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# Morphological and molecular characterization of flax (Linum usitatissimum L.) accessions obtained from different locations in Turkey

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# Abstract

Flax (*Linum usitatissimum* L.) is an important crop for source of with the different uses of oil and fiber. In addition, flax, which has an important place in the world and our country, has a multi-purpose use area. The objectives of the current research were to assess genetic diversity and patterns of relationships among the relative cultivar/genotypes through morphological traits and microsatellite (SSR) markers. The present study evaluated genetic diversity and association patterns among 63 cultivar/genotypes through 19 morphological traits, oil yield (18.033%) and fatty acid compositions. The relative agromorphological traits as well as, assessed genetic diversity through 16 loci strong amplicons SSR markers. According to the findings agronomic parameters; quality analyzes were performed to for promising varieties. The quality characteristics of the related fiber varieties were determined with the help of Scanning Electron Microscopy (SEM) and strength devices. As a result; It has been observed that the Eckendorfi can be promising. Principal Component Analysis (PCA) (JAMOVI 2022) performed to relevant parameters. Additionally, UPGMA (Arithmetic Mean Unweighted Double Group Method) is an individual marker system used to create the dendrogram. The average Polymorphic Information Content (PIC) values were recorded as (0.689), while the least and largest loci with allele dimensions were Lu9 (2) and Lu19-Lua613(6) respectively. These findings of the present study were supported by the results of the principal coordinate analysis. Morphological markers made use of in the study were found to be complementary to microsatellite-based markers in decoding, the genetic diversity and population structure of the flax germplasm.

# Introduction

The flax (*Linum usitatissimum* L.) is a worldwide distributed as one of the oldest domesticated crops with many applications, as well as the plant is herbaceous, self-pollinated, and annual crop species belongs to family Linaceae (Tork et al. 2019; Saroha et al. 2022). Flaxs, which are distributed in the temperate regions of the world, belongs to the genus Linum and includes diploid species with varying chromosomes (2n = 15, 16, 18, 30, 36, 60, 72) (Saroha et al. 2022). *L. usitatissimum* L., whose primary center of origin is believed to be Ethiopia, Central Asia and India; It has a distribution areas between the Middle East, the North and Southwest of America, and the Mediterranean basin (Vavilov 1951; Zohary and Hopf 2000; Goudenhooft et al. 2018; Xie et al. 2020; Ataii et al. 2021). The relative plant is one of the first cultivated plants such as wheat and barley, which has 22 genera and about 300 species worldwide distribution (Banjare et al. 2019; Talebi and Matsyura 2021). Additionally, it is stated that the wild type is the oldest in archeological research in Tell Abu Hureyra in Northern Syria, and was cultivated by the ancient Egyptians and Somaris about 10,000 years ago (Zohary and Hopf 2000). Herein flax also called common flaxseed or linseed, is true, its species name linum is in Celtic "lin" meaning "fibre" and "usitatissimum" (most useful in Latin) (Vaisey-Genser and Morris 2003; Anurag et al. 2020).

According to ancient sources, flax has been used and which has name given to in many country; China was called "Huma" and "Yama" (Liu et al. 2011). In addition to, the flax is called with different names according to the regions (biziktan, bezir, güdün, cimit, sağlek, siyelek and zeyrek) of Turkey (Dumanoğlu 2020). The flaxseed is used as a raw material in many industrial areas; especially oil flax varieties have a wide area of use in the food industry (Xie et al. 2020). Besides has enormous industrial applications, including the preparation of paints, varnishes, soaps, pastes and polymers due to its fast drying properties (Singh et al. 2017; Sulas et al. 2019), and as well as it has been stated that flaxseed an important place in biodiesel production (Bacenetti et al. 2017). Apart from the use of flax as oil; fibers from the stems; It is known it have a use in many different industrial areas with its soft, absorbent and durable properties (Singh et al. 2017). The flaxseed, an alternative oil plant, is known to contain 35–65% oil in its structure. Furthermore, fibers due to their strength and durability, have significantly more uses as composite materials (Yan et al. 2014; Bourmaud et al. 2015; Goudenhooft et al. 2017; Dubey et al. 2021). Additionally, flaxseed contents significant amounts of fatty acid compositions (α-linolenic acid, linoleic acid, oleic acid, palmitic acid and stearic acid), as well as high amounts of lignans, dietary fiber, protein, vitamins, micronutrients and carbohydrates (Pali and Mehta 2014; Wang et al. 2017; Dubey et al. 2020; Deme et al. 2021; Yang et al. 2021). The relative plant is rich in lignans and secoisolariciresinol diglucoside (SDG), as well as a rich source of omega-3 and omega-6 polyunsaturated fatty acids (PUFA) (Touré and Xueming 2010; Parikh et al. 2019; Razmaitė et al. 2021). Flaxseed is valued for its health promoting properties (Dobrowolska and Regulska-llow 2021). While considering genetic relationship and of plant genetic diversity among groups and different agro-m

Different types of molecular markers; these are reported to have conditions such as PCR-based, reproducibility, high polymorphism, cross-species use, cheap or expensive, and different DNA regions for which they are designed (Karaca et al. 2017; Nag et al. 2020). Today, several molecular markers such as (RAPD, AFLP, ISSR and SSR) have been identified in the flax plant using DNA analysis (Sandip et al. 2012; Khlestkina 2014; Thorat et al. 2017; Jing-Yuan et al. 2018). In this context, Simple Sequence Repeats (SSRs) are the most common genetic markers for plant genotyping. It is reported that multiple allelic, high rate of polymorphism, co-dominant markers and widespread in the genome and being species specific, make it more advantageous (Choudhary et al. 2017; Karaca et al. 2017; Rana and Singh 2017; Talebi and Matsyura 2021). The objectives of this study were to assess the genetic diversity among 63 accessions of *L. usitatissimum* L. genotypes characterized based on agro- morphological traits along with 16 SSRs (gSSR) markers using MrBayes method.

# **Material And Methods**

#### Plant material and experimental design

The relevant study; in 2020, it was carried out in Iğdır University Agricultural Application and Research Center in a randomized block design with three replications. The materials used; it was obtained from different agricultural organizations/institutions of Turkey. In addition, a total of 63 flax seeds of the local/foreign registered variety (24) and the genotype of unknown origin (39) obtained from different provinces of Turkey, and 9 of them showed fiber and oily properties. The plot size is  $3x0.8m = 2.4 \text{ m}^2$ , and the block size is  $3mx0.8m \times 80 = 192 \text{ m}^2$  and the total of 3 blocks is  $192x3 = 576 \text{ m}^2$ . Agro-morphological traits were determined 103 days after sowing by randomly selecting 10 plants from each plot. In addition, promising flaxseed cultivars/genotypes were identified for oil and natural fiber purposes.

#### Experimental area and soil trait

Experiment were conducted at Igdir University Agricultural Application and Research Center, which is located at (39°55′45″ N, 44°05′31″ E, 850m). The Igdir region is the lowest plain of the Eastern Anatolia Region with microclimate characteristics, and it is one of the largest plains in terms of area compared to the surrounding provinces.

#### Phenotypic evaluation

On ten randomly selected plants, 19 morphological plant traits at 95 and 103 DAS were determined for fiber and seed traits, respectively. These descriptors largely conform to the Ministry of Agriculture and Rural Affairs Technical Instruction on Trials of Measuring Agricultural values for flax/flxseed; plant height (cm/plant) (PH), number of flowering days (days) (NFD), ripening days (days) (RD), first branch height (cm)(FBH), number of siblings (number/plant) (NS), number of branches per plant (number/plant) (NBPP), number of capsule branches in the plant (number)(NCBP), number of capsule per plant (g/plant) (CWPP), number of seeds per plant (number/plant) (NSPP), number of seeds in capsule (number/capsule) (NSC), seed yield per plant (g/plant) (SYPP), stem weight (g/plant) (SW), technical stem length (cm) (TSL), biological weight (g/plant) (BW), number of flowering days (NFD), thousand seed weight (g) (TSW), seed yield (kg/da) (SY), harvestindex (%) (HI)

(https://www.tarimorman.gov.tr/BUGEM/TTSM/Belgeler/Tescil/Teknik%20Talimatlar/End%C3%BCstri%20Bitkileri/aspir,keten.susam,yf%C4%B1st%C4%B1%C-The fibre quality parameters (flax fibre fineness and structure) were aevaluated by following some researcher (Goudenhooft et al. 2018; Pisupati et al. 2021). The variable numbers, codes and frequency of individual morphological trait markers together with quantitative traits are offered in Table 1.

SI. No.	Trait name	Code	Detail
1	Plant height(cm)	PH	Height of main stem from cotyledon scar to top boll
2	Number of flowering days	NFD	The date on which 75% of the plants on the parcel flowered
3	Maturation days	MD	The period until the maturity of 90% of the plants in the parcel
4	First branch height (cm)	FBH	The part from the point where the first branch formation starts to the ground level
5	Number of tillering (number/plant)	NS	Count of the branches
6	Number of branches per plant (number/plant)	NBPP	Primary branches on the plant body
7	Number of encapsulated branches in plant (number)	NEBP	Branches with capsules on the plant
8	Number of capsules per plant (number/plant)	NCPP	Capsules being on each plant
9	Capsule weight per plant (g/plant)	CWPP	Wight of capsules on each plant
10	Number of seeds per plant (number/plant)	NSPP	Seeds in capsule per plant
11	Number of seeds in capsule (number/capsule)	NSC	Seeds in capsule
12	Seed yield per plant (g/plant)	SYPP	Seeds of plants taken from each parcel
13	Stem weight (g/plant)	SW	Weight at stem of plants taken from each parcel
14	Technical handle length (cm)	THL	From the first cotyledon leaves to the first branching
15	Biological weight (g/plant)	BW	Covering the underground and above ground parts of each plant
16	Number of days in flower	NDF	The date on which of the plants flowered
17	1000 Grain weight per plant (g)	1000 GWPP	Weight of 1000 bulk seeds
18	Seed yield (kg/hectare)	SD	Seed yield of whole treatment
19	Harvest index (%)	HI	The plants harvest from the field

Flaxseed oil content and fatty acid profile

The harvested flax seeds were powdered. Oil was taken from the samples with the thanks to Soxhlet apparatus. The flaxseed oil and fatty acid compositions were calculated on a percent basis. After the processes, methyl esters of fatty acids were obtained by using high performance liquid chromatography (GC-FID) at Igdir University Research Laboratory Application and Research Center (ALUM).

High Performance Liquid Chromatography (GC-FID) method

Flaxseed oil (0.2 g) was taken into 15 ml centrifuge tubes and shaken with 10 ml of hexane. The samples were dissolved in 0.2 mL of 1 N-methanol and KOH was added. The tube was shaken and phase separation was observed and it was kept in the dark for 2 hours until the upper phase became clear. Clarification after process, some of the upper phase was taken into vials, and fatty acids were analyzed with the help of Agilent 7820 A GC-FID device with a SP 2560 100m\*0.25mm\*0.2µm capillary column with a flame ionization detector (FID). Injection port and FID temperature is 240 °C, 1/10 split ratio at 400 ml/min pressure in split injection mode. After waiting for 5 minutes at 140 °C, the column temperature increased by 4 °C per minute to reach 250 °C and reached 260 °C after waiting for 15 minutes. Helium carrier gas 41 cm/sec (Hydrogen) was used. Samples injected with 1 µL into the device were compared with the GC-FID chromatogram obtained in the analysis of the "Supelco ® 37 Component FAME Mix-Sigma-Aldrich" standard mixture for a total of 37.75 minutes. As a result of the analysis; α-linolenic acid (C18:3n6), linoleic acid (C18:2n6), oleic acid (C18:1n9c), Palmitic acid (C16:0), stearic acid (C18:0) fatty acids were determined as % (Table 4).

Markers	PIC	Number of patterns	Number of alleles	Allele size
Lu35	0.845	7	4	110-190
Lu9	0.829	9	4	100-210
Lu17	0.911	23	3	290-380
Lu10	0.335	5	3	180-290
Lull	0.860	13	6	190-280
Lu15	0.556	5	3	200-300
Lu19	0.750	12	5	100-210
Lu24	0.676	4	3	200-310
Lu29	0.750	5	3	160-270
Lu38	0.517	3	2	110-190
Lu34	0.667	6	3	200-270
Lu32	0.687	б	3	190-300
Lu840	0.844	10	4	200-290
Lu18	0.615	8	4	210-390
Lu445	0.420	4	3	300-390
Lua613	0.766	7	4	210-420

Varieties	Crude oil rate (%) yield	Oil yield (g)	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	α-Linolenic acid
Sarı-85	45,23 a	2,21 a	22,33 a	11,43 b	18,18 m	8,79 q	36,38 j
lzmir-Karşıyaka-1	41,50 b	2,12 a	6,64 l	5,33 ij	18,86 j	14,14 k	46,07 d
Mapun	24,26 c	1,24 c	14,25 e	8,55 d	26,16 b	17,01 e	33,35 l
Clark	23,98 c	1,36 b	5,64 n	4,19 no	21,42 e	12,23 o	42,36 f
Gap-1	23,14 d	1,19 cd	14,62 d	8,34 de	6,27 w	6,26 u	27,28 p
Omega	22,62 e	1,10 de	4,22 q	6,45 gh	11,50 t	11,71 p	10,84 x
Hat-140	21,67 f	1,03 ef	8,07 j	7,64 f	10,11 u	7,89 r	19,75 t
Eckendorfi	21,60 f	1,03 ef	6,49 l	5,25 ijk	15,02 r	12,81 n	25,04 q
Izmir-Kemeraltı1	21,40 f	1,04 ef	7,19 k	6,06 h	21,15 f	15,10 j	46,75 c
Verne	21,33 f	1,08 e	6,57 l	6,08 h	16,50 o	17,37 d	47,39 b
Clli1523	20,67 g	1,04 ef	18,27 c	8,48 de	11,47 t	6,26 u	19,96 t
Ankara-Ulus-1	20,05 h	1,04 ef	5,28 o	4,61 lmn	17,03 n	15,41 i	41,17 g
Clli 1355	19,34 i	0,95 fg	6,07 m	4,77 lm	14,98 r	12,76 n	22,74 r
Mures	19,26 i	0,92 gh	6,58 l	6,79 g	20,04 g	13,06 m	41,38 g
Beyaz Gelin	17,26 ј	0,85 hi	8,36 j	5,03 jkl	19,07 ij	12,34 o	39,64 h
Clli1392	16,60 k	0,82 hi	6,27 lm	6,12 h	18,54 k	15,92 h	44,72 e
Afyon-1	15,54 l	0,74 ij	4,27 q	3,37 p	16,01 p	20,12 a	22,17 s
Van-1	15,50 l	0,79 i	5,73 n	5,53 i	24,42 c	16,16 g	31,13 m
Mersin-1	13,38 m	0,68 jk	4,64 p	5,05 jkl	13,79 s	12,62 n	29,44 o
Rolin	13,25 m	0,63 kl	13,59 f	12,41 a	4,24 x	6,51 t	17,78 u
lzmir-Karşıyaka-2	12,53 n	0,59 klm	7,08 k	6,38 gh	21,33 ef	16,56 f	34,87 k
Bison	12,39 n	0,67 jk	8,07 j	4,82 klm	23,08 d	16,32 g	33,24
Hermes	12,24 n	0,62 kl	3,21 r	2,60 q	7,07 v	7,21 s	10,03 y
Dakota	11,43 о	0,54 lmn	21,13 b	10,48 c	10,05 u	19,41 b	11,05 x
Royal	11,12 o	0,54 lmn	5,14 o	4,15 o	15,49 q	13,04 m	42,72 f
Aydın-1	9,33 p	0,49 mn	9,29 i	6,50 gh	18,27 lm	8,03 r	13,87 w
lgdir-2	8,96 p	0,43 no	10,17 h	8,10 e	28,42 a	13,35 l	16,71 v
AnkaraÇankaya-1	8,40 q	0,44 no	6,27 lm	3,62 p	18,46 kl	18,36 c	37,34 i
lzmir-Karşıyaka-3	6,40 r	0,31 p	10,54 g	6,39 gh	19,12 i	14,15 k	30,16 n
Crystal	6,30 r	0,36 op	7,21 k	4,45 mno	19,40 h	16,82 e	48,44 a
Pr > F (Model)	< 0,0001	< 0,0001	< 0,0001	< 0,0001	< 0,0001	< 0,0001	< 0,0001
Significantly	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 4

Soxhlet Extraction

Extraction was performed by the soxhlet method (Pradhan et al. 2010; Makkar et al. 2019). Flaxseed (5 g) was weighed and Soxhlete extracted with n-hexane for 6 hours. The flaxseed pulp was then filtered and then the solvent was removed in a rotary evaporator (HEIDOLPH Hei-VAP Core (HL/ML)) under vacuum at 40°C. Oil samples were stored in the refrigerator at 4°C for analysis. The oil obtained after processing was considered as the total oil content of flaxseed. The flaxseed oil yield was calculated as; Flaxseed oil yield (kg/ha) = Oil content(%)xFlaxseed yield (kg/ha) (Xie et al. 2020)

Mechanical structure of flax fibre and scanning electron microscopy (SEM)

To identify significantly fibres parameters and yield analyzes of flax varieties with high fiber properties were carried out in Kahramanmaras Sütcü Imam University University-Industry-Public Cooperation Development Application and Research Center with Zwick/Roell, a test machine with a 5 kN load cell and three diameter measurements (left, right and middle) were made with the help of a MİTUTOYO brand digital caliper. In addition, flax fibers strength analysis and calculations were made and images of fibers structures were taken with ZEISS brand scanning electron microscope (SEM Furthermore, the pathway for analysis; A Field Emission Scanning Electron Microscope, Zeiss Sigma 300 was used with an accelerating voltage of 10 kV to observe the morphology of the leaf samples. Before analysis with FE-SEM, since herbal products are not electrically conductive, the surface of the samples was coated with a gold plating device, Qurom, to ensure electron scattering from the surface. A secondary electron (SE) detector was used to display the morphological information of the samples.

#### **DNA** extraction

The relevant cultivars, It was determined by taking agro-morphological observations. Then, molecular characterization analyzes of 58 cultivars were performed using. Genomic DNA was extracted from the cotyledon leaves of 9-day accession seedings with the modification protocol (Aydın et al. 2018). A total of 16 gSSR (Table 3) markers were selected for genotyping of the whole used for germplasm.

#### Genetic diversity and using SSR markers

In the present study, 80 cultivars/genotypes were used. However, different cultivars and genotypes of a single plant strain were grown under laboratory conditions from a panel of 58 participants reared during the flowering-maturity period in the field trial area, and seedlings were used for DNA isolation. Genomic DNA was extracted from fresh leaf tissues using a plant DNA isolation kit (Thermo Fisher) according to the product manufacturer's protocol.

The purified DNA was checked by gel electrophoresis (Hoefer) with 6 µl of Redsafe (RedSafe<sup>™</sup>) added at a concentration of 0.5% µg/mL in 1X TBE buffer; After the procedure, the gel image was recorded by the DNA Redsafe GEN-BOX Ultra Viole (UV) device. In addition to the quality was checked with nanodrop (MAESTRO). A total of 38 primer pairs were used in the current study, all of which consisted of gSSR primers (Table 6). After preliminary screening of 38 SSR primers, 16 of them generated strong amplicons for PCR amplification and the study was continued with these primers. PCR, 0.5 µM each primer pair, 12 mM Tris-HCl (pH: 9.1), 60 mM KCl, 0.012% Triton X-100, 0.28 mM each dNTP, 2−3 mM MgCl<sub>2</sub> and 1 unit of Taq DNA Polymerase enzyme (Thermo Fisher) and PCR studies were performed. After PCR, the samples to which DNA loading buffer was added were loaded onto 4% agarose gel and 1X TBE solution was used and electrophoresis was performed for 2−4 hours by applying 60V/cm (Saroha et al. 2022). The PCR reaction was performed with a thermal cycler program (Table 7). In PCR processes, SensoQuest GmbH made use of the Labcycler Gradient brand Thermal Cycler. Band/allele size, UV pro ImageER. It was used in the scoring and photographing stages, which were recorded in the computer environment with the Eyes program. In the related study, cluster analysis Bayes statistics and MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) program were used. In addition, analyzes were made by using the Jaccard similarity index in Principal Positioning Analysis (PCoA). At the same time, PCoA analysis results were obtained using the MVSP v.3.13 software.

#### Table 5. Fiber mechanical properties of flax (Linum usitatissimum L.) varieties

Varieties	Series (n=3)	Tensile strength (fmax %)	Tensile strength (fmax %) Puller strength (MPa)		Fiber diameter measurement (mm)	
Rolin	(mean value)	0,62	563,4	1,2	0,682	
Mures	(mean value)	0,71	692,2	0,9	1,357	
Eckendorfi	(mean value)	0,70	605,4	1,3	1,409	
Mapun	(mean value)	0,69	537,5	1,5	0,917	
Hermes	(mean value)	0,64	564,3	0,8	1,655	
Dakota	(mean value)	0,58	704,6	1,2	1,626	
Antares	(mean value)	0,83	587,4	1,2	1,323	

Marker	Forward primers (5'-3')	Reverse primers (5'-3')	Referans
Lua613	AGCAGGGTCCTTCTCAAACTC	AAACAAAAATGGCTGGTGGA	Rana and Singh, 2017
Lu840	ATTCCTTTTTGAGGGCGAGT	ACAGCTGGAACTGGAGAGGA	Rana and Singh, 2017, Pali et al., 2015
Lu18	AGAGGCGGAGGGCATTAC	TTGGAGAGTTGGAATCGAGA	Rana and Singh, 2017; Pali et al., 2015
Lu445	CCCTGTAATGGAAGGGGAAT	TCTCCCCCAATCTGACTGAC	Rana and Singh, 2017
Lu9	TTGCGTGATTATCTGCTTCG	ATGGCAGGTTCTGCTGTTTC	Choudhary et al., 2017; Pali et al., 2015
L10	GCCTAAAGCTGATGCGTTTC	TGTCAGGCTCCTTCTTTGC	Choudhary et al., 2017; Pali et al., 2015
Lu11	ATGGCAGGTTCTGCTGTTTC	TTGCGTGATTATCTGCTTCG	Choudhary et al., 2017; Pali et al., 2015
Lu15	TGGACGACGATGAAGATGAA	CCGCCGGGTACACTACTACT	Pali et al., 2015
Lu17	GCTGGACCTTACAAGCCTCA	TTGGTGGGAGAACAACAAGA	Choudhary et al., 2017; Pali et al., 2015
Lu19	TCTCAGCTCCCTTTTATTTACCC	GCAGTCTCGAGTGCTGAGTG	Pali et al., 2015
Lu24	ATGGCAGGTTCTGCTGTTTC	TTGCGTGATTATCTGCTTCG	Pali et al., 2015
Lu29	GGGCAGTGATTGATTGGTTT	GGCGGCAATTGCTACATT	Pali et al., 2015
Lu32	ACGCGTAAACTTTCCGTTTC	ATAATGTCGGCTGCTTCTGC	Rana and Singh, 2017; Pali et al., 2015
Lu34	GGAAGAATTGGAAGAGGAAGG	CCTTCTCCCATGATCAAACAA	Pali et al., 2015
Lu38	GATCTTGTTGCCTGGGAAAG	TTCGTTTGCAATACGTCAGC	Pali et al., 2015
Lu35	CCAACGGATCATCCTCTAGC	GGAACAGAAAGGGGAAAGGAA	Choudhary et al., 2017

Table 6

Table 7. Polymerase Chain Reaction (PCR) profile used in the study

Hot Start	94°C	4 min	1 cycles	Pre-Denaturation
Pre-PZR	94°C	30 s	10 cycles	Denaturation
	56°C→51°C	30 s		Renaturation
	72°C	1 min	-	Synthesis
PZR.	94°C	30 s	30 cycles	Denaturation
	55°C	30 s		Renaturation
	72°C	1 min		Synthesis
Final	72°C	10 min	1 cycles	Final synthesis
	4°C	1 hour		

#### Data analysis

In the current study, three replications were used for each harvest and application, and a total of ten plants were used for each replication. In order to evaluate the results obtained, one-way analysis of variance (JAMOVI) for unrelated (independent) samples, which reveals whether there is a difference between the averages of the cultivars, was performed ( $\alpha = 0.05$ ). Pearson correlation (r) was used to determine the relationship between the investigated parameters. In addition, due to the large number of varieties and examined parameters; Principal Component Analysis (PCA) (JAMOVI 2022) was performed in order to eliminate the dependency structure of the relevant parameters and to reduce the dimension. In addition, heatmap clustering as applied to visualize and decompose the data related to the examined parameters and to determine their correlation (ClustVis) with each other.Both quantitative propertyes and agromorphological and molecular markers were used for genetic diversity of analysis based on the jaccard coefficient in DARwin 5 software (Perrier and Jacquemoud-Collet 2006). Additionally, UPGMA (Arithmetic Mean Unweighted Double Group Method) is an individual marker system used to create the dendrogram.

### Results

#### Morphological diversity and correlation analysis

In accordance with the experimental design, as we stated in the findings, the growth potential capacities of the region under high alkaline conditions were tested. Amid the 80 flax cultivar/genotypes, sixty-three of the flax genotypes were able to exhibit the growth and development under the such high alkaline conditions. The agro-morphological characteristics of the relevant flax cultivars/genotypes are listed in Table 2. For the characteristics considered, we have used 10 randomly-chosen plants for each plot. As uttered above, the flax is characterized with two characteristics, being used for the fiber and oil content. Herewith the study, we aproximately examined 19 traits of the plant. However, we addressed our comments on the fiber and oil content linked to the parameters, such as plant height, technical stem length, number of seeds per plant and seed yield per plant. Accordingly, the values of the parameters ranged as follows: plant height (25.50–80 (cm/plant)), technical stem length (61.26-77.83cm), number of seeds per plant (87.66–114 number/plant) ve seed yield per plant (0.63–0.72 g/plant) (Table 2). Specifically, the highest plant height was observed for Eckendorfi, while the lowest height was recorded for "Bison" and "Sandra". Regarding comparison to the foreing genotypes, those of plant height of local cultivars were relatively lower. For instance, plant height in local cultivars was found to be as 37.50 cm for "Beyaz Gelin" and 28.00 for "Clili392".

Concerned with the tehcnical stem length, the highest and shortest length were recorded as 77.83 and 22.10 for "Eckendorfi" and "Bison", respectively. Regarding local cultivars, the highest and shortest length values were observed as 58.90 cm and 23.23 cm for "İzmir Karşıyaka" and "Clli1392", respectively.

Number of seeds per plant values ranged from 35.00 to 114.00 for Rolin and Bison, respectively. In comparison to the foreign genotypes, the local cultivars had lower number of seeds per plant. In addition, the highest seed yield was found at Clli 1523 (13.95 kg/da) and the lowest Clli 1392 (1.67 kg/da) in local cultivars. In foreign genotypes, Mures (26.49 kg/da) was the highest and Bison (3.60 kg/da) was the lowest (Table 2). Correlation analysis revealed that plant height was positively correlated with number of seeds per plant (r= 0,857; p < 0,001) and technical stem length (r= 0,997; p < 0,01). Number of seeds per plant was also positively associated with the technical stem length (r= 0,861; p < 0,01). Interestingly, even the positive correlation coefficients were recorded for seed yield per plant with plant height (r= 0,662), number of seed per plant (r= 0,709), and technical stem length (r= 0,680); the relevant coefficients were not significant (p > 0,05; Fig. 4).

Heatmap clustering and Principal Component Analysis (PCA) of plant growth and biomass production traits

In order to visualize, correlate and clarify the agro-morphological traits considered to the cultivars/genotypes, we performed heat map (Fig. 2) clustering and PCA (Fig. 3). Heat map clustering revealed two major clusters. The first cluster was comprised of the number of maturity days, the number of flowering days and the number of days in flower, being linked to the flowering and seed onset. The second major cluster was associated with the growth and development attributes of the flax. Critically, oily varieties were entirely scattered at the same cluster. However, the fiber varieties were not clearly scattered as the case of oily varieties. In order to explain the percentage of variation; PCA was carried out to reveal what kind of relationship and what level of differentiation there is between cultivars and related parameters (Fig. 3). Due to being based on the correlations, the heatmap like scattering was also observed for PCA. For instance; attributes including the number of maturity days, the number of flowering days and the number of days in flower were clearly separated from other agro-morphological parameters. Accordingly, two components with eigen values over 1 were observed. These two components (F<sub>1</sub>: 68.2% and F<sub>2</sub>: 7.7%) explain the total variation of 75.9%. Such high explained ratio might be significant indicator for discrimination of cultvars/genotypes.

Oil yield and fatty acid compositions of flaxseed cultivars with the help of Principal Component Analysis (PCA), heatmap and correlation analysis

In addition to the quantitative analysis of the attributes of the plant, we also screened the oil yield and fatty acid composition of the seeds. For the relevant analysis, the flax cultivar/genotypes characterized with high oil content were assayed. In this context, 15 cultivar/genotypes were used. Accordingly, oil yield ranged from 0.263 to 0.717 g/plant for "Crystal" and "Sarı-85", respectively. In addition, we hypothesized that quality and quantity indicators might always be proportional to each other. For this reason, following extracting the oil, we further analzyed the fatty acid composition of the oils. GC-FID analysis revealed that the major fatty acids components of related flax plant oils are α-linolenic acid (C18:3n-3), linoleic acid (C18:2n-6), palmitic acid (C16:0), oleic acid (C18:1n-9) and stearic acid (C18:0). Considering fatty acid percentage, α-linolenic acid was found to be the highest in Crystal (48.44%) and the lowest in Hermes (10.03%). Linoleic acid (6.260-20.127%) was determined in Clli 1523 and Afyon-1 cultivar and genotypes, respectively. Oleic acid percentage ranged as lğdir-2 (28.427%)-Rolin (4.24%), while while the value for Sarı-85 cultivar was 18.180% (Table 5). Regarding local genotypes, İzmir-Karşıyaka-1 (41.50%) had the highest oil yield in terms of oil and the lowest oil yield was determined as İzmir-Karşıyaka-3 (6.40%). For α-linolenic acid content, İzmir-Karşıyaka-1 (46.07%) and İzmir-Kemeraltı-1 (46.75%) were found to be the highest.

According to the heatmap clustering, both the cultivars and the examined oil properties were divided into two major clusters (Fig. 5). Regarding fatty acid composition, linoleic acid, oleic acid and α-linolenic acid fatty acid components are scattered in a cluster, whereas stearic acid, palmitic acid, crude oil content and oil amount were scattered in the other main cluster. As the case in agronomic attributes, we further carried out PCA (Fig. 6), observing that three components (factors) with eigen values above 1 > were identified. The first two of them ( $F_1$ : 40.1% and  $F_2$ : 29%) explain the total change of 69.1%. Correlation analysis revealed that stearic acid was positively correlated with palmitic acid (r = 0,848; p < 0,001). Linoleic acid was also positively associated with the oleic acid (r = 0,544; p < 0,01). Interestingly, even the positive correlation coefficients were recorded for α-Linolenic acid with oleic acid (r = 0,476), linoleic acid (r = 0,396) the relevant coefficients were not significant (p > 0,05; Fig. 7).

#### Mechanical properties of flax fibre and SEM

Due to the low fiber ratio related thin and strenght of flax, the flax cannot compete other fiber crops such as hemp, jute, ramie, and cotton. That caused gradual decreases in uses of flax for fiber. Regarding fiber content, Arslanoğlu et al. (2017) and Yılmaz and Uzun (2019) reported the fiber content to be as 16–24% and 34–37% in flax stem. In the related study, in line with the results obtained in the analyzes (tensile strength, puller strength, rupture stretched and fiber diameter measurement) performed on the evaluated varieties; in fiber diameter measurement, the lowest Rolin variety (0.682 mm), while the highest Hermes variety (1.655 mm) was evaluated, the lowest (0.8%) in breaking tension. In this context, the highest tensile strength of the Mapun cultivar was determined (1.5%), the tensile strength was the lowest (537.5 MPa) among the varieties, while the highest tensile strength was determined as Dakota (704.6 MPa) (Table 5). In addition to the structural analysis were carried out with the help of Scanning Electron Microscopy (SEM) to determine the difference in the microstructure of the related cultivars. Additionally, it is observed in SEM images that the layers among fiber and matrix are separated (Fig. 8).

#### Experimental area and soil trait

Due to the micro-climate feature of the Iğdır region; It is noteworthy that not all of its lands are suitable for agriculture due to its salty and calcareous soils (Table 8) (Karaoğlu and Celim 2018). The annual average temperature is 12.2°C and the monthly average total precipitation is 261 mm (Celik 2021).

	tics of the field	
Examined trials/analysis types	Value/result	Soil structure
рН	9.7	Strong alkaline
EC (dS/m)	3.050	Moderately tolerant
Total salt (%)	82.44	Too salty
CaCO <sub>3</sub> (%)	9.29	Medium lime
Organic matter (%)	0.425	Very little
P <sub>2</sub> O <sub>5</sub> (ppm)	0.44	Very little
K <sub>2</sub> O (ppm)	42.56	Sufficient
Satürasyon (%)	52.29	Killi-tınlı

Molecular marker polymorphism and genetic diversity analysis

In order to determine the quality and quantity of DNA with the completed isolation, values at wavelengths of A230 nm (in polysaccharides, phenolic compounds),  $A_{260}$  nm (in nucleic acids) and  $A_{280}$  nm (in proteins) were determined by means of the nanodrop device connected to the spectrophotometric method (Table 9).  $A_{260}/A_{280}$  ratios are required to be between 1.8-2.0, and  $A_{260}/A_{230}$  ratios are reported to be great than 0.5. In addition, a ratio of 1.8 is generally accepted as "pure" for DNA; and 2.0 is generally accepted as "pure" for RNA (Karaca ve ark 2005; Abdel-Latif and Osman, 2017; Gupta, 2019). At the end of the analysis; the changed  $A_{260}/A_{280}$  ratio of DNA between 0.921 and 2.782. In addition, the DNA concentration was found to be between 9.78–217.3 ng in  $\mu$ L (Table 10). In the Polymerase Chain Reactions (PCR) study, gDNAs belonging to a total of 63 cultivar/genotypes were used. However, since cultivars such as (Bison, Gap-1, Mcduff, Royal and Rolin) did not form amplicons, the study continued with 58 genotypes. In addition, as a result of analyzing the gels of 16 loci screened in 58 cultivar/genotypes, scoring and calculating the PIC value determined that the lengths of alleles changed between 100–420 bp and the smallest locus was Lu9 and Lu19, while the largest was Lua613. In addition, it was determined that the allele numbers of the loci targeted by the primer pairs differed among 2–6 and that the average number of alleles per locus was 3.56. While the allele frequency (according to the number of patterns) of the existing loci was between 3–23, the mean allele frequency was detected as to be 4.43 (Table 9). In this context, since the markers (loci) used in the studies are SSR markers with codominant and multi-allelic features, some loci have PIC values (0.335–0.911) higher than 0.5 (Table 10). As a result; PIC value of the locus with the lowest polymorphism value belonged to Lu10 with 0.335 pattern number (5 – 3) allele number; the highest PIC value was found to be 0.911

Varieties	A <sub>260/280</sub>	Concentration	Varieties	A <sub>260/280</sub>	Concentration
1	2.123	95,34	35	2.206	73,02
2	2.025	99,36	36	2.782	21,82
3	2.172	84,36	37	2.063	93,76
4	2.229	217,3	38	2.208	58,43
5	1.967	119,18	39	2,062	51,04
6	2.161	124,28	40	2.238	66,8
7	1.131	27,42	41	2.145	163,86
9	1.731	143,02	42	2.163	72,45
10	1.813	120,93	43	2.335	63,93
11	1.988	177,04	44	2.277	96,58
12	2.098	73,43	45	2.316	91,66
13	2.141	51,04	46	2.193	36,92
14	2.191	129	47	2.335	78,5
15	2.104	129,36	48	2.113	82,14
17	2.108	42,53	49	2.327	83,99
18	2.160	72,41	50	2.420	68,18
19	2.200	52,76	51	2.131	86,87
20	2.227	54,35	52	1.928	136,66
22	1.887	156,87	53	2.235	72,37

Table 9

(more) A <sub>260/280</sub> and concentration spectrophotometer readings of some varieties								
Varieties	A260/280	Concentration	Varieties	A260/280	Concentration			
23	2.044	178,93	54	2.190	81,4			
24	2.343	45,46	55	2.240	56,18			
25	2.228	81,09	56	2.217	9,78			

57

58

59

60

61

62

63

2.406

2.215

1.527

2.336

2.214

0,921

2.275

85,68

84,54

31,9

75,86

65,15

28,48

82,89

Table 9

#### Cluster analysis

26

28

30

31

32

33

34

2.130

2.102

1.998

2.355

1.774

2.424

1,638

67,33

119,1

97,43

34,91

28,34

39,81

41,88

Bayesian analyses (BI) were performed using MrBayes v.3.1.2 with (Ronquist and Huelsenbeck 2003). The program is based on "presence (1)" and "absence (0)" scoring and cluster analysis of cultivar/genotypes obtained by analyzing the gels formed by the amplicons of the loci. The results of the analysis made by the MrBayes program took approximately 2 hours and 40 minutes. In addition, as a result of the analysis, the mean standard deviation of the split frequency was found to be 0.007859. Clustering analysis (phylogenetic tree) revealed the differences among the existing flax cultivar/genotypes and divided the phylogenetic tree into 2 main clusters as 100%. It was determined that the cultivars/genotypes with oily and fibrous characteristics showed similarity in different clustering and approximately (55–92%) (Fig. 9). In the current study, the necessary calculations were made using the Jaccard genetic similarity index percentile for the relationships among cultivar/genotypes. However, the most distant relationship was found to be 20.5% (Van-1 with Omega), and the closest relationship was 95.1% (Ankara-Ulus 3 and Ankara-Ulus 2) (Fig. 9). According to the similarity matrix; the similarity rate of Omega variety, known to be of USA origin, with the other 57 cultivar/genotypes was found to be between 20.5% and 34.3%. Since Omega is a variety from growth germination in an older culture medium other than variety, the similarity rate is the least (Mohammed et al. 2017).

The objective of using Principal Coefficient Analysis (PCoA) is to ensure that the flax cultivar/genotypes are positioned on a certain coordinate by preserving the distance relations between them as much as possible. The relevant to the presence and absence scores of the cultivar/genotypes in the current study was analyzed with the help of the Jaccard similarity index, and the results were obtained by using the MVSP v.3.13 program. Accordingly, the cultivars used gave similar results as in the cluster analysis in which Bayesian statistics were made, so the PCoA analysis supported the Bayesian statistics. From this a research perspectives, 3 clusters are seen in the obtained three-dimensional PCoA image (Fig. 10). In addition, *L. usitassimum* cultivar/genotypes were determined, respectively (cluster A (11), B (31), and C (16)).

# Discussion

For plant genetic resource uses, a thorough understanding of genetic diversity is a requirement for the plant genetic study, especially when most of the plants perform a narrow genetic application yield, and adverse effects against different stresses increase (Mishra et al. 2012; Suvi et al. 2020). Genetic diversity analysis at both the agro-morphological and molecular level plays an important role in optimizing breeding practices and yields. In addition, significant genetic variation has been reported in flax plant, agro-morphological traits and molecular level (Khan et al. 2013). For that reason, we have estimated a wide array of agro-morphological attributes and genetic diversity of the flax cultivar/genotypes considered. Present study was carried out as a field experiment in the ecological conditions of lğdır province in 2020. It is desired that the studies carried out in field conditions that cannot be fully or partially controlled, such as greenhouses, climate chambers or grow cabinets, should be at least two years old. Nevertheless, it is useful to state that although our study is not only yield-based, it is a molecular characterization-addressed study. For that reason, the assessment was based on a one-year field study.

Due to expanding world population, the need for raw materials for the agricultural industry is also increasing due to the rapid increase in the food needs of the people. To supply these increasing needs; it is only possible by producing efficiency and high quality varieties (Bennett et al. 2012; Tian et al. 2021). In this context; flax, which is an important industrial plant with both oil, and fiber, properties, has its own distribution area in almost every geography of the world (Ataii et al. 2021; Melelli et al. 2022). In addition, it is reported to be one of the oldest crops plants cultivated (Shivaraj et al. 2019; Cassuto et al. 2022). It is known that flax has the ability to adaptation to extreme conditions and has a culture area (Bourmaud et al. 2013; Goreeva et al. 2020; Li et al. 2022).

With respect to the plantation of the cultivar/genotypes considered, 63 out of 80 flax emerged at the trial conditions. Amid the emerged flax cultivar/genotypes, Bison, Mcduff, Hermes, Eckendorfi, and Mapun genotypes were for the first time assayed in Turkey. Being critical indicator/parameter, the plant height might be the important criteria in the selection of flax grown for fiber purposes (Heller et al. 2015; Goudenhooft et al. 2019; Moudood et al. 2019). According to the present finding, the lowest value for plant height in flax was detected in Bison-Sandra (25.50 cm), which has oily properties, and the highest in Eckendorfi (80.00 cm), which is fibrous, and its average was determined to be 50.88 cm. The present findings are consistent with the former reports (Mirshekari et al. 2012 (58.2–60.3 cm); Rashwan et al. 2016 (85.11–96.74 cm); Goudenhooft 2018 (85.4-123.8 cm); Sheng et al. 2020 (30.7–59 cm). Moreover, it was determined that the lowest value in technical stem length was Bison (22.10 cm) and the highest value was Eckendorfi (77.83 cm) varieties. In addition, our results were found to have higher values than previous studies (EI-Harir 2004: 50,41–77,19 cm; Kurt et al. 2015: 29,4–31,7 cm; Örs and Öztürk 2018: 12,9–25,7 cm; Emam 2020: 61,58-88.91 cm). Generally, it has been stated that agro-morphological traits are dependent on each other and differences especially when evaluated as oily and fiber (Mirshekari et al. 2012; Sheng et al. 2020). In this context, it was founded in our results that the differences determined in the agro-morphological traits taken in the relevant plant may be relation to soil organic matter contents, pH and lime (Pisupati et al. 2021), and it was determined that our results different from previous studies and are compatible with the literature. In addition to being the desired feature for fiber plants, it is expected that low plant height and high number branching are of the desired feature of cultivars/genotypes with industrial value as oil (Zang et al. 2018).

As expressed in present study; the oil yield of the related cultivars was respectively among 6.30-45.23%; this was found to be similar to previous studies (Arslan et al. 2011; 36.8%; Keskin et al. 2020; 34.1%). Considering fatty acid percentage, we observed  $\alpha$ -linolenic acid (10.03-48.44%), linoleic acid (6.260-20.127%) and oleic acid (4.24-28.43%). However,  $\alpha$ -linolenic acid was 48.41% (Hatanaka et al., 2021), linoleic acid was 14.90% (Njembe et al., 2021) and oleic acid was 23.28% (Liang et al. 2021). The present findings are consistent and in the range of the previous studies.

For fibrous flax varieties are desirable high plant height, technical stem length, and less branched structure. In addition, it has been reported that quality parameters such as fiber tensile strength (fmax %), puller strength (MPa), rupture stretched (%) and diameter length are of significant importance (Ramesh and Sudharsan 2018; Korolyov et al. 2019). Furthermore, it was emphasized that quality parameters may be related to the matrix between the fibers (Chuah et al. 2014; Goudenhooft et al. 2018). According to results; fiber diameter data exhibited wide variability. For instance, the values are higher than Ryvolová (2019), Betts et al. (2017) and Goudenhooft et al. (2018). In addition, structural analyzes were carried out with the help of scanning electron microscopy (SEM) to determine the difference in the microstructure of the related cultivars.

It was determined that the fiber rupture stress values of the related plant based on the measurements and SEM images on the technical system (0.8-1.5%) were lower than in the previous studies. In this context, it is stated to the mechanical properties of the matrix structure in the fiber structure of flax are deteriorated due to hydrolysis and the layers between the fiber and the matrix are separated, especially as a result of this separation, the rupture and breakage increase, as well as the breaking stress is significantly adversely affected (Moudood et al. 2019; 1.9-2.54%). Moreover, it is observed in SEM images that the layers among fiber and matrix are separated.

The analysis of genetic diversity at the morphological and molecular level is essential in different crops improvement and development applications as it has a significant impact in optimizing breeding efforts (Anumalla et al. 2015). In the present study; 58 flax cultivar/genotypes were used for molecular analysis and characterization has performed with SSR markers, showing co-dominant, multi-locus and multi-allelic traits. Although, 38 primer pairs consisting of gSSR primers with high polymorphic information content were used, 16 of them continued to work as they formed strong amplicons for PCR amplification. Accordingly, the PIC values of the relevant loci used in the study were found to be higher than in previous studies. The finding of Choudhary et al. (2017)

suggested fifteen gSSR markers produced 47 polymorphic bands with a mean polymeric information content PIC value of 0.326. In addition, ror dominant markers the *PIC* values indicate that low (0 to 0.10), medium (0.10 to 0.25), high (0.30 to 0.40) and very high (0.40 to 0.50) (Serrote et al. 2020). The PIC value indicates population polymorphism depending on the number and frequency of alleles in a marker (Nachimuthu et al. 2015). In this context, it is reported that the use of molecular markers can be better understood the genetic differences in cultivated plants and to reveal their structure at the molecular level can help us to reach more realistic information (Nadeem et al. 2018). For the first time, the molecular characterization of flax has been extensively discussed in the relevant cultivars/genotypes in Turkey. According to the findings, the population used was divided into two main groups and a high level of variation was detected. It is a considerable advantage that the genetic material to be used for flax cultyver/genotypes developed in Turkey has a high level of variation.

# Conclusion

The Igdir region, where this study was conducted, is the lowest plain of the Eastern Anatolia Region with its microclimate characteristics, and it is one of the largest plains in terms of surface area (climate, soil and vegetation, etc.) compared to the surrounding provinces. Within the extent of the results, among the registered varieties with high seed yield that can be used in flax farming in the region (Rolin, Mures, Eckendorfi, Mapun, Royal, Omega); genotypes (Aydın-1, Mersin-1, İzmir Tepecik-2, Bursa-1, Van-2) were determined. In addition, for purposes of cultivation of this fiber, Eckendorfi, Mures, Royal, and Rolin varieties have been seen to the fore with their long plant height. In the context, the molecular characterization of flax in Turkey was performed in this way for the first time in Turkey with the molecular characterization study with 16 SSR markers screened with 38 Simple Sequence Repeat (SSR) markers in existing cultivar/genotypes and with high polymorphism. Thanks to different analysis methods, genetic relationships were determined and the population was separated into two groups and a high level of variation was detected. In addition, it was determined that the *PIC* values of the SSR markers had been used higher than the previous populations and that the distinctive power of the markers was higher. Regarding these results; It is thought that these varieties can be a resource for researchers and contribute to science by demonstrating the usability of groups defined based on both morphological and molecular markers in our country and in foreign countries. In addition, it is predicted that high seed yield can be obtained by growing proprietary varieties with superior genotypic characteristics in ecologically suitable regions, only, by adapting the relevant varieties to the region and by appropriate agronomic practices.

# **Abbreviations**

SSR	Simple Sequence Repeat
RAPD	Random Amplified Polymorphic DNA
ISSR	Inter Simple Sequence Repeat
AFLP	Amplified Fragment Length Polymorphism
PUFA	Polyunsaturated Fatty Acids
SDG	Secoisolariciresinol Diglucoside
SEM	Scanning Electron Microscopy
PCoA	Principal Coefficient Analysis
PCA	Principal Component Analysis
UPGMA	Arithmetic Mean Unweighted Double Group Method
PIC	Polymorphic Information Content

# Declarations

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#### Author contributions

All authors have significantly contributed in finalising the research. M.Z.K., A.M.K. and M.H.A. designed the experimental set-up. M.Z.K. performed the greenhouse experiments and relevant biochemical analyses. M.Z.K. and A.M.K. performed the molecular analyses and performed the statistical analysis.

M.Z.K. analysed the data and wrote the first draft, while the final draft was read and approved by all authors. M.Z.K., A.M.K. and M.H.A. Methodology, Validation, Conceptualization, Supervision, Writing - original draft.

#### Conflict of interest

The authors declare that they have no conflict of interest.

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# Table 2

Table 2 is available in Supplementary Files section.

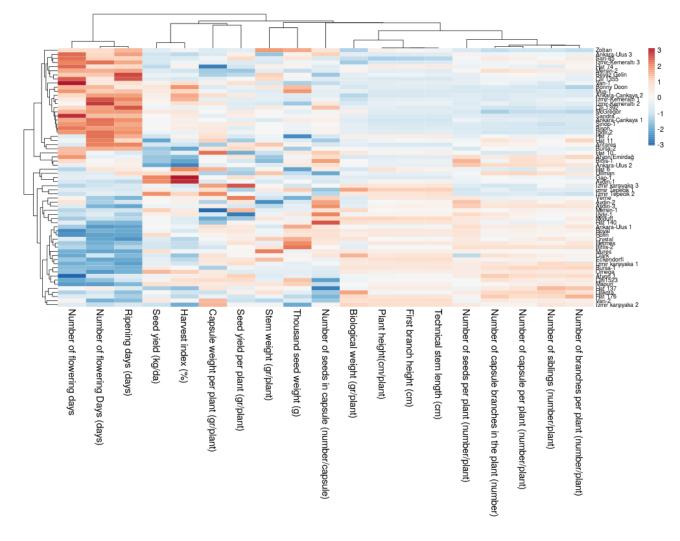
# Table 10

Table 10 is not available with this version

# **Figures**

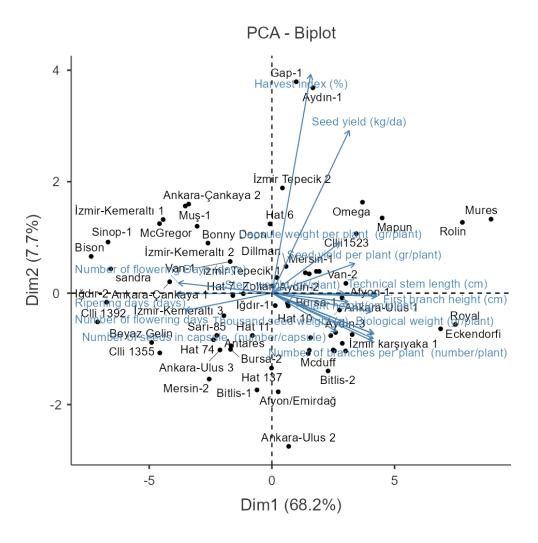


Developmental stages and other processes of flaxseed cultivars/genotypes

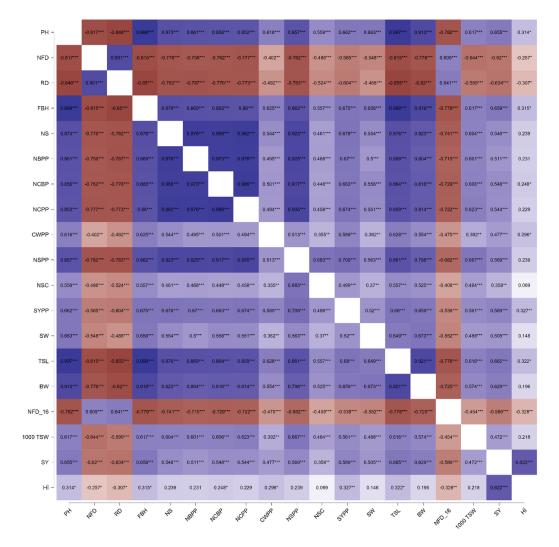


#### Figure 2

Heatmap clustering of morphological and agricultural characteristics of flax cultivar/genotypes



Principal Component Analysis (PCA) of agro-morphological characteristics of flax cultivar/genotypes

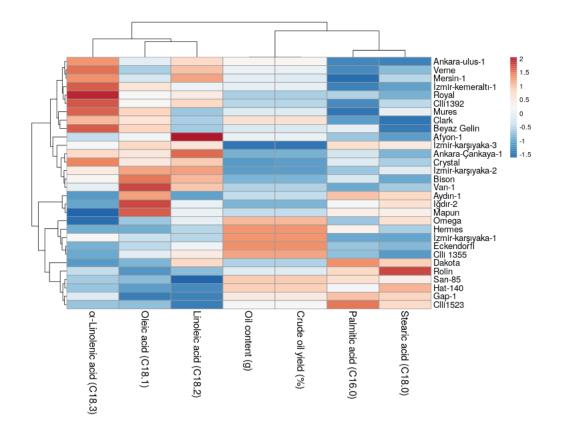


Correlation analysis of agro-morphological characteristics of flax cultivar/genotypes

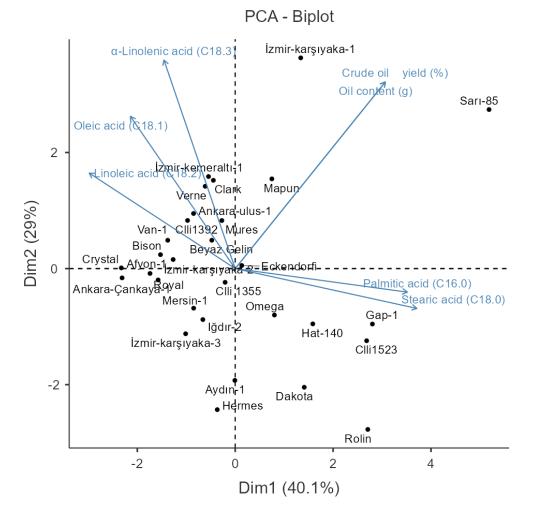
\*\* 0.01 significant at the level

\* 0.05 significant at the level

"Each data is given as the average of three replicates. Values shown with lowercase and different letters in each column indicate the difference (p<0.05) between applications. Evaluation was made according to the Post-Hoc Duncan test. Plant height (cm/plant) (PH), Number of flowering Days (days) (NFD), Ripening days (days) (RD), First branch height (cm) (FBH), Number of siblings (number/plant) (NS), Number of branches per plant (number/plant)(NBPP), Number of capsule branches in the plant (number) (NCBP), Number of capsule per plant (number/plant) (NCPP), Capsule weight per plant (g/plant) (CWPP), Number of seeds per plant (number/plant) (NSPP), Number of seeds in capsule (number/capsule) (NSC), Seed yield per plant (g/plant) (SYPP), Stem weight (g/plant) (SW), Technical stem length (cm) (TSL), Biological weight (g/plant) (BW), Number of flowering days (NFD), Thousand seed weight (g) (TSW), Seed yield (kg/da) (SY), Harvest index (%) (Hi)."



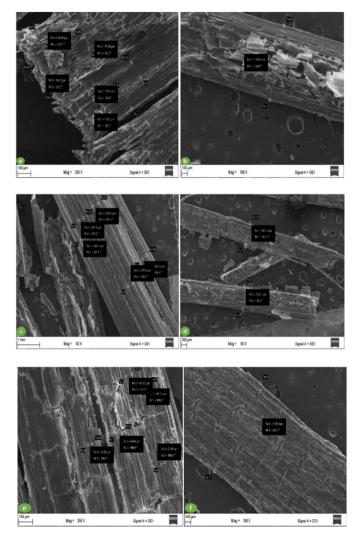
Heatmap clustering of oil and fatty acids in flax cultivar/genotypes



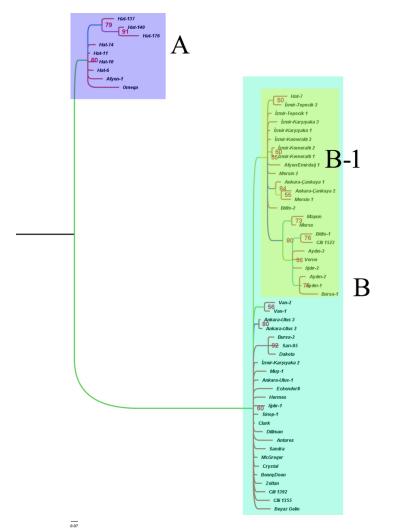
Principal Component Analysis (PCA) of oil and fatty acids of flax cultivar/genotypes

Oil content –		1***	0.285	0.287	-0.02	-0.234	0.234	
Crude oil _ yield	1***		0.286	0.287	-0.02	-0.233	0.234	
Palmitic acid (C16.0) -	0.285	0.286		0.848***	-0.166	-0.256	-0.226	
Stearic acid (C18.0) -	0.287	0.287	0.848***		-0.226	-0.352	-0.288	
Oleic acid (C18.1) -	-0.02	-0.02	-0.166	-0.226		0.544**	0.476**	
Linoleic acid (C18.2) -	-0.234	-0.233	-0.256	-0.352	0.544**		0.396*	
$\alpha$ -Linolenic acid (C18.3) –	0.234	0.234	-0.226	-0.288	0.476**	0.396*		
Oil content Crude vield C16.0) C18.0) C18.2) Oil content Crude vield C16.0) C18.2) C18.2) C18.2) Oil content C18.2) Oil content card C18.2) Oil conten								

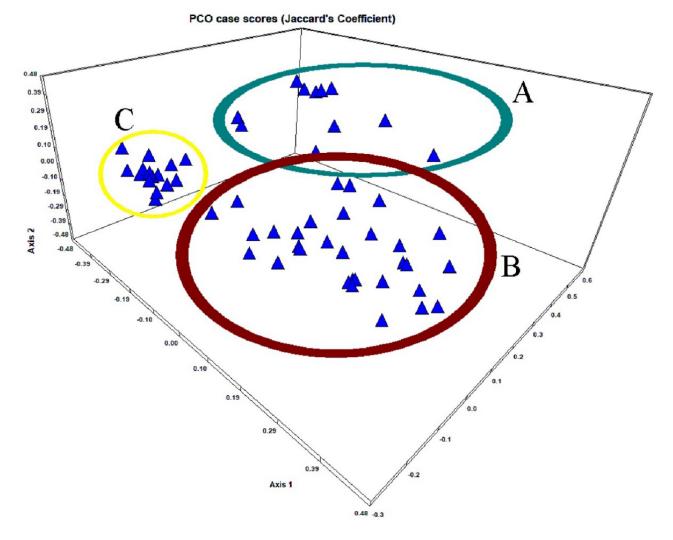
Correlation analysis of oil and fatty acids of flax cultivar/genotypes.



Scanning Electron Microscopy (SEM) fibre images in flax cultivars (a. Rolin, b. Mures, c. Eckendorfi, d. Hermes, e. Mapun, f. Dakota).



Consensus tree formed by Bayesian statistics of flax cultivar/genotypes used in the study. The polar tree is presented in unrooted format, the values shown in the nodes are the sequent probability values, and the bar at the bottom of the tree represents the base change scale.



Principal Coefficient Analysis (PCoA) analysis with Jaccard similarity index values.

# **Supplementary Files**

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• Table2.docx