification, assessment of genotypes and marker aided breeding (Baack *et al.,* 2005; Paniego *et al.,* 2002 and Barbara *et al.,* 2007).ISSR (Inter Simple Sequence e repeats) is one of the molecular markers which are fast, affordable, highly discriminative, and confidential (Safari *et al.,* 2013). ISSR loci have a high polymorphism ratio; a lot of alleles can be observed (Moghaddam *et al.,* 2009). Because of that ISSRs are ideal tools to determine the similarity and differences among genotypes (Abdelmigid, 2012) so it used for genetic diversity analysis and molecular characterization of different plants like bread wheat (El-Sherbeny *et al.,* 2020), flax (Ahmed *et al.,* 2019). ISSR offers several advantages such as high reproducibility, high polymorphism, low DNA requirements, easy handling and high genomic distribution (Heidari *et al.,* 2016). SCOT (Start codon targeted (SCOT) marker) is one of the reliable techniques as it is considered an efficient, informative and inexpensive tool (Osman *et al.,* 2021). SCOT marker technique is one of powerful tools as molecular markers based on the short conserved region flanking the ATG start codon in plant genes. It was developed by designing single 18-mer primers complementary to a short conserved region flanking the ATG start codon that is conserved for all plant genes (Sawant *et al.,* 1999). It is widely used in recent days for phylogenetic hypotheses generation, cultivar identification and several genetic variation studies. The main goals of this study were to assess genetic diversity and examine the effectiveness of start codon targeted (SCOT) markers for analysis of genetic diversity of twelve genotypes flax cultivar (*Linum Usitatissimum* L.) and to study genetic relationships between them. Mao *et al.,* (2018) used a combination of ISSR and SCOT markers to offer detailed results than a single analysis of ISSR and SCOT to study genetic diversity and population structure analyses in *Senna obtusifolia* L. The main aim of the current investigation was to evaluate the scope and spread of genomic variation across different linseed accessions by employing molecular markers ISSR and SCOT.

The main goals of this study were to perform a comparative evaluation among some new Egyptian flax genotypes regarding yield, yield components, technological characters, genetic diversity and examine the effectiveness of start codon targeted (SCOT) markers for analysis of genetic diversity of twelve flax genotypes (*Linum Usitatissimum* L.) and study genetic relationships among them to evaluate the scope and spread of genomic variation across different linseed accessions by employing molecular markers ISSR and SCOT.

MATERIALS AND METHODS

The existing investigation was once performed at Giza agricultural research station, Giza governorate, Egypt, during 2018/2019 and 2019/2020 winter seasons to find out about the overall performance of twelve flax genotypes regarding straw, fiber, seed and oil yields as properly as their associated characters. The genotypes included ten promising strains (S.2419/1, S.2467/1, S. 894/13/4/3,S.889/50/5/9,S.541/C/6,S.808/58/2,S.888/22, S.881/10/9, S.651, S.620/3/5 and two commercial varieties Sakha 5 and Sakha 3) (Table 1).

The experiments trials have been organized in a randomized complete block design with three replications. Sowing date was once in the first week of November in each season, the plot size was 6 m^2. Each plot contains ten rows spaced 20 cm apart. Normal cultural practices for flax production as recommended have been followed. The yield used to be decided on the foundation of trying out all plants harvested from every plot. Morphological measurements had been made on 10 randomly chosen plants from every plot. Studied characters were plant height (cm), technical length (cm), straw yield/plant (g), straw yield ha^{-1}(ton), fiber yield ha^{-1} (kg), number of capsules/plant, number of seeds/capsule, seed yield/plant (g), seed index (g), seed yield ha^{-1}(kg), oil yield ha^{-1}(kg), fiber length (cm), total fiber percentage (%) and oil percentage (%).

Table 1: Sources of different flax genotypes (oil types(O) and fiber types (F)

Genotypes	Source	Classifications
Strain 2419/1	Selection from I. Humpata (Hungary)	O
Strain 2467/1	Introduction from India	O
Strain894/13/4/3	Romania 14 x S.10	O
Strain 889/50/5/9	S.2465/1/3 x S.10	O
Strain 541/C/6	Giza 8 x S.2419/1	O
Sakha 5	I.370 x I.2561	O
Strain808/58/2	Belinka x S. 2467/1	F
Strain888/22	Romania 20 x I. Daniel	F
Strain881/10/9	Marlin x Giza 4	F
Strain651	(I.1563) x (S. 402/1 x Iriana)	F
Strain620/3/5	Giza 7 x S.422	F
Sakha 3	Belinka x 1.2096	F

Analysis of variance was carried out for each trait according to Snedecor and Cochran (1980); differences between means were tested by using LSD at the levels of 0.05 of probability.

Genomic DNA extraction

Genomic DNA was extracted from young leaves of the flax plants according to https://primerdigital.com/dna.html using CTAB solution (2% CTAB, 1.5 M NaCl, 10 mM Na3EDTA, 0.1 M HEPES-acid; 100 ml: 2 g CTAB, 2.4 g HEPES-acid, 2 ml 0.5 M Na3EDTA, 30 ml 5 M NaCl, Chloroform-isoamyl alcohol mix (24:1), 100% isopropanol (isopropyl alcohol, 2-propanol), 70% ethanol and 1xTE (10 mM Tris-HC1, pH 8.0; 1 m M EDTA).

PCR amplification of ISSR and SCOT markers

Sixteen primers (8 ISSR and 8 SCOT) were used in this study (Table 2). PCR were carried out in 20 µl for both markers containing 10X PCR buffer, 25 mM $MgCl_2$, 10 mMdNTPs, 2 µM primer, 5 U Taq DNA polymerase and 100 ng templates DNA. All PCR reactions were carried out in an eppendorf and perkin elmer Thermal Cycler. PCR programmed: 95 ºC for 5 min; 35 cycles (95ºC for 30 sec, 50ºC for ISSRs and 45ºC for SCOT for 45 sec, 72ºC for 1:30 min) and 72ºC for 5min. The amplification products were resolved by electrophoresis in a 1.2% agarose gel containing ethidium bromide (0.5ug/ml) in 1X THE buffer at 80 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

Table 2: SCOT and ISSR primer names, sequences used for genetic diversity among 12 flax genotypes

	Primer	sequence
1	SCOT 1	CAACAATGGCTACCACCA
2	SCOT 2	CAACAATGGCTACCACCC
3	SCOT 5	CAACAATGGCTACCACGA
4	SCOT 7	CAACAATGGCTACCACGG
5	SCOT 8	CAACAATGGCTACCACGT
6	SCOT 11	AAGCAATGGCTACCACCA
7	SCOT 12	ACGACATGGCGACCAACG
8	SCOT14	ACGACATGGCGACCACGC
9	ISSR825	ACACACACACACACT
10	UBC827	ACACACACACACACACG
11	UBC808	AGAGAGAGAGAGAGAGC
12	UBC901	CACACACACACACACA
13	ISSR-807	AGAGAGAGAGAGAGAGT
14	ISSR-810	GAGAGAGAGAGAGAGAT
15	ISSR-835	AGAGAGAGAGAGAGAGYC
16	ISSR-841	GAGAGAGAGAGAGAGAYC

Data analysis

Data were scored using MEGA 5.10 (Molecular Evolutionary Genetics Analysis) version 7 (http: //www.megasoftware. net).The band profiles were scored only for distinct, reproducible bands as present (1) or absent (0) for each ISSR and SCOT marker analysis. The banding patterns generated by eight SCOT-PCR and

8 ISSR-PCR primers were compared to determine the genetic relatedness of the samples under study. 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). Polymorphism information content (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 according to Smith *et al*. (2000). PIC values range from 0 (monomorphic) to 1 (very highly) discriminative, with many alleles each in equal and low frequency. The genetic similarities were calculated using Jaccard's similarity coefficient (Jaccard, 1908). The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal, 1973). 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.

Online Marker Efficiency Calculator (IMEC)

The analysis was conducted using IMEC software program online on an example data set taken from Poczai *et al*. (2011) for using SCOT and ISSR markers on the twelve flax genotypes. The data set is available for download and the resulting calculations are summarized in Table 7. The D parameter (discriminating power of primer) described by Tessier *et al.,* (1999) evaluated the efficiency of the primers in identification of several flax genotypes. The program Online Marker Efficiency Calculator (IMEC software) according to Amiryousefi *et al*., (2018) was 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 (R). 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/. Also we used heat map for analyzing our data.

RESULTS AND DISCUSSION

Straw yield and its related characters

The tested flax genotypes were not similar in all straw yield and its traits, data introduced in Table (3) revealed substantial variations among the flax genotypes in straw yield and its components strain 620/3/5 surpassed the other genotypes. Strain 620/3/5 significantly outperformed Sakha 3 (commercial fiber variety) in plant height by 6.71% and 10.74%, technical length by 18.84% and 22.18%, straw yield/plant by 36.50% and 56.69%, straw yield ha^{-1} by31.33% and 33.19% and fiber yield ha^{-1} by 32.78% and 31.91% in both seasons, respectively. The variations among flax genotypes ought to be due to the genetic factor. The above noted effects are due to the higher vegetative growth of 620/3/5 in contrast with different genotypes. These results agreed with those noticed by Bakry *et al.*, (2012), Chandvawati *et al.,* (2017), El sayed *et al.,* (2018) and Sayed *et al.,* (2019).

Seed yield and its related characters

Data in table (4) indicated point out that flax genotypes significantly differed in no. of capsules/plant, no. of seeds/capsule, seed yield/plant, seed index, seed yield $ha^{-1}$and oil yield ha^{-1}in each season.

Table 3: Mean values of straw yield and its attributes in the two different seasons (2018 / 2019 and 2019 / 2020) for twelve flax genotypes

Genotypes	Plant height (cm)		Technical length (cm)		Straw yield /Plant (g)		Straw yieldha^{-1}(ton)		Fiber yieldha^{-1}(kg)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Strain2419/1	91.370	93.912	76.525	74.266	1.376	1.351	5.426	6.166	883.853	995.374
Strain 2467/1	90.708	94.266	73.896	77.983	1.605	1.635	5.316	5.222	762.156	745.810
Strain894/13/4/3	94.219	98.329	75.914	77.783	1.676	1.782	5.304	5.054	700.181	656.971
Strain 889/50/5/9	93.419	92.336	73.186	74.428	1.491	1.536	5.150	5.690	768.902	826.474
Strain 541/C/6	79.892	73.000	69.695	67.601	1.441	1.358	4.109	3.816	532.870	487.075
Sakha 5	80.692	84.217	63.817	67.915	1.214	1.219	4.885	4.978	689.767	702.588
Strain808/58/2	101.696	100.543	86.650	81.650	2.470	2.550	7.325	7.498	1361.388	1379.784
Strain 888/22	104.273	103.916	87.563	84.563	2.746	2.753	7.322	7.558	1401.727	1429.596
Strain 881/10/9	112.581	115.816	95.143	94.143	2.296	2.463	8.551	8.170	1596.168	1507.291
Strain 651	116.170	116.743	94.156	104.156	2.621	2.886	9.398	9.799	1926.953	2020.104
Strain 620/3/5	121.120	123.993	110.683	111.350	3.160	3.593	11.407	11.510	2290.908	2236.817
Sakha 3	113.501	111.972	93.140	91.137	2.315	2.293	8.686	8.645	1725.307	1695.763
LSD (0.05)	2.936	3.932	3.944	4.1701	0.297	0.301	0.175	0.317	57.280	54.029

Table 4: Mean values of seed yield and its attributes in the two different seasons (2018 / 2019 and 2019 / 2020) for twelve flax genotypes

Genotypes	Number of capsules/plant		Number of Seeds/Capsule		Seed yield /plant (g)		Seed index (g)		Seed yield ha^{-1}(kg)		Oil yield ha^{-1}(kg)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Strain2419/1	34.540	38.200	8.308	8.033	1.707	1.788	9.986	10.071	1225.397	1259.023	515.165	531.670
Strain 2467/1	20.244	21.189	7.349	8.922	1.628	1.827	9.250	9.695	1107.998	1142.599	446.021	464.009
Strain894/13/4/3	23.408	21.766	8.408	7.630	1.850	1.641	10.153	9.959	1253.515	1147.438	513.103	472.121
Strain 889/50/5/9	32.047	43.184	9.198	9.556	2.376	2.727	11.025	11.459	1432.039	1474.555	621.062	630.883
Strain 541/C/6	19.047	20.713	7.600	7.416	1.990	1.882	11.034	11.110	1272.710	1400.002	541.800	604.248
Sakha 5	20.217	21.164	7.339	8.914	1.611	1.805	9.244	9.683	1086.542	1123.500	436.954	456.331
Strain808/58/2	13.3	16.266	6.812	6.800	0.716	0.736	6.563	6.300	645.482	618.607	241.126	230.374
Strain 888/22	12.981	14.716	6.500	7.166	0.950	0.753	6.481	6.231	705.456	638.270	264.194	238.349
Strain 881/10/9	13.904	12.810	7.531	7.863	0.820	0.973	7.200	7.086	647.918	659.791	242.008	248.158
Strain 651	13.293	13.3830	7.334	7.166	0.973	0.993	5.723	5.366	562.752	594.480	203.578	216.806
Strain 620/3/5	14.506	14.213	7.833	7.666	1.180	1.110	6.500	6.340	559.574	529.392	203.186	191.777
Sakha 3	13.115	12.618	7.201	7.131	0.928	0.917	6.163	6.152	548.206	514.917	202.366	189.989
LSD (0.05)	2.546	3.616	0.569	0.682	0.111	0.245	0.668	0.370	51.393	29.491	21.363	15.362

Strain 889/50/5/9 gave the highest values in all previous characters except no. of capsules / plant in the first season where strain 2419/1 achieved the highest value in no. of capsules / plant but without significant differences with strain 889/50/5/9. Regarding Strain 889/50/5/9 significantly outperformed Sakha 5 (commercial oil variety) in seed yield and its traits i.e., no. of capsules / plant by 58.52% and 104.04%, no. of seeds / capsule by 25.33% and 7.20%, seed yield / plant by 47.49% and 51.08%, seed index by 19.27% and 18.34%,seed yield ha^{-1} by 31.80% and 31.25% and oil ha^{-1} by 42.13% and 38.25% in both seasons, respectively.

Ghanem, (1990), showed that the 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. The above cited results are due to the higher reproductive growth of strain 889/50/5/9. These results are harmony with those obtained by Dikshit and Shivaraj (2015), Nag *et al.,* (2015) and Elsorady *et al.* (2022).

Technological characters

The mean performance of the studied genotypes for technological characters was shown in Table (5). Strain 620/3/5 significantly outperformed Sakha3 in fiber length by 17.54% and 21.86% in both seasons, respectively. These results may be due to the superiority of 620/3/5 in plant height and technical length characters. While Strain 651 significantly outperformed Sakha3 in total fiber percentage by 3.22% and 5.09% in both seasons, respectively. Concerning oil percentage, S.889/50/5/9 surpassed sakha5 genotype in oil percentage by7.85% and 6.77% in both seasons, respectively. Similar results of mean performance for technological characters were obtained by Abd El – Fatah (2007), Worku *et al*. (2015) and Praczyk and Wielgusz (2021).

Table 5: Mean values of technological characters in the two different seasons (2018 / 2019 and 2019 / 2020) for twelve flax genotypes

Genotypes	Fiber length (cm)		Total fiber percentage		Oil percentage	
	1st	2nd	1st	2nd	1st	2nd
Strain 2419/1	74.108	71.766	16.288	16.144	42.041	42.227
Strain 2467/1	71.479	75.483	14.337	14.281	40.255	40.610
Strain 894/13/4/3	73.497	74.283	13.201	12.998	40.929	41.142
Strain 889/50/5/9	70.769	71.928	14.929	14.524	43.371	43.365
Strain 541/C/6	67.278	65.101	12.969	12.764	42.567	43.155
Sakha 5	61.422	65.413	14.123	14.115	40.215	40.617
Strain 808/58/2	84.233	79.150	18.586	18.403	37.360	37.240
Strain 888/22	85.146	82.063	19.143	18.916	37.446	37.341
Strain 881/10/9	92.726	91.643	18.666	18.450	37.341	37.595
Strain 651	91.740	101.656	20.503	20.615	36.170	36.470
Strain 620/3/5	107.266	108.850	20.083	19.433	36.311	36.226
Sakha 3	91.258	89.327	19.864	19.616	36.914	36.897
LSD (0.05)	3.945	4.170	0.532	0.695	0.399	0.560

Principal components analysis

PCA was employed to study the relationship among the flax genotypes and traits, as displayed in (Figures 1 and 2).The first two PCAs exhibited 95.46% and 92.90% of the variability. The PCA1accounted for 83.76% and 82.55% in both seasons respectively, of the variation and was associated with the flax genotypes 620/3/5, 881/10/9, 651, 888/22, 808/58/2 and Sakha 3 on the extreme left and 889/50/9, 2419/1, 894/13/4/3, 2467/1, 541/C/6 and sakha 5 on the extreme right (Figure 1 and 2).The evaluated traits, No. of capsules/plant, No. of seeds/capsule, seed yield/plant, oil%, seed index, seed yield ha^{-1} and oil yield ha^{-1} traits were positively associated with the flax genotypes 889/50/9, 2419/1, 894/13/4/3, 2467/1, 541/C/6 and Sakha 5, while the evaluated traits, fiber length, technical length, plant height, straw yield/plant, straw yield ha^{-1}, fiber yield ha^{-1} and total fiber percentage were positively associated with the flax genotypes 620/3/5, 881/10/9, 651, 888/22, 808/58/2 and Sakha 3 on the PCA1, which is consistent with the obtained results in (Tables 2 – 4). Thereupon, the PCA biplot is emphasizing the foregoing displayed results (Figure 1 and 2).

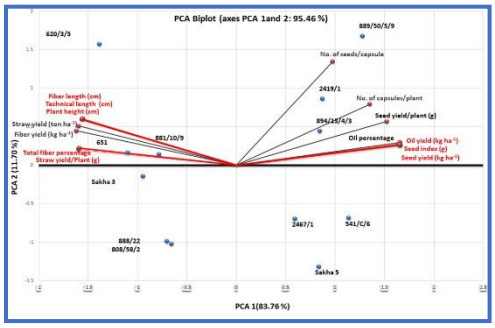

Figure 1: Biplot of the principal components analysis representing the relationship among the evaluated straw and seed yield and its traits and technological traits of flax genotypes in the first season

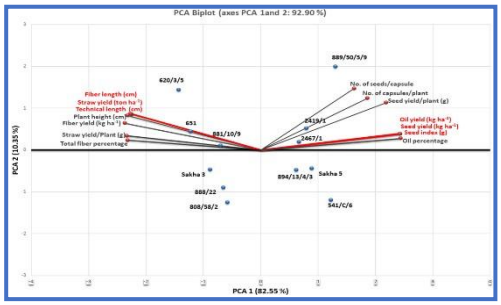

Figure 2: Biplot of the principal components analysis representing the relationship among the evaluated straw and seed yield and its traits and technological traits of flax genotypes in the second season

Genetic diversity for 12 flax genotypes

We measured the genetic diversity among flax genotypes at the molecular level through two molecular parameters SCOT and ISSR markers (Figures 3, 4). This work focused on their effect on the strength of the molecular parameter. Sixteen primers (8 SCOT and 8 ISSR) were used to find relationship among 12 flax genotypes.

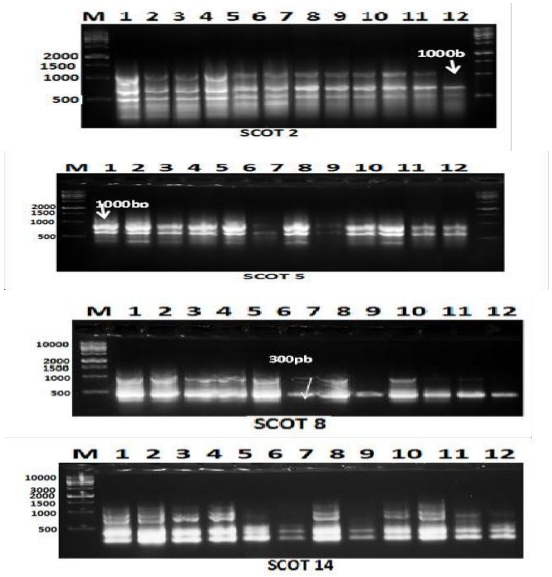

Figure 3: SCOT markers for flax genotypes

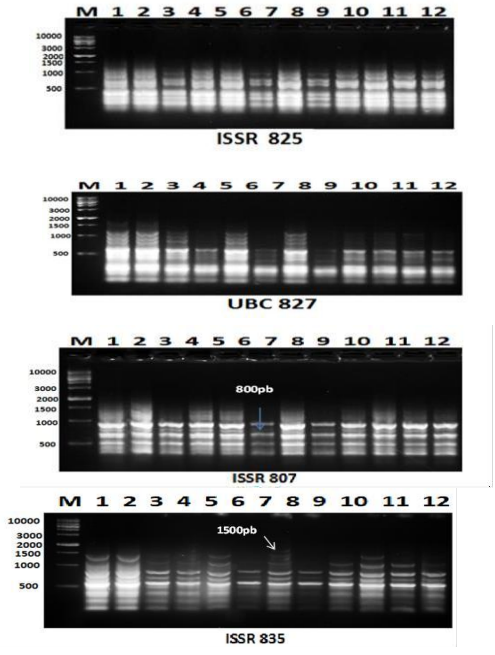

Figure 4: I SSR markers for ISSR

Totally, 106 amplicons were produced. 48 were polymorphic bands and 58 were monomorphic (Table 6). Bands produced by primers SCOT-11 and SCOT-12 to 6 bands produced by primers SCOT-8 and SCOT-14. Fragment size for SCOT primers ranged from 200 to 1100 bp.

Table 6: Total amplicons, polymorphic, monomorphic, unique bands produced by SCOT and ISSR marker in 12 flax genotypes

SCOT	Total bands	Poly morphic	Mono morphic	Poly morphism %	Unique	Positive	Negative	Genotype	Band Size
SCOT2	4	1	3	25.0	1	0	1	12	1000
SCOT5	4	2	2	50.0	1	1	0	1	1000
SCOT7	5	4	1	80.0	3	3	0	12	200,300.10
SCOT8	6	5	1	83.3	0	0	0	0	0
SCOT11	2	1	1	50.0	1	0	1	8	600
SCOT12	2	0	2	0.0	0	0	0	0	0
SCOT14	6	4	2	66.7	0	0	0	0	0
SCOT1	3	2	1	66.7	1	1	0	5	300
	32	19	13	60	7	5	2	5	7
ISSR-825	10	4	6	40.0	0	0	0	0	0
UBC-827	12	5	7	41.7	0	0	0	0	0
UBC-808	8	3	5	37.5	1	1	0	10	1600
ISSR807	10	4	6	40.0	1	0	1	6	800
ISSR810	7	4	3	57.1	0	0	0	0	0
ISSR841	9	3	6	33.3	0	0	0	0	0
UBC-901	5	2	3	40.0	0	0	0	0	0
ISSR835	13	4	9	30.8	1	1	0	7	1500
TOTAL	74	29	45	40	3	2	1	3	3
Total bands	106	48	58	46	10	7	3	7	

SCOT

Out of 36 SCOT primers, 8 primers SCOT produced amplicons to find out the relationships between 12 Flax genotypes. Thirty two amplicons produced from SCOT, 19 were polymorphic with polymorphism 60 % and 13 were monomorphic. Eight SCOT primers produced 7 unique bands (Five positive unique bands and two negative from SCOT), four genotypes were characterized with unique bands by SCOT, genotypes 2419/1 (oily), 889/50/5/9 (oily), 888/22 (fibrous) and Sakha 3 (fibrous).Genotype12 have the largest number of unique bands 4 (1000, 1000, 200 and 300bp). SCOT primers produced lowest values then ISSR primers. Heterozygosity for SCOT primers ranged from 0.57 by primer SCOT-5, SCOT-8 and SCOT-14 while the lowest value obtained by primer SCOT-2 (0.19). PIC ranged from 0.52 produced by primer SCOT-5 and SCOT-8, while the lowest value produced by primer SCOT-2 (0.19). H.av and MI ranged from 0.19 obtained by SCOT-2 to 0.57 produced by primer SCOT-5, SCOT-8 and SCOT-14. Total bands for SCOT primers ranged from 2 bands produced by primers SCOT-11 and SCOT-12 to 6 bands produced by primers SCOT-8 and SCOT-14. Fragment size for SCOT primers ranged from 200 to 1100 bp.

ISSR

Out of 15 ISSR primers eight primers used to find out the genetic relationship among 12 Flax genotypes. Seventy four amplicons were produced. Twenty nine of them were polymorphic and with polymorphism 40% and 45 bands were monomorphic. Three unique bands (two positive and one negative) were produced. Three genotypes were characterized with unique bands (sakha 5 (oil type), S. 808/58/2 (fiber type) and genotype 651 (fibrous).
Heterozygosity for ISSR primers ranged from 0.64 to 0.40. Prime rUBC-827 gave the highest value for H followed by primer ISSR-835 while primer UBC-901 gave the lowest heterozygosity (0.40). PIC ranged from 0.57 for primer UBC-827 to 0.53 by ISSR-825. While primer UBC-901 recorded the lowest PIC value (0.37). H.av and MI for primer ISSR-6 gave the highest value (0.64) while primer UBC-901 gave the lowest value (0.40). Primer UBC-827 and ISSR-835 produced the highest number of bands (12-13 respectively), while primer UBC-901 and ISSR-810 produced the lowest number of bands (5-7 respectively). Fragment size for all ISSR primers ranged from 100 to 1000bp.

Dendrogram

Dendrogram have two clusters (Figure 5), the first cluster have genotypes541/C/6 and 888/22.The genetic similarity between the two genotypes 888/22 and 541/C/6 may due to the genetic sources and these data similar to Cullis, 1881 who revealed that, the overall pattern of similarity between the oil seed and fiber genotypes in the earlier

identification of the distribution of repetitive sequences with the flax genome, Povkhova *et al.(* 2021) who study the genome sequences data for 191 flax genotypes (79 fiber flax and 112 linseed flax) and found that there are no association between flax types and polymorphisms in FAD3A and FAD3B genes this may be due to the fact

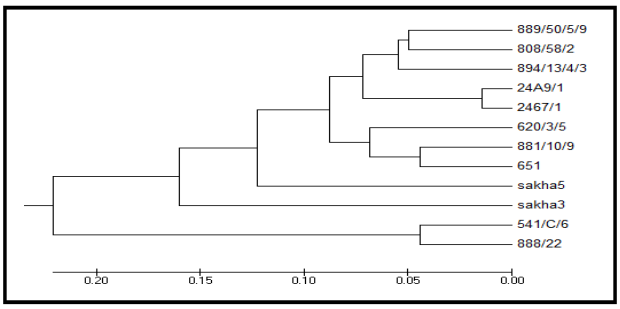

Figure 5: Cluster illustrating the genetic distance, based on the analysis of 16 primers for 12 flax genotypes using the Euclidean distance and the UPGMA algorithm in the PAST software

that most linseed genotypes similar to fiber ones did not carry the key polymorphisms in FAD3 genes that determine a lower content of linolenic acid (kezimana *et al.,* 2020), and the used sample set did not include a significant number of low-linolenic genotypes that could form a separate cluster (Povkhova *et al., 2021*).Second cluster have two subclusters, genotype Sakha 3 found in separated subcluster, second subcluster have two subclusters Sakha 5 separated from all the other genotypes. The last subcluster have two groups, one have genotypes 620/3/5, 881/10/9 and 651, while the second group included genotypes 889/50/5/9, 808/58/2, 894/13/4/3, 2419/1 and 2467/1.

Genetic distances

Genetic distance between flax genotypes ranged between 0.029 and 0.620. The Lowest genetic distance value was 0.029 between genotypes 2419/1 and 2467/1, followed by 0.089 between genotypes 541/C/6 and 888/22, while genetic distance 0.099 among genotypes 808/58/2, 894/13/4/3 and 889/50/5/9. The highest genetic distance value 0.620 was between genotypes 2419/1 and 888/22 followed by 0.603 between genotypes 2467/1 and 888/22.

Principle component Analysis

PCA showed clear differentiation between oilseed and fiber flax (Figure 6), it separated oil seeds and fiber flax to 4 groups genotype Sakha 5 and Sakha 3 found in separated group from all genotypes, while genotypes 541/C/6 and 888/22 found in one group closed to each other, the last group have genotype 2467/1 and genotype 2791/1 oilseed closed to each other. Genotypes 894/13/4/3 and 889/50/5/4 oilseed closed to each other, while genotypes 808/58/2, 881/10/9, 620/3/5

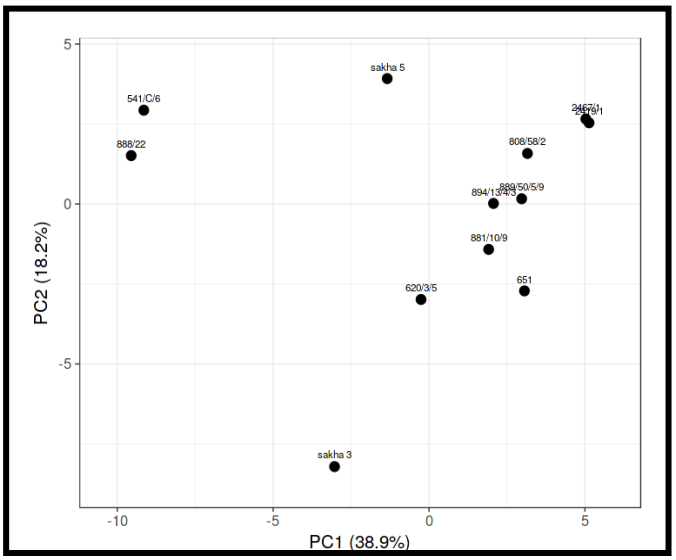

Figure 6: PCA analysis for flax genotypes

and 651 scattered in one group (Figure 6).
Caption example: Unit variance scaling is applied to rows; SVD with imputation is used to calculate principal components. X and Y axis show principal component 1 and principal component 2 that explain 38.9% and 18.2% of the total variance, respectively. N = 12 data points.

Heat map

Heat map divided flax genotypes to two clusters (Figure 7), first cluster have genotypes 888/22 and 541/C/6 in one subcluster and genotype sakha 5 in another subcluster. Second cluster have two subcluster, genotypes 881/10/9 and 651 separated and genotypes 620/3/5 and sakha 3 which found in separated subcluster, while the last subcluster have two groups, genotypes 2419/1 and

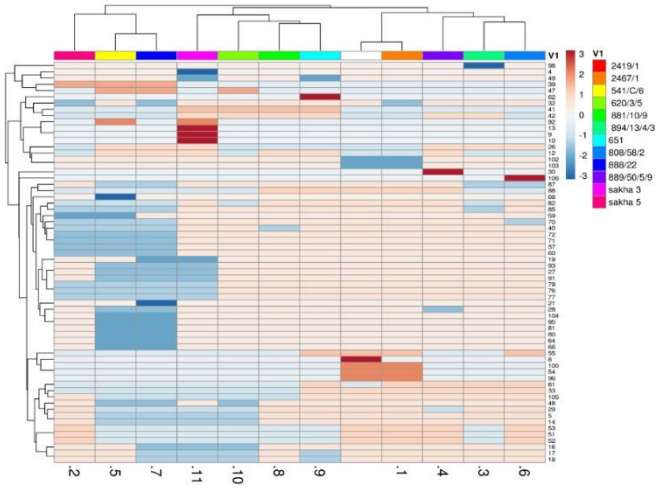

Figure 7: Heat map for flax genotypes

Table 7: IMEC analysis for ISSR and SCOT primers,,heterozygosity index (H), polymorphism information content (PIC), discriminating power (D), effective multiplex ratio (E), marker index (MI), arithmetic mean heterozygosity (Havp) and resolving power (R)

	Primer	H_0	PIC_0	E_0	H.av_0	MI_0	D_0
1	ISSR-825	0.59	0.53	1.00	0.59	0.59	0.16
2	UBC-827	0.64	0.57	1.00	0.64	0.64	0.16
3	UBC-808	0.53	0.48	1.00	0.53	0.53	0.12
4	UBC-901	0.40	0.37	1.00	0.40	0.40	0.09
5	ISSR 807	0.56	0.48	1.00	0.56	0.56	0.13
6	ISSR 810	0.48	0.42	1.00	0.48	0.48	0.10
7	ISSR 835	0.61	0.55	1.00	0.61	0.61	0.11
8	ISSR 841	0.56	0.49	1.00	0.56	0.56	0.15
9	SCOT1	0.30	0.28	1.00	0.30	0.30	0.07
10	SCOT 2	0.19	0.19	1.00	0.19	0.19	0.13
11	SCOT 5	0.57	0.52	1.00	0.57	0.57	0.12
12	SCOT 7	0.56	0.51	1.00	0.56	0.56	0.13
13	SCOT 8	0.57	0.52	1.00	0.57	0.57	0.19
14	SCOT 11	0.31	0.29	1.00	0.31	0.31	0.07
15	SCOT 12	0.31	0.29	1.00	0.31	0.31	0.06
16	SCOT 14	0.57	0.51	1.00	0.57	0.57	0.17

Table 8. Genetic distances between 12 flax genotypes using SCOT and ISSR primers

Genotype	2419/1	2467/1	Sakha5	894/13/4/3	889/50/5/9	541/C/6	808/58/2	888/22	881/10/9	651	620/3/5	Sakha3
2419/1												
2467/1	0.029											
Sakha5	0.245	0.233										
894/13/4/3	0.186	0.175	0.221									
889/50/5/9	0.142	0.131	0.245	0.120								
541/C/6	0.569	0.586	0.269	0.387	0.444							
808/58/2	0.120	0.110	0.198	0.099	0.099	0.444						
888/22	0.620	0.603	0.307	0.429	0.489	0.089	0.489					
881/10/9	0.164	0.175	0.221	0.120	0.120	0.387	0.164	0.429				
651	0.175	0.164	0.307	0.131	0.131	0.520	0.153	0.505	0.089			
620/3/5	0.257	0.269	0.294	0.164	0.209	0.359	0.233	0.320	0.120	0.153		
Sakha3	0.415	0.429	0.401	0.307	0.307	0.359	0.359	0.320	0.233	0.245	0.186	

2467/1 in one group separated to genotypes 889/50/5/9, 894/13/4/3 and 808/58/2 which found in second group (Figure 7). All data PCA, heat map, genetic distances and dendrogram represent similarity between flax genotypes.

Genotypes 889/50/5/9 and 894/13/4/3 (oilseed) have similar data to (fiber) flax genotype 808/58/2. Genotypes 2467/1 and 2419/1 (oil type) represented similarity between each other. Genotype 541/C/6 (oilseed) had similar data with fiber flax genotype888/22. While sakha 5 and sakha 3 separated to other genotypes in all analysis (Figure 7).

Conclusion

The differences in yield and its components among flax genotypes were concluded to be due to genetic and environmental factors. S.620/3/5 outperformed in straw and fiber yield ha^{-1}, as well as other characteristics. Meanwhile, S.889/50/5/9 surpassed the other genotypes in seed, oil yield ha^{-1} and its components. All data PCA, heat map, genetic distances and dendrogram represent similarity between flax genotypes. Genotypes 889/50/5/9 and 894/13/4/3 (oil type) have similar data to (fiber) flax genotype 808/58/2. Genotypes 2467/1 and 2419/1 (oil type) represented similarity between each other. Genotype 541/C/6 (oil type) had similar data with fiber flax genotype 888/22. In every analysis, Sakha 5 and Sakha 3 were distinguished from other genotypes.

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