Agro-morphological traits and iPBS-ISSR markers based genetic characterization of flax genotypes (Linum usitatissimum L.)

Mehmet Zeki KOCAK (mehmetzekikocak@gmail.com)
Igdir University: Igdir Universitesi https://orcid.org/0000-0002-8368-2478
Mustafa Guven Kaysim
Iğdır University: Igdir Universitesi
Adnan Aydın
Igdir University: Igdir Universitesi
Ceknas Erdinc
Van Yuzuncu Yil University: Van Yuzuncu Yil Universitesi
Muhittin Kulak
Igdir University: Igdir Universitesi

Research Article

Keywords: Linseed, Genetic diversity, Molecular markers, Crops

Posted Date: April 20th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2828900/v1

Abstract

Flax is one of the significant crops owing to oilseed and fibre attributes, exhibiting a wide distribution in many parts of the world. The present study was designed to reveal the genetic diversity of 29 genotypes of flax through morphological traits and 12 iPBS and 33 ISSR markers. According to the UPGMA-based dendogram, genotypes of the flax were classified into the two major groups (A and B) and group B was composed of two sub-groups (B1 and B2) according to the six phenotypic attributes considered for analysis. Concerning molecular relationships of the genotypes, individual and combined UPGMA-based dendograms were constructed with respect to the ISSR and iPBS markers. According to the Jaccard similarity coefficients for ISSR data, flax genotypes were divided into two main groups (A and B) and the “Van-1” genotype was in group A alone. Also, group B was divided into two separate subgroups. “Afyon-1” and “İzmir-Kemeraltı” genotypes were located in group B1, while all the remaining genotypes were located in group B2. In addition, the average genetic similarity was 0.755 regarding the findings of iPBS-retrotsposon markers, flax genotypes were classified into two main groups (A and B), and these main groups formed two subgroups among themselves. While “Konya-1” is located alone in A1 subgroup, “Iğdır”, “İzmir-Kemeraltı”, “Mardin”, “Bitlis” and “Afyon-1” genotypes are located in A2 subgroup. While “Van-1” genotype was located in the B2 subgroup alone, other genotypes were found in the B2 subgroup. The average genetic similarity was determined as 0.578 according to Jaccard binary similarity coefficient. According to the combined data of two markers, two separate groups (A and B) were revealed, similar to the dendrogram constructed with ISSR data. “Van-1” genotype was located in group A alone. Group B was divided into two subgroups (B1 and B2). The average genetic similarity was 0.722 according to the Jaccard similarity coefficient of matrix.

Introduction

Flax or linseed (*Linum usitatissimum* L.) is one of the oldest crops cultivated, with evidence of its use dating back to ancient Egypt and Mesopotamia (Saha and Hazra, 2004). Asia, Ethiopia, and India are considered as secondary centres of the plant (Choudhary et al., 2017; Chen, 2022). However, flax is now a worldwide spread annual, herbaceous and self-pollinating crop species belonging to the Linaceace family with 300 species (Đurić and Spasić, 2019; Akram et al., 2021). Concerning the orders of plants used for oil and fiber, the plant ranked at the third in terms natural fiber and major oil crops in the World (Ahmed et al., 2019). Today, major producers of flax include Canada, China, Russia, and India. Flax is also grown in smaller quantities in many other parts of the World (Jhala and Hall, 2010; Mishra and Awasthi, 2020; Stavropoulos et al., 2023). In the case of Türkiye, Anatolia is considered as homeland of many wild flax species, being as of the first cultivation regions of flax (Şahin and Yıldız, 2022). Türkiye has ideal environmental conditions for the cultivation and production of fiber and oil flax (Arslanoglu et al., 2022) and flax is cultivated in different regions of Türkiye. The flax is locally named as “bızıktan, bezir, güdün, cimit, sağlek, siyelek and zeyrek” (Dumanoğlu, 2020). However, it has been determined that the amount of flax production in Türkiye decreased significantly between 1990 and 2020 (1,570-8 tons), respectively (TUIK, 2019; FAOSTAT, 2020). In cultivated plants, the genetic relationship and revealing of the phenotypic

and genetic diversity between plants form the basis of successful breeding studies. Therefore, evaluation of genetic diversity is important for the selection, conservation, and evaluation of the most productive genotypes. Different morphological, biochemical features and molecular markers were used to evaluate the diversity in genotypes (Rana and Singh, 2017; Soto-Cerda et al., 2019; Landoni et al., 2020).

Amid the marker systems adopted, Inter Simple Sequence Repeat (ISSR) marker system is based on amplification of DNA segments located between regions of identical but opposite microsatellite repeats at a distance that allows amplification (Zietkiewicz et al., 1994). Primers used in this technique are also known as microsatellites and can be di-, tri- and tetra- or penta-nucleotide repeats. In this technique, long primers with a size of 15–30 bases are normally used (Gupta et al. 1994). ISSR shows higher reproducibility, levels of variability, and simplicity compared to other dominant marker systems (Wolfe and Liston, 1998). Therefore, ISSR has several advantages such as high reproducibility, high polymorphism, low DNA requirements, easy handling and high genomic distribution (Heidari et al., 2016). It has been widely used in the characterization of plant gene resources and has been reported to be effective in many studies (Erdinc et al., 2013; Ekincialp et al., 2019; Ibrahim and Erdinc, 2020). DNA sequences that can move in the genome and cause mutation are called transposons (Capy et al. 2000). Transposons make up a large part of the genome (Mansour, 2007) and markers obtained from them are widely used to determine genetic diversity (Roy et al. 2015). Since there is a universal tRNA complement as the primary binding site of reverse transcriptase in long terminal repeat (LTR) retrotransposons, Interspersed Repeats-based PCR (iPBS) marker system can be used in all plant species without the need for sequence information (Kalendar et al. 2010). iPBS has been successfully applied in many plant species including wild chickpeas (Andeden et al. 2013), grapes (Guo et al. 2014), peas (Baloch et al. 2015a), beans (Nemli et al. 2015; Öztürk et al. 2020), okra (Yildiz et al. 2015), Leonurus cardiaca (Borna et al. 2017), Fagaceae (Coutinho et al. 2018), *Ranunculaceae* (Hossein-Pour et al. 2019), oregano (Karagoz et al. 2020), pepper (Yıldız et al. 2020), and wild rhubarb (Erdinç et al. 2021).

Both ISSR and iPBS markers are highly reproducible and require only small amounts of DNA for analysis. They are also relatively easy and cost-effective to use, making them popular choices for population genetic studies, phylogenetic analysis, and biodiversity conservation. Overall, ISSR and iPBS markers are useful tools for genetic comparison because they can provide insights into the evolutionary relationships and genetic diversity of populations, which can be used to inform conservation strategies and other applications (Demirel 2020; Kumlay et al. 2021; Eren et al. 2022). For those reason, we herein aimed the potential differences when individual or combined use of ISSR and iPBS for characterization of flax. Also, some phenotypic attributes such as technical stem length, technical stem fresh weight, technical stem dry weight, root length, root fresh weight and root dry weight were recorded. Finally, the scattering of the genotypes with respect to the molecular and phenotyic data were carried out.

Material And Methods

Plant material and experimental design

Plant materials were obtained from Türkiye and a total of 29 flax genotypes were used (Table 7). The experiments were carried out at the greenhouses of Agricultural Research and Application Centre, Igdir University of Türkiye. The study was performed as a factorial experiment using a completely randomized design with three replicates. Prior to sowing, the seeds were surface-sterilized using 1% (*v/v*) hypochlorite for 2–4 min and then the seeds were rinsed with distilled water to remove the residues of the disinfectant. Then, the seeds were sown in 2,5 L-plastic pots containing peat and grown with a 26 – 30°C/day relative in the experimental area.

Morphological evaluation

All plants were harvested after 76 days and some morphological attributes including technical stem length, technical stem fresh weight, technical stem dry weight, root length, root fresh weight and root dry weight were recorded.

Genomic DNA extraction

The genomic DNA was extracted from the cotyledon leaves in 8-day seedlings according to the protocols of Aydin et al. (2018) with slight modifications. Also, 12 iPBS and 33 ISSR markers were selected for genotyping. The concentration and quality of the genomic DNA samples were determined using nanodrop spectrophotometer (MAESTRO).

iPBS-Retrotransposon Amplification

In total, 50 iPBS-retrotransposon primers were screened in eight randomly selected flax genotypes, and the 12 most polymorphic primers were selected for testing in all genotypes (Table 2). PCR amplicafications were made according to the conditions proposed by Kalendar et al. (2020). Briefly, the PCR reaction was carried out in a total volume of 25 µl, containing 3X Dream Taq Green PCR buffer, 5 mM dNTPs, 10 µM primer, 1.75 units Dream Taq DNA polymerase and 10 ng DNA. Concerning PCR amplification, it was firstly carried out starting with 4 min denaturation at 95 ^{o}C, then followed by 15 sec denaturation at 95 ^{o}C, 1 min binding at 50–65 ^{o}C (depending on primer), and 1 min at 68 ^{o}C. The final extension phase was carried out by keeping it at 72 ^{o}C for 5 minutes.

ISSR Amplification

First, a total of 63 ISSR primers were screened in eight randomly selected flax genotypes, and 33 primers were selected for amplification of all genotypes, with the clearest readable bands and high polymorphism (Table 3). PCR reaction was carried out with 2.5 µl of 10 × buffer (Tris–HCl (pH 8.3) 100 mM; KCl 500 mM), 0.1 mM dNTP, 3.0 mM $MgCl_2$, 10 pmol primer, 0.75 U Taq DNA polymerase (Takara Biotech Co., Ltd.) and 30 ng of template DNA. Regarding PCR amplification, the reactions started with 5 min denaturation at 94 ^{o}C, and were followed by 35 cycle-1 min denaturation at 94 ^{o}C, 1 min binding at 50–59,7 ^{o}C (depending on primer), 1,5 min elongation at 72 ^{o}C, and final extension of 7 minutes at 72 ^{o}C (Ahmed et al. 2019).

Gel-Electrophoresis and Image Analysis

The purified DNA was checked by gel electrophoresis (Hoefer) with 6µl of Redsafe (RedSafeTM) added at a concentration of 0.5% µg/mL in 1X TBE buffer. Then, the gel image was recorded by the DNA Redsafe GEN-BOX Ultra Viole (UV). 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 $MgCl_2$ and 1 unit of Taq DNA Polymerase enzyme (Thermo Fisher) and PCR studies were carried out. The next of 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 2–5 hours by applying 60V/cm (Saroha et al., 2022). Additionally, 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. Cluster analysis Bayes statistics and MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003) program were used. In addition to the analyzes were made by using the Jaccard similarity index in Principal Positioning Analysis (PCoA). Therewithal, PCoA analysis results were obtained using the MVSP v.3.13 software.

Data analysis

Three replicates (each replicated corresponded ten plants) were used for data analysis. The flax plants were compared for their agronomic and morphological attributes using one-way variance analysis ($p < 0.05$) (SPSS 22). Pearson correlation (*r*) was used to determine the relationship between the investigated parameters. Also, principal component analysis (JAMOVI) and heatmap clustering (ClustVis) were employed to cluster the dependent variable parameters corresponding to the genotypes (independent variables). Concerning the statistics of molecular analysis, scoring was made according to the binary data system and recorded as "1" in the presence of the band and "0" in the absence of it. Molecular data was analyzed using PAST3. The similiarity between molecular and phenotypic data were determined using Jaccard similarity coefficient and Euclidean coefficient, respectively. The dendogram showing the genetic link between the flax genotypes was constructed by the UPGMA method using the similarity coefficients. The polymorphic information content (PIC) was calculated according to Powell et al. (1996) and Smith et al. (1997). The effective number of alleles (*ne*), gene diversity (*h*) and Shannon information index (*I*) (Yeh et al., 2000) were calculated using POP-GENE 1.32. The population structure of the flax germplasm was determined with Bayesian clustering model using STRUCTURE 2.3.2 (Pritchard, 2000). Most likely number of clusters (K = number of subpopulations) was determined according to Evanno et al. (2005) and the number of clusters (K) was plotted against the logarithm probability according to the standard deviation (ΔK). Finally, Mantel test (Mantel, 1967) was applied to determine the consistency between the molecular and morphological data using GenAlEx (6.51) (Peakall and Smouse, 2006).

Results

Genetic diversity by morphological traits and correlation analysis

In accordance with the experimental design, as we stated in the findings, growth potential capacities were tested by artificially growing in organic soil under controlled conditions. In addition, a total of 29 genotyps registered varieties and unknown genotypes of flaxseeds obtained from different provinces of Türkiye were used. The morphological characteristics of the relevant flax genotypes are listed in Table 1. All characteristics measurements were performed in Agricultural Research and Application Center, Igdir University, and randomly-chosen to twenty plants for each plot. In addition, in the study, we approximately examined 6 traits selected from flax plants and we addressed our comments. Accordingly, the values of the parameters ranged as follows: technical stem length (19.32-45.39cm), technical stem fresh weight (0.13-0.82g), root length (4.07-7.84cm), and root fresh weight (0.03-0.13g) (Table 1). Specifically, the highest technical stem length was observed for "Ankara-Çankaya", while the lowest height was recorded for “Royal”. In addition to the highest root length was observed for "Afyon-2", while the lowest was recorded for "Eckendorfi". Concerned with the technical stem fresh weight, the highest and lowest weight were recorded as 0.82g and 0.13g for “Ankara-Çankaya” and “Royal”, respectively. Accordingly, the root fresh weight, the highest and lowest weigh values were observed as 0.13g and 0.03g for “Van-1” and “Konya-1, Sarı-85”, respectively. Correlation analysis revealed that plant technical stem length was positively correlated with technical stem fresh weight (r = 0,520; p < 0,05). Technical stem fresh weight (r = 0,810; p < 0,05) was positively correlated with root dry weight. In addition, the relevant coefficients were not significant (Fig. 3).

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

For the visualize, correlate and clarify the morphological traits considered to the genotypes, we performed heatmap (Fig. 1) clustering and PCA (Fig. 2). Heatmap clustering revealed two major clusters. The first cluster was comprised of technical stem length, root length. Additionally, the second major cluster was associated with the development attributes of the flax. This cluster was comprised of technical stem fresh weight, technical stem dryweight, root fresh weight and root dry weight. 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. 2). Due to being based on the correlations, as the heatmap scattering was also observed for PCA. For instance; attributes including the technical stem length technical stem fresh weight and technical stem dry weight were clearly separated from other morphological (root length, root fresh weight and root dry weight) parameters. In addition, two components with Eigen values over 1 were observed. These two components (F_1: 44.9% and F_2: 20%) explain the total variation of 64.9% (Fig. 2).

Phenotypic characterization

According to the six phenotypic attributes considered for analysis, a UPGMA-based dendogram was constructed (Fig. 4). The genotypes of the flax were classified into the two major groups (A and B) and group B was composed of two sub-groups (B1 and B2). Of the genotypes, only Ankara-Çankaya genotype was located in the A group, whilst 9 genotypes (Dakota, Antares, Sarı-85, Rolin, Bursa, Iğdır, Muş, Samsun-

1 and İzmir-Kemeraltı) and 13 genotypes (Mardin, Mersin-1, Afyon-1, Bitlis, Konya-1, Afyon-2, Sinop, Van-1, Siirt, Kilis, Diyarbakır, İzmir-Karşıyaka and İzmir-Tepecik) were included in the B1 subgroup and B2 subgroup, respectively. According to the Euclidean similarity coefficient, the average similarity coefficient of the genotypes was determined as 13.47. The closest genotypes pairs were as Sinop and Konya-1 (2.56), Dakota and Sarı-85 (2.73) and Rolin and Samsun-1 genotypes (2.87). On the other hand, the most distant genotypes from each other were as Ankara-Çankaya and Bitlis (44.20), Ankara-Çankaya and Mardin (42.20) and Ankara-Çankaya and Konya-1 (41.10). According PCoA, the phenotypes were classified into three groups. The scattering of the genotypes was largely similar to cluster analysis. In this context, while the Ankara-Çankaya genotype was located at a single point, it was determined that the İzmir-Kemeraltı genotype was located in the third group, unlike the dendogram in the cluster analysis in the second group (Fig. 5).

iPBS and ISSR amplification

A total of 91 scoreable bands were obtained from 13 iPBS primers used to reveal the genetic variation in a population of 23 flax genotypes, and 77 of the obtained bands showed polymorphism (Table 2). Accordingly, the lowest polymorphic band production per primer was obtained from primer 2085 with 1 band, while the highest band production was obtained from primer 2232 with 9 bands. Primers 2228, 2375 and 2376 showed 100% polymorphism. While the average polymorphism % of the primers studied was 82.55, the lowest polymorphism rate was found to be 33% in the 2085 primer. The mean polymorphism information content (PIC) value was calculated as 0.90 for 23 flax genotypes. The minimum PIC value was obtained from 0.09 from 2239 primer, while the highest PIC value (0.93) was obtained from 2220 primer (Table 2). The *ne* value for the thirteen iPBS primers ranged from 1.33 (2085) to 1.73 (2230). The average *ne* value was calculated as 1.58. The average *h* value was calculated as 0.34. The lowest and highes *h* value were as 0.09 (2085 primer) and 0.47 (2415 primer), respectively. I values ranged from 0.19 (2085 primer) to 0.67 (2415 primer) with an average value of 0.50 (Table 2).

In the characterization of flax genotypes with ISSR markers, a total of 309 scoreable bands were obtained from 33 primers, and 212 of the obtained bands were found to be polymorphic bands (Table 3). The lowest polymorphic band production was obtained from the 812 primer with 2 bands, while the highest band production was obtained from the Sola-5 primer with 13 bands, and the average number of polymorphic bands was found to be 6.42.

Primers 8f and Sola-5 showed 100% polymorphism, primer PHV-6 had the lowest polymorphism rate with 33%. At the same time, the polymorphism average of the primers was determined as 67.91. The mean PIC value of the primers was calculated as 0.73, and these values ranged from 0.23 (5f) to 0.93 (I12 and LOL-8) (Table 3). While the mean *ne* value was found to be 1.49 for 33 ISSR primers used in the study, Sola-4 had the highest ne value with 1.89 and 5f had the lowest ne value (1.23). The mean *h* value of the primers was calculated as 0.30. The lowest *h* value was obtained from the B10 primer with 0.15 and the highest *h* value was obtained from the Sola-4 primer with 0.47. The highest Shannon index was 0.66 in

the Sola-4 primer, the lowest value was found in the B10 primer with 0.27, and the average of the primers was calculated as 0.46 (Table 3).

Phylogenetic analysis with molecular data

The Jaccard similarity coefficient was used to determine the genetic relationships between flax genotypes with iPBS-retrotsposon markers and a UPGMA-based dendogram was obtained (Fig. 6). Accordingly, flax genotypes were divided into two main groups (A and B), and these main groups formed two subgroups among themselves. While Konya-1 is located alone in A1 subgroup, Iğdır, İzmir-Kemeraltı, Mardin, Bitlis and Afyon-1 genotypes are located in A2 subgroup. While the Van-1 genotype was located in the B2 subgroup alone, other genotypes were found in the B2 subgroup (Fig. 6). According to the matrix values obtained with the Jaccard binary similarity coefficient, the average genetic similarity was determined as 0.578. The closest genetic similarity was between Mersin-1 and İzmir-Tepecik (0.936), followed by İzmir-Tepecik and Siirt (0.895) and Mersin-1 and Siirt genotype pairs (0.886). The lowest genetic similarity was determined between Afyon-1 and Ankara-Çankaya genotypes with 0.308, Ankara-Çankaya and Iğdır and Van-1 and Afyon-1 genotypes were determined as other distant genotypes (0.309 and 0.310, respectively).

Principal coordinate analysis (PCoA) was also performed to determine the genetic diversity and four main groups were formed. According to the scattering, Van-1 genotype is located at a different point while, as in the cluster analysis, it was noted that Konya-1, Iğdır, İzmir-Kemeraltı, Mardin, Bitlis and Afyon-1 genotypes, which are in the same main group, are located close to each other in the dendrogram. In this context, it was observed that the results of cluster analysis and PCoA analysis performed to examine genetic variation of iPBS-retrotsposon markers were largely compatible with each other (Fig. 7).

The UPGMA-based dendrogram was obtained using the Jaccard similarity coefficient for ISSR data (Fig. 8). Accordingly, the flax genotypes were divided into two main groups (A and B) and the Van-1 genotype was in group A alone. Also, group B was divided into two separate subgroups. Afyon-1 and İzmir-Kemeraltı genotypes were located in group B1, while all the remaining genotypes were located in group B2 (Fig. 8). According to Jaccard similarity coefficient, the average genetic similarity was determined as 0.755. While the closest genetic distance was found between Mersin-1 and Afyon-2 (0.941), Siirt and Afyon-2 and Sinop and Afyon-2 genotype pairs followed these genotypes with a similarity coefficient of 0.915. On the other hand, İzmir-Kemeraltı and Antares were the most distant genotypes with a similarity coefficient of 0.585, while Muş and İzmir-Kemeraltı (0.589) and Ankara-Çankaya and İzmir-Kemeraltı (0.591) genotypes were other distant genotype pairs.

According to PCoA of ISSR data, Van-1 genotype was located at a different point, similar to the cluster analysis, and the İzmir-Kemeraltı and Afyon-1 genotypes, which were in a different group in the cluster analysis, were also close to each other. It was also determined that Konya-1, Mardin, Bitlis, Muş and Ankara-Çankaya, which are in the third group, are located at more distant points than other genotypes (Fig. 9). In ISSR data, as in iPBS data, cluster and PCoA results were found to be compatible with each other.

In the UPGMA-based dendogram obtained by combining iPBS and ISSR marker data, two separate groups, A and B, were formed, similar to the dendrogram constructed with ISSR data, and Van-1 was in group A alone. Group B was divided into two subgroups (B1 and B2). Afyon-1 and İzmir-Kemeraltı genotypes were in group B1 and the remaining genotypes were in group B2 (Fig. 10). Considering the matrix formed according to the Jaccard similarity coefficient, it was determined that the average genetic similarity was 0.722. Of the genotypes, Mersin-1 and Afyon-2 genotypes had the closest genetic distance (0.928), Mersin-1 and İzmir-Tepecik and Siirt and Afyon-2 genotype pairs followed this genotype pair with similarity coefficients of 0.908 and 0.906, respectively.

While Ankara-Çankaya and İzmir-Kemeraltı (0.554) genotypes were the most distant from each other, it was determined that İzmir-Kemeraltı and Antares (0.556) and Kilis and İzmir-Kemeraltı (0.562) genotype pairs were the other distant genotype pairs. PCoA of molecular data revealed four groups. Accordingly, While Van-1 genotype was in a single group, İzmir-Kemeraltı and Afyon-1 genotypes and Ankara-Çankaya and Muş genotypes were located in two different groups. The remaining genotypes were found to be in a different group (Fig. 11). In this context, it is noteworthy that there is a great deal of similarity with the distribution of genotypes in the dendogram obtained as a result of the cluster analysis. In the Mantel analysis using two different marker systems and genetic distance matrices obtained from phenotypic traits, it was determined that the correlation between ISSR and iPBS was high and this correlation was significant (Fig. 12). Although the correlation between the morphological marker and ISSR was low, this correlation was found to be significant, but the correlation between iPBS and the morphological marker was low and not significant (Fig. 12).

Population structure analysis

The population structure was analyzed using molecular data with STRUCTURE. In the STRUCTURE analysis performed with the data of iPBS marker system, the highest Delta K was found as 8 (Fig. 13). The population consisting of 23 genotypes was divided into 8 subpopulations (Fig. 14). Membership coefficients were especially high in VI and VIII subpopulations (9 and 8 genotypes, respectively), and high membership coefficients were not determined in IV and V subpopulations (Table 3). According to the population structure performed with ISSR data, the highest Delta K value was 2 (Fig. 13), and it was determined that 2 subpopulations were formed accordingly (Fig. 15). Concerning the membership coefficients, there were 7 genotypes in the first subpopulation and 13 genotypes in the second subpopulation. The membership coefficients of the remaining three genotypes (Ankara-Çankaya, Iğdır and Bitlis) were found to be close to each other (Table 5). Finally, we carried out Structure analysis using the combined molecular (iPBS + ISSR) data and the highest Delta K value was found to be 2 (Fig. 13). Accordingly, 2 subpopulations were formed (Fig. 16). Regarding membership coefficients, there were 11 genotypes in the first subpopulation and 12 genotypes in the second subpopulation (Table 6).

Discussion

For the use of plants as genetic resources, a comprehensive understanding of plant genetic diversity is required, as in general many plants achieve a narrow yield of genetic treatments and adverse impacts are increased against different stresses (Mishra et al., 2012; Suvi et al., 2020). Genetic diversity analysis at the morphological and molecular level has an important role in optimizing breeding practices and yields. In addition, significant genetic variation has been reported in the flax plant at the morphological and molecular level (Khan et al., 2013). Herewith the study, we addressed our targets on revealing the morphological and molecular variations. The molecular characterization and discrimination were based on the used two different markers systems including iPBS and ISSR. Of the morphological attributes considered, the lowest and highest values of technical stem length were recorded in Royal (19.32cm) and Ankara-Çankaya (45.39cm), respectively. Our current findings are in parallel with previous reports (Brunšek et al., 2022 (49.10-73.63cm); Emam, 2019 (66.97-82.36cm); Korolev and Bome, 2017 (59.24cm)). The highest value of fresh stem weight (0,82g) was recorded in “Ankara-Çankaya” genotype whilst the lowest value (0,13g) was recorded in “Royal” genotype. The highest and lowest root length values were 7,84cm and 4,07cm for “Afyon-2” and “Eckendorfi”, respectively. Those values were relatively lower than the values (0,56 – 0,60cm) reported by Yıldız and Özgen (2004). Regardin fresh weight of root, the highest and lowest values were as 0,13g for “Van-1” and 0,03g for “Konya-1” and “Sarı-85”. The manifested significant differences might be attributed to the quality of the seeds, environmental factors in cultivation media.

Concerning molecular analysis, a total of 289 polymorphic bands (N = 77 in iPBS and N = 212 in ISSR) were recorded and and the average number of polymorphic bands per primer was calculated as 5.92 and 6.42, respectively. Lancíková and Žiarovská (2020) reported the genetic diversity in flax with IRAP and iPBS marker systems. IRAP primers revealed 38 (mean 6.3) and 46 (mean 7.7) polymorphic bands in two different populations, whereas all of the bands obtained from iPBS primers were monomorphic. Corresponding to the reports using different retrotransposon marker systems in flax, Holasou et al. (2016) reported 41 (mean 5.86) and 78 (mean 6.0) polymorphic bands from IRAP and REMAP primers, respectively. Average number of bands per primer for iPBS in grape varieties was 5.7 and 6.75 for grape varieties (Guo et al., 2014) and peas (Baloch et al., 2015a), respectively.

The relevant values of mean number of polymorphic bands are not entirely related to the marker technique but is, in general, related to the species investigated. With respect to the uses of ISSR marker for genetic characterization of flax gene resources, Ahmed et al. (2019) observed 4.7 polymorphic bands per primer from 47 polymorphic bands, and Atri and Kumar (2018) recorded 11.5 polymorphic bands from 344 bands. The polymorphism rate of iPBS and ISSR markers were found to be 82.55% and 67.95%, respectively, for the present study.

Regarding reports on uses different retrotransposon markers in flax, Lancíková, and Žiarovská (2020) found the mean polymorphism rate in the range of 30.8 to 24.6%, while Habibollahi et al. (2015) determined the highest rate as 54.53%. Similarly, Smýkal et al. (2011) obtained 36.70% in the IRAP marker system, Holasou et al. (2016) obtained polymorphism rates of 53.25% and 58.92% from two different retrotransposon marker systems. In another study, the rate was found to be as 100% (Žiarovská

et al., 2022). In this respect, a higher polymorphism rate was obtained than the iPBS primers used to determine the genetic variation among the flax genotypes in the present study. While high polymorphism rates such as 90% with 18 polymorphic bands (Pali and Mehta, 2016), 89% with 66 polymorphic bands (Atri et al., 2018), 95% from 298 bands (Uysal et al., 2010) were recorded from ISSR primers, El Sayed et al. (2018) obtained a low polymorphism rate (21.41%). The polymorphism rates of the present study are consistent with the reports of Rajwade et al. (2010) and Osman et al. (2021) (63.90% and 64.40%, respectively).

PIC values (Shete et al. 2000), which are commonly used to indicate the level of polymorphism of a marker locus used in linkage analysis in genetic studies, provide a broader table for evaluating diversity compared to the number of bands obtained because it takes into account the relative frequencies of each band (Cömertpay et al., 2012). Therefore, PIC results allow the selection of larger polymorphic markers to reduce the number of loci required for precise genotype characterization. In the study, averages of 0.75 from iPBS primers and 0.73 from ISSR primers were obtained. Of the former reports, the mean PIC value was 0.42 in the IRAP marker system (Smýkal et al., 2011), while in another study, 0.37 in IRAP primers and 0.31 in REMAP primers (Holasou et al., 2016). In comparison to the previous reports concerned with uses of retrotransposon marker systems in flax, it is noteworthy that high PIC values were obtained in the present study. PIC values of ISSR marker system were found to be as 0.88 (Ahmed et al., 2019), 0.68 (Pali and Mehta, 2016), and 0.86 (Atri et al., 2018) in flax gene resources. In this context, it is seen that the PIC value obtained from ISSR primers in the study is relatively compatible with the previous reports.

The mean *ne* value was found to be as 1.58 and 1.49 in the iPBS and ISSR primers, respectively. Habibollahi et al. (2015) reported the *ne* value between 1.15 and 1.37 with IRAP markers in the flax gene resources. Similarly, Holasou et al. (2016) also reported average effective allele counts (*ne*) of 1.19 and 1.23 in different retrotransposon markers, which are lower than the present study. Average *h* and *I* values were 0.34 and 0.50 for iPBS, 0.30 and 0.46 for ISSR, respectively. Concerning the studies with uses of IRAP and REMAP, it was determined that average values of *h* were between 0.10 and 0.21 and I averages were between 0.16 and 0.31 values. In this study, higher average values were recorded with iPBS in genetic diversity indices.

Indeed, retrotransposon-based DNA marker systems, which have been used for many plant species, are stated to be a powerful tool for the analysis of different aspects of natural genomic variability of higher plants (Andeden et al. 2013; Guo et al. 2014; Baloch et al. 2015a; Nemli et al. 2015; Yildiz et al. 2015; Borna et al. 2017; Coutinho et al. 2018; Hossein-Pour et al. 2019; Karagoz et al. 2020; Yıldız et al. 2020; Erdinc et al., 2021). Regarding uses of ISSR markers in the characterization of the flax genetic resources, there were mostly no findings related to the genetic diversity indices. In a study conducted by Talebi and Matsyura, (2021), they reported the value of *I* and *ne* as 0.098 and 1.11, respectively. It was determined that the genetic diversity indices of the ISSR primers used in the present study were higher than these averages. In addition, the results obtained from iPBS and ISSR were highly consistent ($r = 0.78$), but iPBS and ISSR data and morphological data were not so highly consistent ($r = 0.07$ and $r = 0.15$, respectively). Baloch et al. (2015b) reported that these two marker systems showed high correlation in comparison of

the wild lentil genotypes (r = 0.91). Also, the relevant correlation coefficients were found to be as r = 0.89 in chickpea (Andeden et al., 2013), and r = 0.44 in watermelon (Coşkun and Gülşen, 2022). However, no findings were reported for the correlation of molecular and morphological data in flax but different molecular and morphological marker data showed low correlation in species such as olive (Laaribi et al., 2017) and watermelon (Coşkun and Gülşen, 2022). Similarly, in the dendogram and PCoA of morphological attributes, it was observed that Ankara-Çankaya genotype was located in a different place and separated from other genotypes. In the iPBS and ISSR analyzes, it was noted that the Van-1 genotype was located in a different place and the same results were obtained in both cluster and PCoA analysis. In general, it was determined that genotypes were basically divided into two groups in both marker systems.

Bayesian-based population structure analysis performed with iPBS and ISSR data divided the genotypes into 8 and 2 populations, respectively, and in the analysis performed with combined molecular data, it was determined that 2 subpopulations were formed. According to the findings of the present study, geographical differences might not be predictor factors in dividing the genotypes into the populations. Regarding membership coefficients, it is noteworthy that there are genetic differences between populations, especially in the iPBS results. The presence of parts with different colors in each genotype indicates that each genotype has low gene flow with other genotypes. This means that the introduction of new genes into populations and genetic variability within populations increase. In the study by Holasou et al. (2016), the flax genotypes were divided into two subpopulations with the uses of IRAP and REMAP markers, whereas Habibollahi et al. (2015) reported that they were divided into 3 subpopulations. At the same time, it was stated that gene transfers between these populations were low. In population analysis studies with ISSR markers in flax genotypes, Nag et al. (2021) 8, Kumari et al. (2018) 2, Talebi and Matsyura (2021) reported that 6 subpopulations were formed.

In population structure analysis, individuals with a membership coefficient of 0.8 or higher in a subpopulation are considered pure, while individuals with a lower membership coefficient are considered a mixture of at least two different subpopulations (Fukunaga et al. 2005). Accordingly, in the present study, 8 genotypes (iPBS markers) (Mersin-1, Afyon-1, Afyon-2, İzmir-Tepecik, Siirt, İzmir-Kemeraltı, Bitlis and Mardin) and 14 genotypes (ISSR markers) (Van-1, Afyon-1, Afyon-2, İzmir-Kemeraltı, Konya-1, Sarı-85, Sinop, Siirt, Kilis, Bursa, Samsun-1, Mersin-1, İzmir-Karşıyaka and İzmir-Tepecik) were higher than 0.8 and those can be described as pure.

Conclusion

We here, for the first time, revealed the genetic diversity and population structure of 29 flax genotypes using individual and combined data of iPBS-ISSR markers. According to the Mantel analysis concerning genetic distance matrices of morphological traits, the correlation between ISSR and iPBS was high and significant. According to the structure analysis of combined data of iPBS + ISSR, 2 subpopulations were obtained. Concerning the membership coefficients, there were 11 genotypes in the first subpopulation and 12 genotypes in the second subpopulation.

Declarations

Author contributions

All authors have significantly contributed in finalising the research. M.Z.K., A.A. designed the experimental set-up. M.Z.K. performed the greenhouse experiments. M.Z.K. Ç.E. and A.A. performed the molecular analyses and performed the statistical analysis. M.Z.K., Ç.E., M.G.K. and M.K. analysed the data and wrote the first draft, while the final draft was read and approved by all authors.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Ahmed MZ, Masoud IM, & Zedan SZ (2019) Molecular characterization and genetic relationships of cultivated flax (*Linum usitatissimum* L.) genotypes using ISSR markers. Middle East Journal of Agriculture Research, 8(3), 898-908. ISSN 2077-4605.
2. Akram NA, Shafiq F, Ashraf M, Iqbal M, & Ahmad P (2021) Advances in salt tolerance of some major fiber crops through classical and advanced biotechnological tools: A Review. Journal of Plant Growth Regulation, 40, 891-905. https://doi.org/10.1007/s00344-020-10158-5
3. Andeden EE, Baloch FS, Derya M, Kilian B, Özkan H (2013) iPBS-Retrotransposons-based genetic diversity and relationship among wild annual Cicer species. Journal of Plant Biochemistry Biotechnology 22(4): 453-466. https://doi.org/10.1007/s13562-012-0175-5
4. Ataii E, Mirlohi A, Sabzalian MR, Sharif-Moghaddam N, Sadri N, Jafari P, Mohammadi Mirik A (2021) Genetic characterization of a diversity panel, selected from IPK linseed (*Linum usitatissimum*) world collection. Plant Breeding, 140(5), 919-932. https://doi.org/10.1111/pbr.12962
5. Atri C, & Kumar H (2018) Assessment of Genetic Diversity in Germplasm Collection of Linseed (Collection Of Linseed (*Linum usitatissimum* L.). *The Bioscan*, *13*(1), 95-99. https://doi.org/10.20546/ijcmas.2020.905.057
6. Atri C, Kumar H, & Sharma S (2018) Agro-Biochemical Based Characterization and Molecular Profiling of Linseed (*Linum usitattissimum* L.) Cultivars. *Applied Biological Research*, *20*(2), 137-145. https://doi.org/10.5958/0974-4517.2018.00018.6
7. Aydin A, Ince AG, Uygur Gocer E, & Karaca M (2018) Single cotton seed DNA extraction without the use of enzymes and liquid nitrogen. Fresenius Environmental Bulletin, 27(10), 6722-6726.
8. Baloch FS, Alsaleh A, de Miera LE, Hatipoğlu R, Çiftçi V, Karaköy T, Yıldız M, Özkan H (2015a) DNA based iPBS-retrotransposon markers for investigating the population structure of pea (*Pisum sativum*) germplasm from Turkey. Biochemical Systematics and Ecology 61: 244-252. https://doi.org/10.1016/j.bse.2015.06.017

9. Baloch FS, Derya M, Andeden EE, Alsaleh A, Cömertpay G, Kilian B, & Özkan H (2015b) Inter-primer binding site retrotransposon and inter-simple sequence repeat diversity among wild Lens species. *Biochemical Systematics and Ecology*, *58*, 162-168. http://dx.doi.org/10.1016/j.bse.2014.12.002
10. Borna F, Luo S, Ahmad NM, Nazeri V, Shokrpour M, Trethowan R (2017) Genetic diversity in populations of the medicinal plant *Leonurus cardiaca* L. revealed by inter-primer binding site (iPBS) markers. Genetic Resources and Crop Evolution 64: 479-492. https://doi.org/10.1007/s10722-016-0373-4
11. Brunšek R, Butorac J, Augustinović Z, & Pospišil M (2022) Effect of Nitrogen on the Properties of Flax (*Linum usitatissimum* L.) Plants and Fibres. Polymers, 14(3), 558. https://doi.org/10.3390/polym14030558
12. Capy P, Gasperi G, Biemont C, Bazin C (2000) Stress and transposable elements: co-evolution or useful parasites? Heredity 85: 101-106. https://doi.org/10.1046/j.1365-2540.2000.00751.x
13. Chen M (2022) China and the World in the Liangzhu Era. Springer. https://doi.org/10.1007/978-981-16-6897-5
14. Choudhary SB, Sharma HK, Kumar AA, Chowdhury I, Maruthi RT, & Kak A (2017) Genetic diversity spectrum and marker trait association for agronomic traits in global accessions of *Linum usitatissimum* L. Industrial Crops and Products, 108, 604-615. https://doi.org/10.1016/j.indcrop.2017.07.003
15. Cömertpay G, Baloch FS, Kilian B, Ülger AC and Özkan H (2012) Diversity assessment of Turkish maize landraces based on fluorescent labelled SSR markers. Plant Mol. Biol. Rep., 30: 261—274. https://doi.org/10.1007/s11105-011-0332-3
16. Coşkun ÖF, & Gülşen O (2022) Determination of Correlation Variation of Morphological and Some Molecular Marker Techniques in Watermelon. In Proceedıngs of Iv. Internatıonal Agricultural, Biological & Life Science Conference Agbiol 2022 (P. 488).
17. Coutinho JP, Carvalho A, Martín A, Lima-Brito J (2018) Molecular characterization of Fagaceae species using inter-primer binding site (iPBS) markers. Molecular Biology Reports 45: 133–142. https://doi.org/10.1007/s11033-018-4146-3.
18. Demirel F (2020) Bazı siyez buğdaylarının ISSR Markörleri ile karakterizasyonu. Journal of Agriculture, 3(2), 33-39. (in Turkish). https://doi.org/10.46876/ja.809882
19. Dumanoğlu Z (2020) Keten (*Linum usitatissimum* L.) Bitkisi tohumlarının genel özellikleri. Bütünleyici ve Anadolu Tıbbı Dergisi, 2, 3-9. https://dergipark.org.tr/en/pub/batd/issue/59277/800008 (in Turkish).
20. Đurić N, & Spasić M (2019) Introduction of alternative oil plants in production on small farms. Sustainable Agriculture and Rural Development in Terms of the Republic of Serbia Strategic Goals Realization within the Danube Region: sustainability and multifunctionality, 605-622. https://hdl.handle.net/21.15107/rcub_rivec_327
21. Ekincialp A, Erdinç Ç, Turan S, Cakmakci O, Nadeem MA, Baloch FS, & Sensoy S (2019) Genetic characterization of Rheum ribes (wild rhubarb) genotypes in Lake Van basin of Turkey through ISSR

and SSR markers. International Journal of Agriculture and Biology, 21(4), 795-802. https://doi.org/10.17957/ijab/15.0958

22. El Sayed A, Ezzat SM, Mostafa S, Zedan S, Abdel-Sattar E, & El Tanbouly N (2018) Inter simple sequence repeat analysis of genetic diversity and relationship in four egyptian flaxseed genotypes. https://doi.org/10.4103/pr.pr_126_17
23. Emam S (2019) Cultivars response of flax (*Linum usitatissimum* L.) to different nitrogen sources in dry environment. Egyptian journal of agronomy, 41(2), 119-131. https://doi.org/10.21608/AGRO.2019.10947.1157
24. Erdinç Ç, Ekincialp A, Turan S, Koçak M, Baloch FS, Şensoy S (2021) The first report about genetic diversity analysis among endemic wild rhubarb (*Rheum ribes* L.) populations through iPBS markers, Turkish Journal of Agriculture and Forestry: 45(6): 784-796. https://doi.org/10.3906/tar-2102-12
25. Erdinc C, Ekincialp A, Yildiz M, Kabay T, Turkmen O, & Sensoy S (2013) Molecular genetic diversity in Lake Van basin melons (*Cucumis melo* L.) based on RAPD and ISSR markers. Yuzuncu Yıl University Journal of Agricultural Sciences, 23(3), 264-270. https://dergipark.org.tr/en/pub/yyutbd/issue/21975/235966
26. Eren B, Keskin B, Demirel F, Demirel S, Türkoğlu A, Yilmaz A, & Haliloğlu K (2022) Assessment of genetic diversity and population structure in local alfalfa genotypes using iPBS molecular markers. Genetic Resources and Crop Evolution, 1-12. https://doi.org/10.1007/s10722-022-01450-2
27. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol 14: 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x
28. Fróna D, Szenderák J, Harangi-Rákos M (2019) The challenge of feeding the world. Sustainability, 11(20), 5816. https://doi.org/10.3390/su11205816
29. Fukunaga K, Hill J, Vigouroux Y, Matsuoka Y, Sanchez GJ, Liu K, Buckler ES, Doebley J (2005) Genetic diversity and population structure of Teosinte. Genetics 169, 2241-2254. https://doi.org/10.1534/genetics.104.031393
30. Ghonaim M, Kalendar R, Barakat H, Elsherif N, Ashry N, & Schulman AH (2020) High-throughput retrotransposon-based genetic diversity of maize germplasm assessment and analysis. Molecular Biology Reports, 47, 1589-1603. https://doi.org/10.1007/s11033-020-05246-4
31. Guo DL, Guo MX, Hou XG, Zhang GH (2014) Molecular diversity analysis of grape varieties based on iPBS markers. Biochemical Systematics and Ecology 52: 27-32. https://doi.org/10.1016/j.bse.2013.10.008
32. Gupta M, Chyi YS, Romero-Severson J, Owen JL (1994) Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theoretical and applied genetics, 89, 998-1006. https://doi.org/10.1007/BF00224530
33. Habibollahi H, Noormohammadi Z, Sheidai M, & Farahani F (2015) Genetic structure of cultivated flax (*Linum usitatissimum* L.) based on retrotransposon-based markers. Genetika, 47(3), 1111-1122. https://doi.org/10.2298/GENSR1503111H

34. Hasan N, Choudhary S, Naaz N, Sharma N, & Laskar RA (2021) Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. Journal of Genetic Engineering and Biotechnology, 19(1), 1-26. https://doi.org/10.1186/s43141-021-00231-1
35. Heidari EF, Rahimmalek M, Mohammadi S, and Ehtemam MH (2016) Genetic structure and diversity of ajowan (*Trachysper mumammi*) populations based on molecular morphological markers and volatile oil content. Ind. Crops and Prod., 92:186-196. https://doi.org/10.1016/j.indcrop.2016.08.014
36. Holasou HA, Mandoulakani BA, Jafari M, & Bernousi I (2016) Use of IRAP and REMAP markers to interpret the population structure of *Linum usitatissimum* from Iran. *Biologia*, *71*(3), 305-315. https://doi.org/10.1515/biolog-2016-0042
37. Hossein-Pour A, Karahan F, İlhan E, İlçim A, Haliloğlu K (2019) Genetic structure and diversity of Adonis L. (Ranunculaceae) populations collected from Turkey by inter-primer binding site (iPBS) retrotransposon markers. Turkish Journal of Botany 43(5): 585-596. https://doi.org/10.3906/bot-1810-1
38. Ibrahim AS, & Erdin Ç (2020) Determination of genetic relations among tomato accessions in Sulaymaniyah region through ISSRs markers genetic relations in tomato accessions. Yuzuncu Yıl University Journal of Agricultural Sciences, 30(4), 810-820. https://doi.org/10.29133/yyutbd.755083
39. Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bulletin de la Societe Vaududoise des Sciences Naturelles 44: 223-270. (in French).
40. Jhala AJ, & Hall LM (2010). Flax (*Linum usitatissimum* L.): current uses and future applications. Aust. J. Basic Appl. Sci, 4(9), 4304-4312. ISSN:1991-8178.
41. Kalendar R, Antonius K, Smýkal P, Schulman AH (2010) iPBS: A universal method for DNA fingerprinting and retrotransposon isolation. Theorotical and Applied Genetics 121: 1419-30. https://doi.org/10.1007/s00122-010-1398-2
42. Karagoz H, Cakmakci R, Hosseinpour A, Ozkan G, Haliloglu K (2020) Analysis of genetic variation and population structure among of oregano (*Origanum acutidens* L.) accessions revealed by agro-morphological traits, oil constituents and retrotransposon-based inter-primer binding sites (iPBS) markers. Genetic Resources and Crop Evolution 67: 1367-1384. https://doi.org/10.1007/s10722-020-00887-7
43. Khan MA, Mirza MY, Amjad M, Nawaz N, Nawaz MS, and Baig D (2013) Assessment of genetic diversity in germplasm of linseed (*Linum usitatissimum* L.). Pak. J. Agric. Res. 26, 178-184. ISSN: 0251-0480
44. Korolev KP, & Bome NA (2017) Evaluation of flax (*Linum usitatissimum* L.) genotypes on environmental adaptability and stability in the North-Eastern Belarus. Sel'skokhozyaistvennaya biologiya [Agricultural Biology], 3(52), 615-621. https://doi.org/10.15389/agrobiology.2017.3.615eng
45. Kumari A, Paul S, & Sharma V (2018) Genetic diversity analysis using RAPD and ISSR markers revealed discrete genetic makeup in relation to fibre and oil content in *Linum usitatissimum* L. genotypes. The Nucleus, 61(1), 45-53. http://dx.doi.org/10.1007%2Fs13237-017-0206-7

46. Kumlay AM, Barış E, Demirel S, Demirel F, & Yildirim B (2021) Bazı Pamuk Çeşitlerinde iPBS Analiziyle Genetik Varyasyonun Ortaya Çıkarılması. Avrupa Bilim ve Teknoloji Dergisi, (21), 67-73. (in Turkısh). https://doi.org/10.31590/ejosat.809479
47. Laaribi I, Gouta H, Ayachi MM, Labidi F, Mars M (2017) Combination of morphological and molecular markers for the characterization of ancient native olive accessions in Central-Eastern Tunisia. Comptes Rendus Biologies, 340(5), 287-297. http://dx.doi.org/10.1016/j.crvi.2017.03.003
48. Lancíková V, & Žiarovská J (2020) Inter-retrotransposon amplified polymorphism markers revealed long terminal repeat retrotransposon insertion polymorphism in flax cultivated on the experimental fields around Chernobyl. Journal of Environmental Science and Health, Part A, 55(8), 957-963. https://doi.org/10.1080/10934529.2020.1760016
49. Landoni B, Viruel J, Gómez R, Allaby RG, Brennan AC, Picó FX, Pérez-Barrales R (2020) Microsatellite marker development in the crop wild relative Linum bienne using genome skimming. Applications in Plant Sciences, 8(5), e11349. https://doi.org/10.1002%2Faps3.11349
50. Mansour A (2007) Epigenetic activation of genomic retrotransposons. Journal of Cell and Molecular Biology, 6 (2): 99107. 63.
51. Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Research, 27(2), 209–220. ISSN: 1538-7445
52. Mishra DK, & Awasthi H (2020) Quality evaluation of flaxseed obtained from different locations. In Biology and Life Sciences Forum (Vol. 4, No. 1, p. 70). MDPI. https://doi.org/10.3390/IECPS2020-08754
53. Mishra RP, Pandey M, Tripathi UK, Singh M (2012) Genetic diversity within the genus Pleurotus determined by random amplified polymorphism DNA (RAPD) analysis. Arch. Phytopathol. Pflanzenschutz. 45, 873-880. https://doi.org/10.1080/03235408.2011.599143
54. Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, & Baloch FS (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnology & Biotechnological Equipment, 32(2), 261-285. https://doi.org/10.1080/13102818.2017.1400401
55. Nag S, Mandal R, & Mitra J (2021) Genetic characterization of global collection of flax (*L. usitatissimum* L.) utilizing molecular marker. Ecological Genetics and Genomics, 19, 100084. https://doi.org/10.1186%2F1471-2229-13-78
56. Nemli S, Kianoosh T, Tanyolaç MB (2015) Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) accessions through retrotransposon-based interprimer binding sites (iPBSs) markers. Turkish Journal of Agriculture and Forestry 39: 940-894. https://doi.org/10.3906/tar-1505-59
57. Oomah BD, Kenaschuk EO, Cui W, & Mazza G (1995) Variation in the composition of water-soluble polysaccharides in flaxseed. Journal of Agricultural and Food Chemistry, 43(6), 1484-1488. https://doi.org/10.1021/jf00054a013

58. Osman MH, Badawı M, Zayed E, Abd El-Maksoud RM, & Radwan K (2021) Genetic Diversity of Selected Flax Genotypes (*Linu-Musitatissimum* L.) Based on Scot, Issr and Ramp Markers. Egyptian Journal of Genetics and Cytology, 50(2), 95-117.
59. Öztürk Hİ, Dursun A, Hosseinpour A, Haliloğlu K (2020) Genetic diversity of pinto and fresh bean (*Phaseolus vulgaris* L.) germplasm collected from Erzincan province of Turkey by inter-primer binding site (iPBS) retrotransposon markers," Turkish Journal of Agriculture and Forestry: Vol. 44: No. 4, Article 10. https://doi.org/10.3906/tar-2002-9
60. Pali V, & Mehta N (2016) Genetic diversity assessment of flax (*Linum usitatissimum* L.) germplasm using molecular and morphological markers. Electronic Journal of Plant Breeding, 7(4), 986-995. https://doi.org/10.5958/0975-928X.2016.00135.6
61. Peakall R, & Smouse PE (2006) Genalex 6: Genetic analysis in excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6(1), 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x
62. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, et al. (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2: 225-238. https://doi.org/10.1007/BF00564200
63. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945-959. https://doi.org/10.1093/genetics/155.2.945.
64. Rajwade AV, Arora RS, Kadoo NY, Harsulkar AM, Ghorpade PB, & Gupta VS (2010) Relatedness of Indian flax genotypes (*Linum usitatissimum* L.): an inter-simple sequence repeat (ISSR) primer assay. Molecular biotechnology, 45(2), 161-170. https://doi.org/10.1007/s12033-010-9256-7
65. Rana MK, Singh S (2017) Assessment of genetic diversity and DNA profiling of linseed (*Linum usitatissimum* subsp. *usitatissimum* L.) germplasm using SSR markers. Journal of Plant Biochemistry and Biotechnology, 26(3), 293-301. https://doi.org/10.1007/s13562-016-0391-5
66. Ravensdale M, Nemri A, Thrall PH, Ellis JG, Dodds PN (2011) Co-evolutionary interactions between host resistance and pathogen effector genes in flax rust disease. Molecular plant pathology, 12(1). https://doi.org/93-102. 10.1111/j.1364-3703.2010.00657.x.
67. Riehl K, Riccio C, Miska EA, & Hemberg M (2022) TransposonUltimate: software for transposon classification, annotation and detection. Nucleic Acids Research, 50(11), e64-e64. https://doi.org/10.1093/nar/gkac136
68. Ronquist F and Huelsenbeck JP (2003) Mrbayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 19, 1572-1574. https://doi.org/10.1093/bioinformatics/btg180
69. Roy NS, Choi JY, Lee NSK (2015) Marker utility of transposable elements for plant genetics, breeding, and ecology: A review. Genes and Genomes 37: 141-151. https://doi.org/10.1007/s13258-014-0252-3
70. Saha D, & Hazra CR (2004) Agri-historical Genesis of Flax (*Linum usitatissimum* L.).
71. Şahin G, & Yıldız B (2023) Türkiye'de Keten Üretimi ve Karakteristik Keten Dokumaların Durumu. Erciyes Akademi, 36(1), 169-185. https://doi.org/10.48070/erciyesakademi.1055957 (in Turkısh)

72. Saroha A, Pal D, Kaur V, Kumar S, Bartwal A, Aravind J, Wankhede DP (2022) Agro-morphological variability and genetic diversity in linseed (*Linum usitatissimum* L.) germplasm accessions with emphasis on flowering and maturity time. Genet. Resour. Crop Evol., 69, 315-333. https://doi.org/10.1007/S10722-021-01231-3
73. Shete S, Tiwari H, & Elston RC (2000) On estimating the heterozygosity and polymorphism information content value. Theoretical population biology, 57(3), 265-271. https://doi.org/10.1006/tpbi.2000.1452
74. Smith J, Chin E, Shu H, Smith O, Wall S, et al. (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): Comparisons with data from RFLPs and pedigree. Theoretical and Applied Genetics 95: 163-173. https://doi.org/10.1007/s001220050544
75. Smýkal P, Bačová-Kerteszová N, Kalendar R, Corander J, Schulman AH, & Pavelek M (2011) Genetic diversity of cultivated flax (*Linum usitatissimum* L.) germplasm assessed by retrotransposon-based markers. Theoretical and Applied Genetics, 122(7), 1385-1397. https://doi.org/10.1007/s00122-011-1539-2
76. Soto-Cerda BJ, Cloutier S, Gajardo HA, Aravena G, Quian R (2019) Identifying drought-resilient flax genotypes and related-candidate genes based on stress indices, root traits and selective sweep. Euphytica, 215(3), 41. https://doi.org/10.1007/s10681-019-2362-0
77. Stavropoulos P, Mavroeidis A, Papadopoulos G, Roussis I, Bilalis D, & Kakabouki I (2023) On the Path towards a “Greener” EU: A Mini Review on Flax (*Linum usitatissimum* L.) as a Case Study. Plants, 12(5), 1102. https://doi.org/10.3390/plants12051102
78. Suvi WT, Shimelis H, Laing M, Mathew I, Shayanowako AIT (2020) Assessment of the genetic diversity and population structure of rice genotypes using SSR markers. Acta Agric. Scand. Section B – Soil & Plant Sci. 70, 76-86. https://doi.org/10.1371/journal.pone.0259883
79. Talebi SM, & Matsyura A (2021) Genetic structure of some Iranian, new and old worlds *Linum usitatissimum* L. populations. Iranian Journal of Science and Technology, Transactions A: *Science*, *45*(4), 1143-1153. https://doi.org/10.1007/s40995-021-01074-8
80. Uysal H, Fu YB, Kurt O, Peterson GW, Diederichsen A, & Kusters P (2010) Genetic diversity of cultivated flax (*Linum usitatissimum* L.) and its wild progenitor pale flax (*Linum bienne* Mill.) as revealed by ISSR markers. Genetic resources and crop evolution, 57(7), 1109-1119. http://doi.org/10.1007/s10722-010-9551-y
81. Wolfe AD, and Liston A (1998) Contributions of PCR-based methods to plant systematics and evolutionary biology. In: "Molecular systematics of plants", (Eds.): Soltis DE, Soltis PS, and Doyle JJ, 2nd Edition. Kluwer Academic Publishers, Boston, PP. 43-86. https://doi.org/10.1007/978-1-4615-5419-6_2
82. Yang W, Bai Z, Wang F, Zou M, Wang X, Xie J, & Zhang F (2022) Analysis of the genetic diversity and population structure of Monochasma savatieri Franch. ex Maxim using novel EST-SSR markers. BMC genomics, 23(1), 597. https://doi.org/10.1186/s12864-022-08832-x

83. Yeh FC, Yang R, Boyle TJ, Ye Z (2000) Pop Gene 32, Microsoft Windows-based Freeware for Population Genetic Analysis. Version 1.32. Edmonton, Canada: University of Alberta.
84. Yildiz M, & Özgen M (2004) The effect of a submersion pretreatment on in vitro explant growth and shoot regeneration from hypocotyls of flax (*Linum usitatissimum*). Plant Cell, Tissue and Organ Culture, 77, 111-115. https://doi.org/10.1023/B:TICU.0000016493.03592.c3
85. Yildiz M, Koçak M, Baloch FS (2015) Genetic bottlenecks in Turkish okra germplasm and utility of iPBS retrotransposon markers for genetic diversity assessment. Genetics and Molecular Research 14(3): 10588-10602. https://doi.org/10.4238/2015.September.8.20
86. Yıldız M, Koçak M, Nadeem MA, Cavagnaro P, Barboza K, et al. (2020) Genetic diversity analysis in the Turkish pepper germplasm using iPBS retrotransposon based markers. Turkish Jornal of Agriculture and Forestry 44: 1-14. https://doi.org/10.3906/tar-1902-10
87. Žiarovská J, Spevákova I, Klongová L, Farkasová S, & Rashydow N (2022) Transposable Elements in the Revealing of Polymorphism-Based Differences in the Seeds of Flax Varieties Grown in Remediated Chernobyl Area. Plants, 11(19), 2567. https://doi.org/10.3390/plants11192567
88. Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20(2):176–183. https://doi.org/10.1006/geno.1994.1151

Tables

Table 1
Morphological characteristics of flax (*Linum usitatissimum* L.) genotypes in One-way analysis of variance (ANOVA).

Variety	Technical Stem Length	Technical Stem Fresh Weight	Technical Stem Dry Weight	Root Length	Root Fresh Weight	Root Dry Weight
Van-1	25.59 ± **g-l**	0.42 ± **e-h**	0.11 ± **d-g**	5.57 ± **b-h**	0.13 ± **a**	0.03 ± **a-c**
Mersin-1	23.55± **ı-l**	0.37 ± **g-k**	0.08 ± **h-k**	5.48 ± **b-h**	0.05 ± **g-k**	0.01 ± **de**
Afyon-1	26.55 ± **f-l**	0.19 ± **lm**	0.05 ± **l-n**	4.64 ± **f-h**	0.08 ± **d-f**	0.01 ± **e**
Afyon-2	23.89± **ı-l**	0.31 ± **h-l**	0.07 ± **j-l**	7.84 ± **a**	0.06 ± **f-ı**	0.01 ± **de**
Muş	31.92 ± **de**	0.38 ± **f-j**	0.07 ± **j-l**	5.57 ± **b-h**	0.11 ± **b**	0.02 ± **de**
Ankara-Çankaya	45.39 ± **a**	0.44 ± **d-g**	0.09 ± **f-k**	6.36 ± **b-e**	0.06 ± **f-ı**	0.02 ± **de**
İzmir-Kemeraltı	29.74 ± **d-h**	0.60 ± **bc**	0.12 ± **d-f**	6.11 ± **b-f**	0.09 ± **b-d**	0.03 ± **a-d**
İzmir-Karşıyaka	25.06 ± **h-k**	0.44 ± **e-g**	0.09 ± **e-j**	5.77 ± **b-g**	0.11 ± **bc**	0.02 ± **b-d**
İzmir-Tepecik	24.93 ± **h-k**	0.63 ± **b**	0.13 ± **cd**	5.92 ± **b-g**	0.09 ± **b-d**	0.03 ± **a-d**
Iğdır	33.52 ± **b-d**	0.82 ± **a**	0.17 ± **b**	6.98 ± **a-c**	0.06 ± **e-h**	0.03 ± **ab**
Bitlis	20.60 ± **kl**	0.29± **ı-l**	0.04 ± **mn**	5.67 ± **b-g**	0.04 ± **h-k**	0.01 ± **de**
Sinop	22.92 ± **j-l**	0.41 ± **f-h**	0.08 ± **h-k**	5.67 ± **b-g**	0.06 ± **f-ı**	0.02 ± **c-e**
Diyarbakır	26.64 ± **f-j**	0.45 ± **d-g**	0.09 ± **g-k**	5.72 ± **b-g**	0.08 ± **d-f**	0.02 ± **de**
Siirt	28.51 ± **d-ı**	0.39 ± **f-ı**	0.09 ± **e-j**	6.11 ± **b-f**	0.04± **ı-k**	0.01 ± **de**
Konya-1	22.90 ± **j-l**	0.14 ± **m**	0.03 ± **n**	6.53 ± **a-d**	0.03 ± **k**	0.01 ± **de**
Eskişehir	23.86± **ı-l**	0.43 ± **e-h**	0.10 ± **e-j**	5.16 ± **d-h**	0.06 ± **e-h**	0.02 ± **c-e**

Different letters indicate significant difference according to a Duncan's multiple range test ($p < 0.05$).

Variety	Technical Stem Length	Technical Stem Fresh Weight	Technical Stem Dry Weight	Root Length	Root Fresh Weight	Root Dry Weight
Samsun-1	31.19 ± **d-f**	0.28±**ı-l**	0.07 ± **j-l**	5.15 ± **d-h**	0.04 ± **j-k**	0.01 ± **de**
Mardin	22.03 ± **j-l**	0.28± **ı-l**	0.06 ± **k-m**	4.50 ± **gh**	0.04± **ı-k**	0.01 ± **de**
Kilis	26.56 ± **f-j**	0.26 ± **kl**	0.05 ± **l-n**	5.31 ± **d-h**	0.04± **ı-k**	0.01 ± **de**
Bursa	38.01 ± **b**	0.77 ± **a**	0.15 ± **bc**	7.01 ± **ab**	0.09 ± **cd**	0.04 ± **a**
Hermes	23.04 ± **j-l**	0.54 ± **b-e**	0.11 ± **d-g**	4.56 ± **gh**	0.08 ± **d-f**	0.02 ± **b-d**
Rolin	30.29 ± **d-g**	0.50 ± **c-f**	0.34 ± **a**	4.93 ± **e-h**	0.04 ± **j-k**	0.02 ± **de**
Antares	37.29 ± **bc**	0.59 ± **bc**	0.12 ± **de**	4.63 ± **f-h**	0.04±**ı-k**	0.02 ± **c-e**
Royal	19.32 ± **l**	0.13 ± **m**	0.08± **ı-k**	6.67 ± **a-d**	0.04 ± **j-k**	0.02 ± **de**
Dakota	32.94 ± **cd**	0.54 ± **b-e**	0.11 ± **d-h**	4.73 ± **f-h**	0.07 ± **d-g**	0.02 ± **c-e**
Eckendorfi	27.26 ± **e-j**	0.56 ± **b-d**	0.10 ± **e-ı**	4.07 ± **h**	0.06 ± **g-j**	0.02 ± **de**
Sarı-85	32.44 ± **d**	0.35 ± **g-k**	0.07 ± **j-l**	5.47 ± **c-h**	0.03 ± **k**	0.01 ± **de**
Beyaz Gelin	20.93 ± **kl**	0.27 ± j-**l**	0.07 ± **j-l**	5.88 ± **b-g**	0.08 ± **de**	0.01 ± **de**
Karakız	32.77 ± **cd**	0.44 ± **e-g**	0.08±**ı-k**	5.90 ± **b-g**	0.04 ± **h-k**	0.02 ± **de**
Df	28	28	28	28	28	28
F-value	14.99	21.00	44.87	3.70	14.98	3.03
p-value	.000	.000	.000	.000	.000	.000

Different letters indicate significant difference according to a Duncan's multiple range test ($p < 0.05$).

Table 2
Number of total bands, polymorphic bands, and some genetic diversity parameters of the iPBS-retrotransposon primers used in this study.

Primer	Amplified bands		% Polymorphism	*PIC*	*ne*	*h*	*I*
	Total	Polymorphic					
2076	8	6	75.00	0.85	1.44	0.28	0.44
2077	9	8	88.89	0.90	1.56	0.32	0.48
2085	3	1	33.33	0.09	1.10	0.09	0.19
2095	8	7	87.50	0.91	1.68	0.38	0.56
2228	3	3	100.00	0.59	1.59	0.33	0.49
2232	11	9	81.82	0.93	1.76	0.42	0.60
2239	7	6	85.71	0.69	1.42	0.29	0.46
2277	9	7	77.78	0.83	1.52	0.30	0.45
2374	8	7	87.50	0.88	1.68	0.39	0.58
2375	7	7	100.00	0.80	1.72	0.42	0.61
2376	6	6	100.00	0.83	1.49	0.31	0.48
2390	9	8	88.89	0.88	1.63	0.36	0.53
2415	3	2	66.67	0.63	1.89	0.47	0.67
Total	91	77					
Average	7.00	5.92	82.55	0.75	1.58	0.34	0.50

Effective number of alleles (*ne*), gene diversity (*h*), Shannon information index (*I*), and polymorphism information content (*PIC*)

Table 3
Number of total bands, polymorphic bands, and some genetic diversity parameters of the ISSR primers used in this study.

Primer	Amplified bands		% Polymorphism	*PIC*	*ne*	*h*	*I*
	Total	Polymorphic					
1	12	10	83.33	0.84	1.55	0.32	0.49
4	9	6	66.67	0.87	1.49	0.30	0.46
6	12	5	41.67	0.75	1.26	0.18	0.30
7	14	7	50.00	0.92	1.62	0.36	0.53
11	7	5	71.43	0.62	1.40	0.28	0.45
3f	11	7	63.64	0.79	1.47	0.29	0.45
5f	4	3	75.00	0.23	1.23	0.18	0.32
7f	13	9	69.23	0.92	1.54	0.31	0.47
8f	11	11	100.00	0.92	1.39	0.26	0.43
10f	5	4	80.00	0.69	1.63	0.35	0.52
11f	11	10	90.91	0.90	1.50	0.30	0.47
13f	11	10	90.91	0.84	1.59	0.35	0.52
808	10	5	50.00	0.56	1.26	0.19	0.33
810	6	5	83.33	0.67	1.42	0.25	0.39
812	4	2	50.00	0.46	1.60	0.37	0.56
816	6	4	66.67	0.80	1.70	0.39	0.57
825	5	3	60.00	0.74	1.71	0.41	0.60
826	11	7	63.64	0.85	1.50	0.30	0.46
834	10	6	60.00	0.87	1.57	0.35	0.52
B2	5	4	80.00	0.54	1.30	0.22	0.38
B10	10	4	40.00	0.24	1.17	0.15	0.27
I12	12	11	91.67	0.93	1.52	0.30	0.46
ISSR-6	8	4	50.00	0.44	1.27	0.21	0.36
LOL-7	12	9	75.00	0.76	1.47	0.31	0.49

Effective number of alleles (*ne*), gene diversity (*h*), Shannon information index (*I*), and polymorphism information content (*PIC*)

Primer	Amplified bands		% Polymorphism	*PIC*	*ne*	*h*	*I*
	Total	Polymorphic					
LOL-8	11	10	90.91	0.93	1.60	0.35	0.53
PHV-6	9	3	33.33	0.70	1.52	0.31	0.48
PHV-7	10	6	60.00	0.81	1.40	0.25	0.40
Sola-2	14	11	78.57	0.89	1.44	0.28	0.44
Sola-4	9	4	44.44	0.91	1.89	0.47	0.66
Sola-5	13	13	100.00	0.81	1.36	0.22	0.36
Sola-7	11	6	54.55	0.86	1.54	0.33	0.50
Sola-9	7	3	42.86	0.52	1.39	0.25	0.41
Sola-11	6	5	83.33	0.70	1.75	0.42	0.60
Total	309	212					
Average	9.36	6.42	67.91	0.73	1.49	0.30	0.46

Effective number of alleles (*ne*), gene diversity (*h*), Shannon information index (*I*), and polymorphism information content (*PIC*)

Table 4
Distribution of flax (*Linum usitatissimum* L) genotypes to subpopulations according to membership coefficient obtained from structure analysis of iPBS-retrotransposon markers.

Genotypes	Subpopulations							
	I	II	II	IV	V	VI	VII	VIII
Van-1	0.166	0.242	0.038	0.138	0.129	0.004	0.201	0.083
Mersin-1	0.003	0.004	0.003	0.004	0.004	0.977	0.003	0.002
Afyon-1	0.012	0.011	0.011	0.012	0.012	0.004	0.011	0.927
Afyon-2	0.025	0.025	0.021	0.026	0.028	0.845	0.027	0.004
Muş	0.174	0.221	0.060	0.142	0.141	0.009	0.247	0.008
Ankara-Çankaya	0.185	0.259	0.037	0.150	0.127	0.021	0.219	0.002
İzmir-Kemeraltı	0.022	0.024	0.020	0.023	0.021	0.004	0.025	0.861
İzmir-Karşıyaka	0.042	0.033	0.132	0.062	0.094	0.166	0.043	0.428
İzmir-Tepecik	0.006	0.006	0.007	0.007	0.008	0.956	0.006	0.003
Iğdır	0.021	0.020	0.102	0.034	0.051	0.003	0.014	0.756
Bitlis	0.005	0.004	0.006	0.005	0.006	0.004	0.005	0.964
Sinop	0.046	0.036	0.253	0.061	0.054	0.369	0.030	0.151
Diyarbakır	0.034	0.024	0.041	0.027	0.033	0.713	0.029	0.099
Siirt	0.009	0.009	0.021	0.018	0.021	0.909	0.009	0.004
Konya-1	0.041	0.037	0.026	0.041	0.033	0.064	0.038	0.720
Samsun-1	0.062	0.035	0.397	0.138	0.241	0.064	0.049	0.015
Mardin	0.019	0.023	0.014	0.020	0.020	0.011	0.020	0.871
Kilis	0.070	0.075	0.025	0.065	0.049	0.647	0.066	0.003
Bursa	0.033	0.019	0.557	0.117	0.183	0.031	0.024	0.036
Rolin	0.043	0.051	0.225	0.062	0.061	0.383	0.044	0.130
Antares	0.084	0.067	0.056	0.086	0.082	0.552	0.065	0.008
Dakota	0.057	0.052	0.232	0.090	0.079	0.020	0.044	0.426
Sarı-85	0.187	0.151	0.097	0.158	0.133	0.009	0.158	0.107

Table 5
Distribution of flax (*Linum usitatissimum* L) genotypes to subpopulations according to membership coefficient obtained from structure analysis of ISSR markers.

Genotypes	Subpopulations	
	I	II
Van-1	0.988	0.012
Mersin-1	0.011	0.989
Afyon-1	0.984	0.016
Afyon-2	0.021	0.979
Muş	0.660	0.340
Ankara-Çankaya	0.572	0.428
İzmir-Kemeralti	0.984	0.016
İzmir-Karşıyaka	0.160	0.840
İzmir-Tepecik	0.148	0.852
Iğdır	0.469	0.531
Bitlis	0.593	0.407
Sinop	0.089	0.911
Diyarbakır	0.240	0.760
Siirt	0.047	0.953
Konya-1	0.920	0.080
Samsun-1	0.048	0.952
Mardin	0.352	0.648
Kilis	0.169	0.831
Bursa	0.193	0.807
Rolin	0.277	0.723
Antares	0.275	0.725
Dakota	0.701	0.299
Sarı-85	0.950	0.050

Table 6
Distribution of flax (*Linum usitatissimum* L) genotypes to subpopulations according to membership coefficient obtained from structure analysis of combined molecular data

Genotypes	Subpopulations	
	I	II
Van-1	0.991	0.009
Mersin-1	0.007	0.993
Afyon-1	0.991	0.009
Afyon-2	0.028	0.972
Mus	0.714	0.286
Ankara-Cankaya	0.634	0.366
Izmir-Kemeralti	0.991	0.009
Izmir-Karsiyaka	0.323	0.677
Izmir-Tepecik	0.103	0.897
Igdir	0.804	0.196
Bitlis	0.801	0.199
Sinop	0.198	0.802
Diyarbakir	0.259	0.741
Siirt	0.034	0.966
Konya-1	0.967	0.033
Samsun-1	0.197	0.803
Mardin	0.644	0.356
Kilis	0.207	0.793
Bursa	0.321	0.679
Rolin	0.337	0.663
Antares	0.293	0.707
Dakota	0.817	0.183
Sari-85	0.957	0.043

Table 7
The origin and source of flax (*Linum usitatissimum* L.) genotypes

Genotypes	Origin	Obtained
Van-1	Turkey	Van (commercial seed)
Mersin-1	Turkey	Mersin (commercial seed)
Afyon-1	Turkey	Afyon (commercial seed)
Afyon-2	Turkey	Afyon (commercial seed)
Muş	Turkey	Muş (commercial seed)
Ankara-Çankaya	Turkey	Ankara-Çankaya(commercial seed)
İzmir-Kemeraltı	Turkey	İzmir-Kemeraltı(commercial seed)
İzmir-Karşıyaka	Turkey	İzmir-Karşıyaka(commercial seed)
İzmir-Tepecik	Turkey	İzmir-Tepecik(commercial seed)
Iğdır	Turkey	Iğdır (commercial seed)
Bitlis	Turkey	Bitlis (commercial seed)
Sinop	Turkey	Sinop (commercial seed)
Diyarbakır	Turkey	Diyarbakır (commercial seed)
Siirt	Turkey	Siirt (commercial seed)
Konya-1	Turkey	Konya (commercial seed)
Eskişehir	Turkey	Eskişehir (commercial seed)
Samsun-1	Turkey	Samsun (commercial seed)
Mardin	Turkey	Mardin (commercial seed)
Kilis	Turkey	Kilis (commercial seed)
Bursa	Turkey	Bursa (commercial seed)
Hermes	France	Trakya Agricultural Research Institute
Rolin	Romania	Trakya Agricultural Research Institute
Antares	France	Karadeniz Agricultural Research Institute
Royal	Canada	Trakya Agricultural Research Institute
Dakota	ABD	Trakya Agricultural Research Institute
Eckendorfı	Hungary	Trakya Agricultural Research Institute
Sarı-85	Turkey	Karadeniz Agricultural Research Institute

Genotypes	Origin	Obtained
Beyaz Gelin	Turkey	Trakya Agricultural Research Institute
Karakız	Turkey	Trakya Agricultural Research Institute

Figures

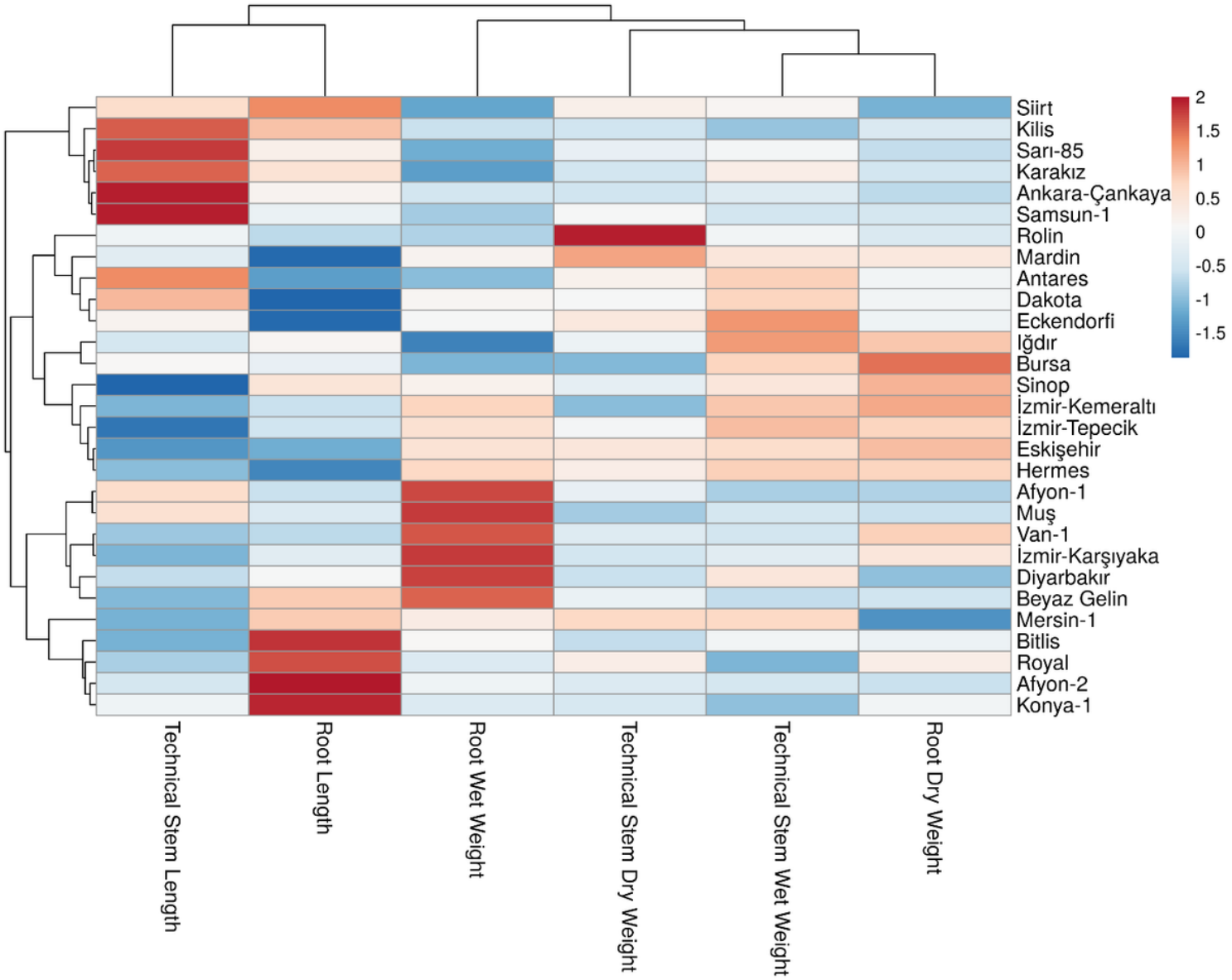

Figure 1

Heatmap clustering of morphological characteristics of flax (*Linum usitatissimum* L.) genotypes

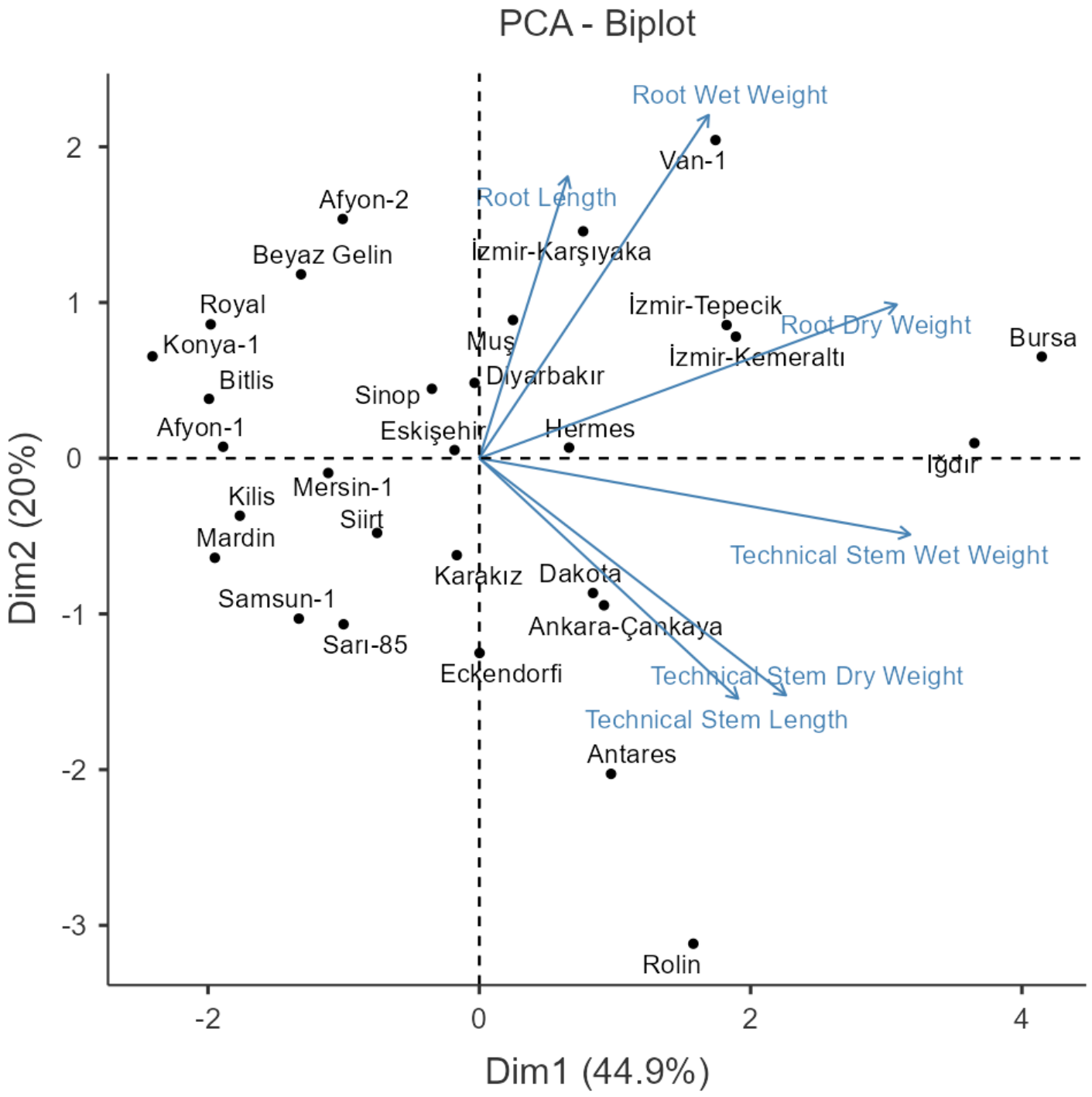

Figure 2

Principal Component Analysis (PCA) of morphological characteristics of flax (*Linum usitatissimum* L) genotypes

Figure 3

Correlation analysis of morphological characteristics of flax (*Linum usitatissimum* L) genotypes

Figure 4

UPGMA based genetic clustering of 23 flax (*Linum usitatissimum* L) genotypes obtained from morphologic characters using Euclidean similarity coefficient

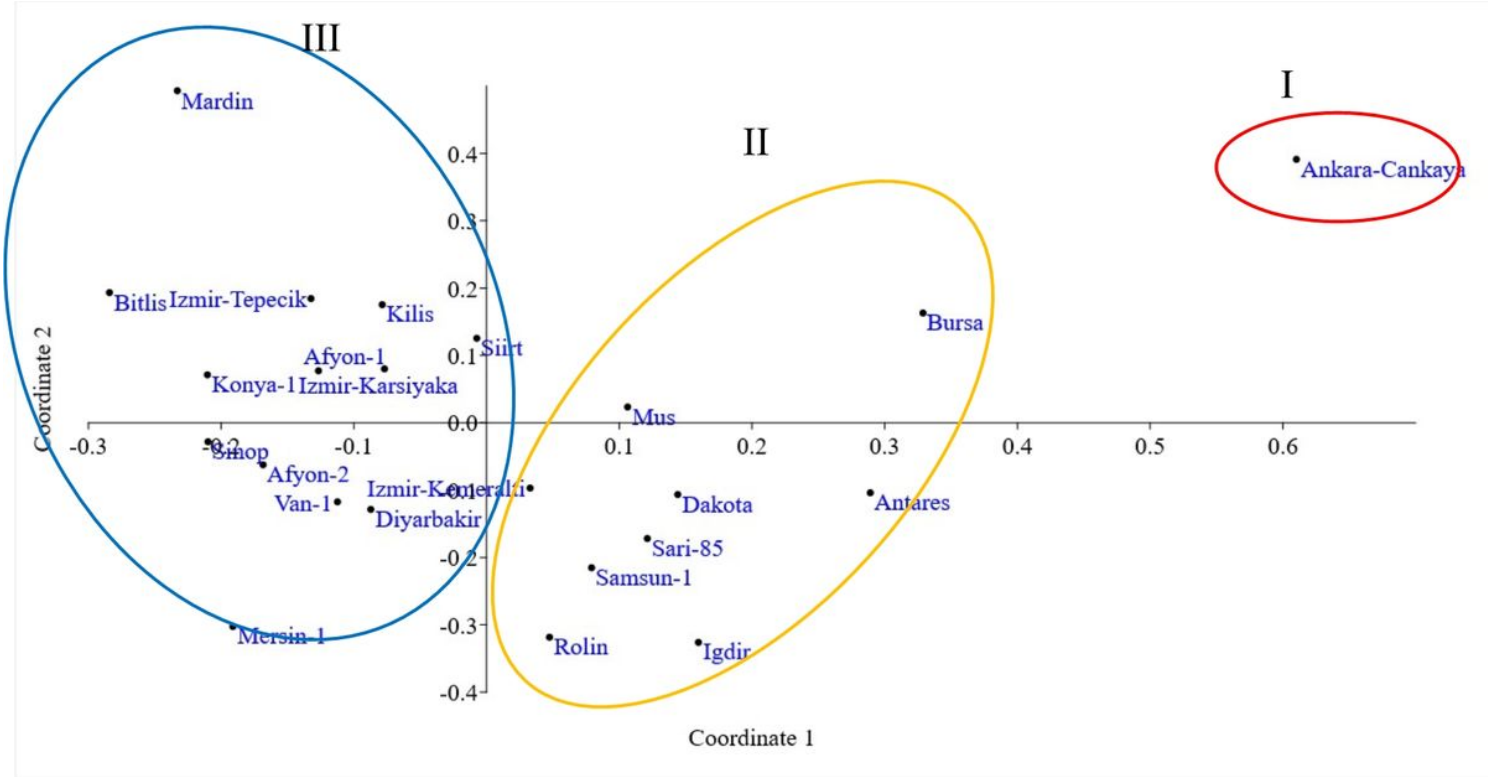

Figure 5

Phenotypic clustering of 23 flax (*Linum usitatissimum* L) genotypes based on Principal Coordinate Analysis (PCoA)

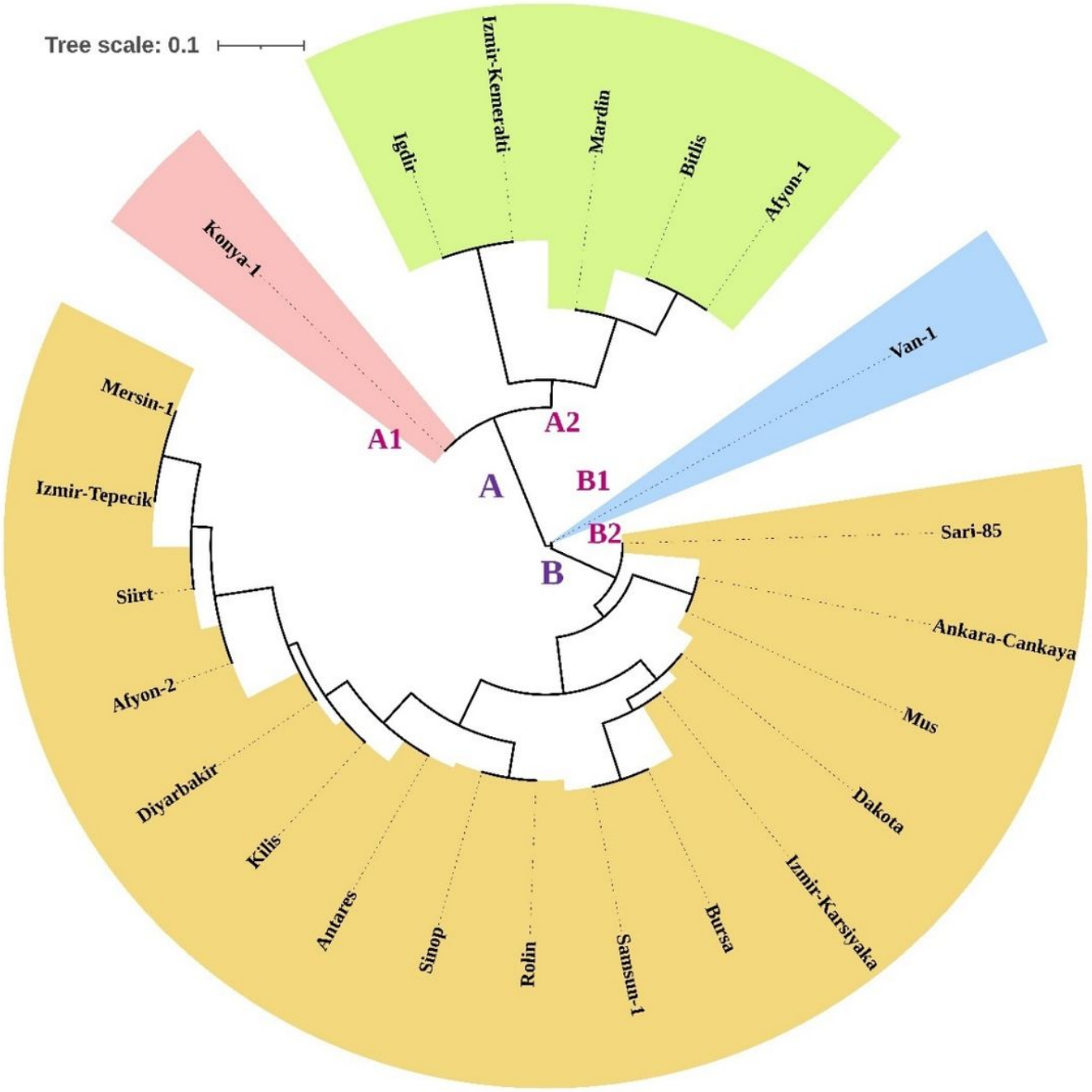

Figure 6

UPGMA based genetic clustering of 23 flax (*Linum usitatissimum* L) genotypes obtained from iPBS-retrotansposon markers

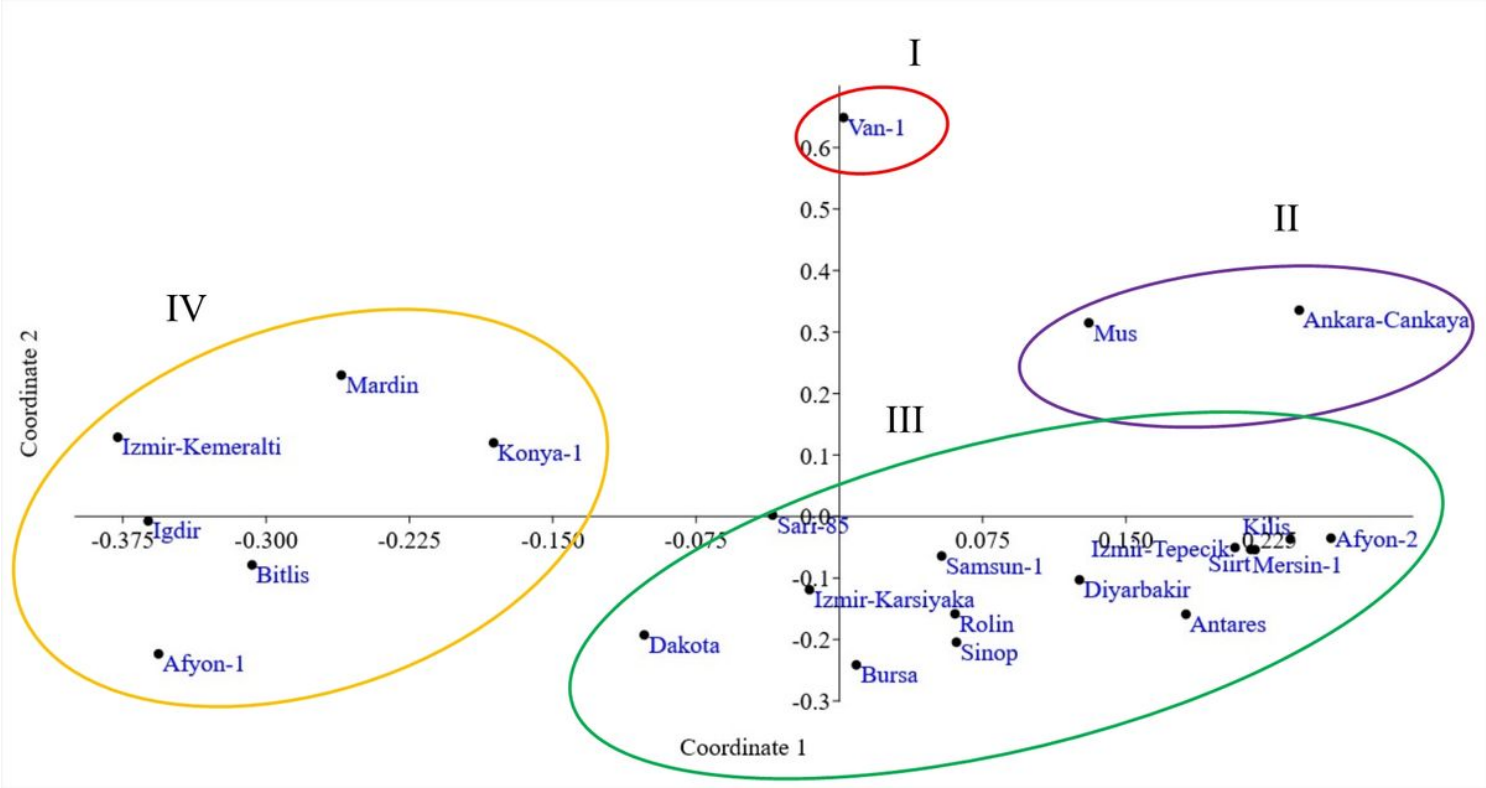

Figure 7

Genetic clustering of 23 flax (*Linum usitatissimum* L) genotypes based on Principal Coordinate Analysis (PCoA) using iPBS-retrotransposon markers

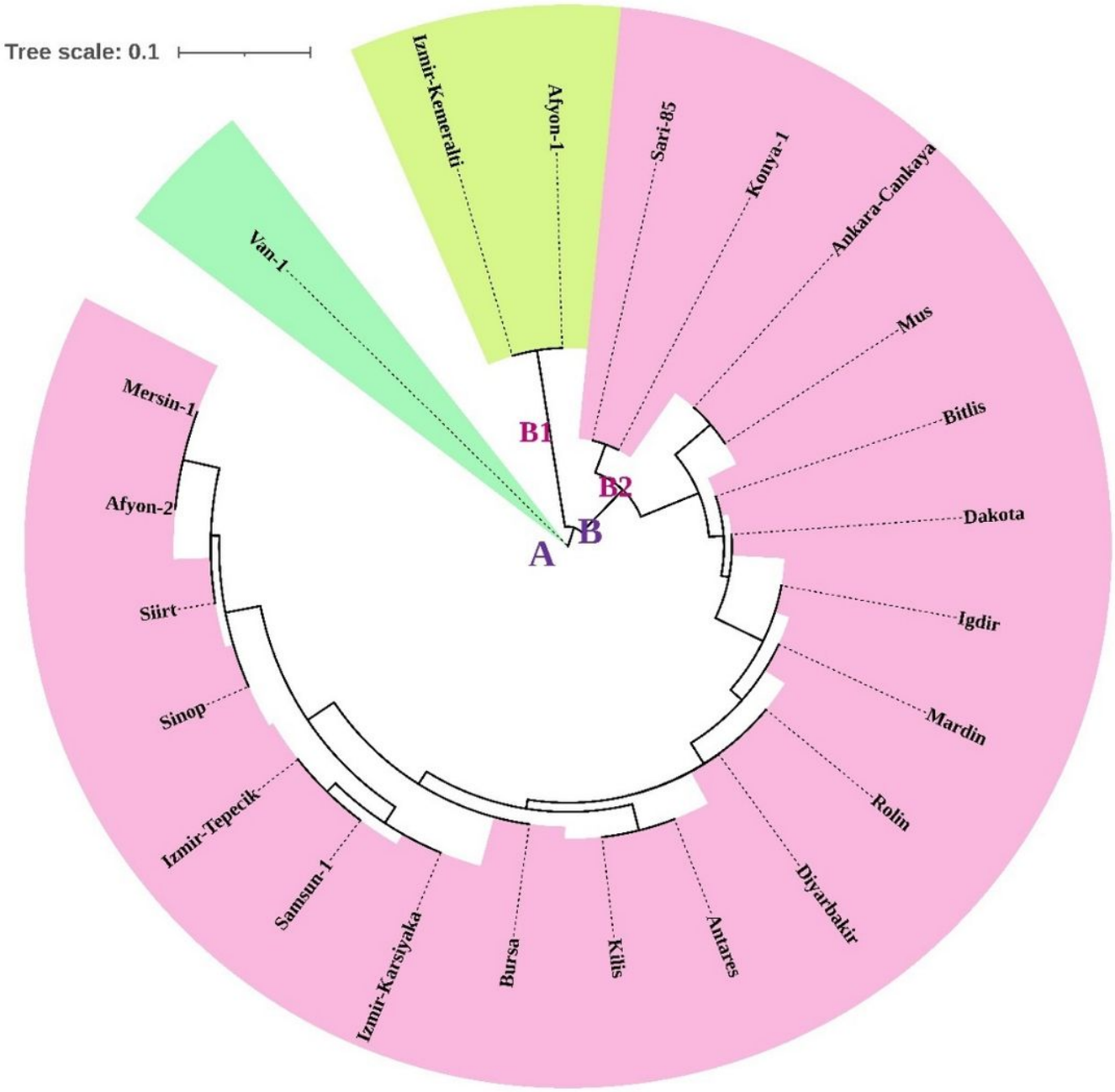

Figure 8

UPGMA based genetic clustering of 23 flax (*Linum usitatissimum* L) genotypes obtained from ISSR markers

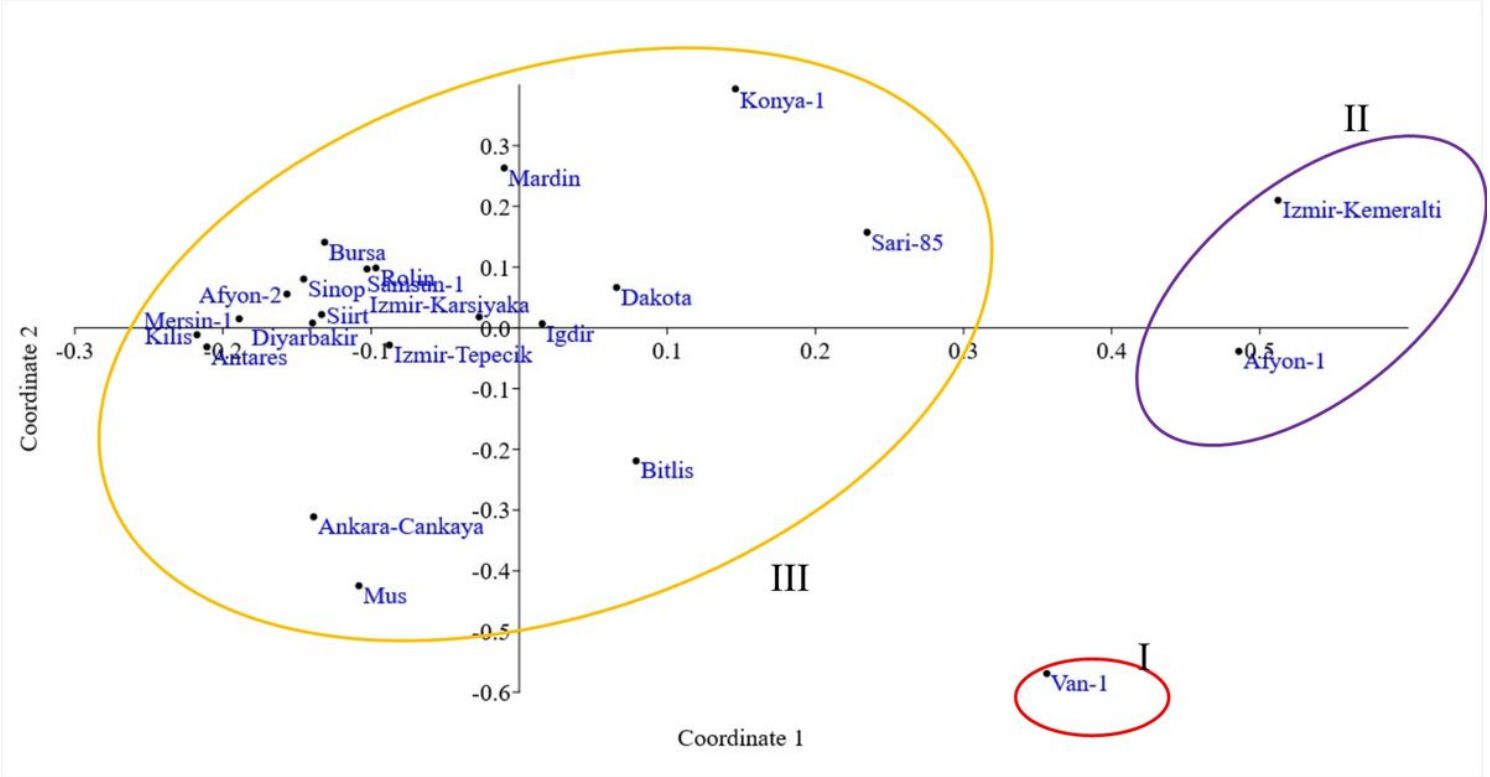

Figure 9

Genetic clustering of 23 flax (*Linum usitatissimum* L) genotypes based on Principal Coordinate Analysis (PCoA) using ISSR markers

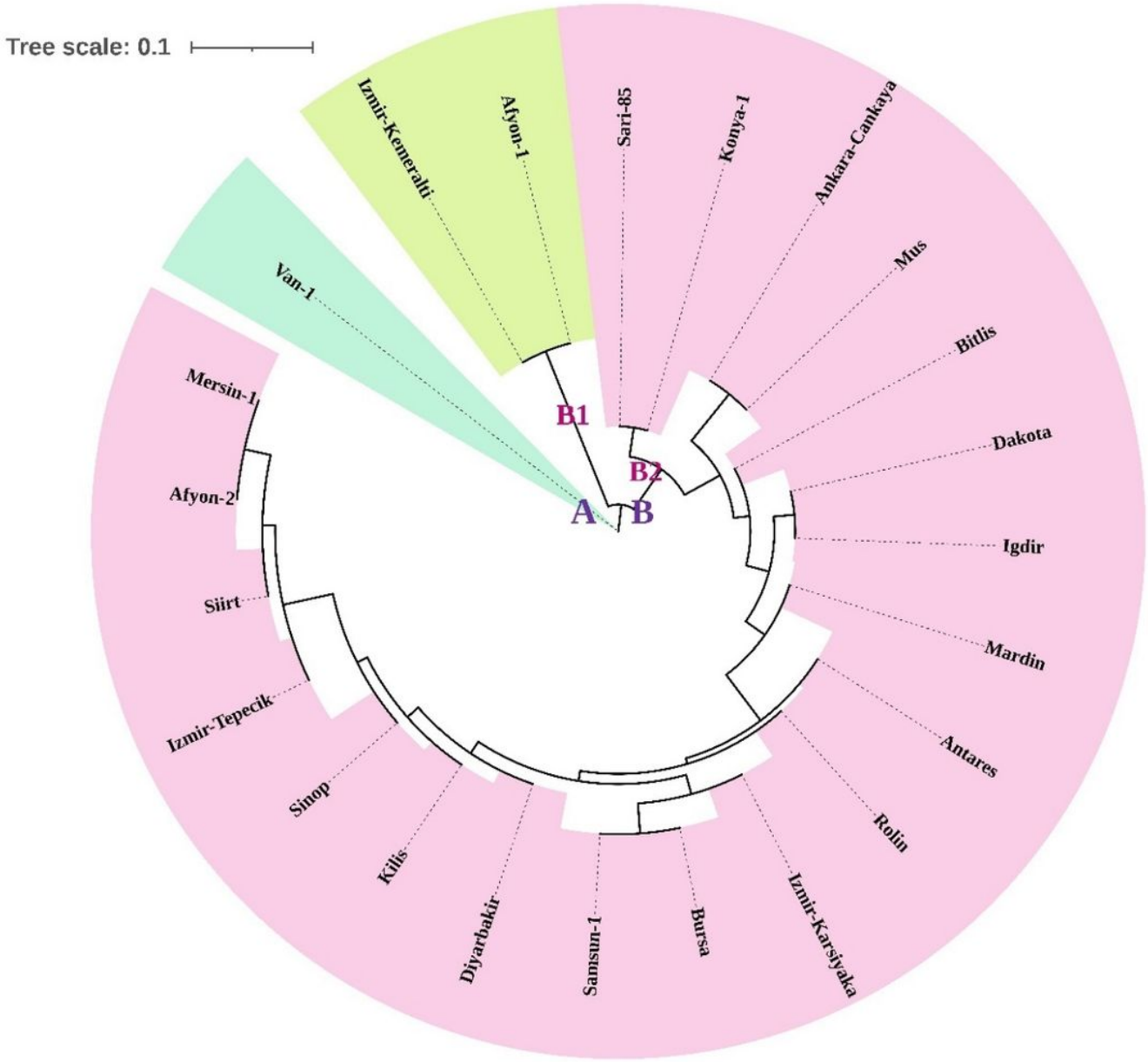

Figure 10

UPGMA based genetic clustering of 23 flax (*Linum usitatissimum* L) genotypes obtained from combined molecular data

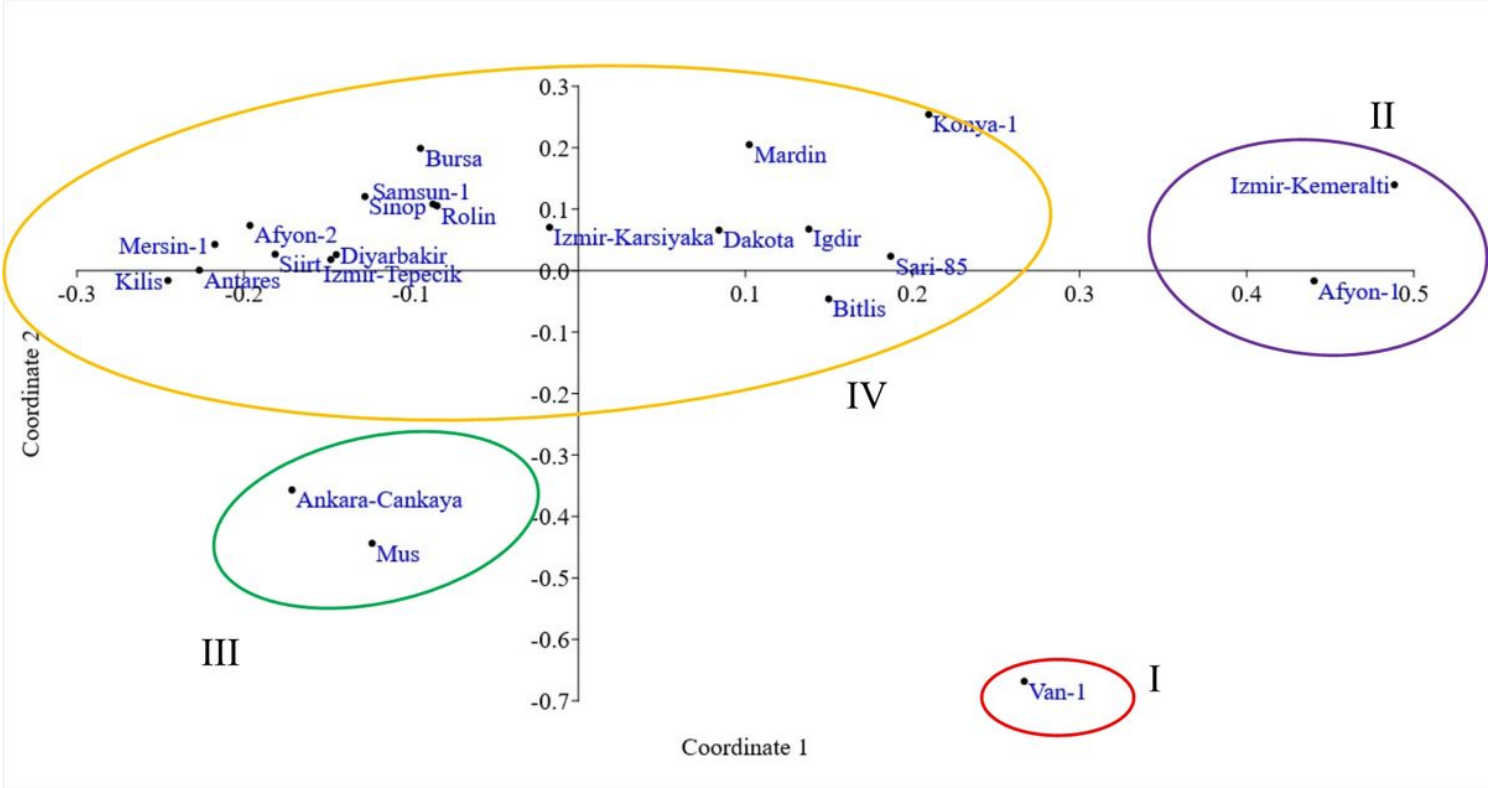

Figure 11

Genetic clustering of 23 flax (*Linum usitatissimum* L)genotypes based on Principal Coordinate Analysis (PCoA) using combined molecular data

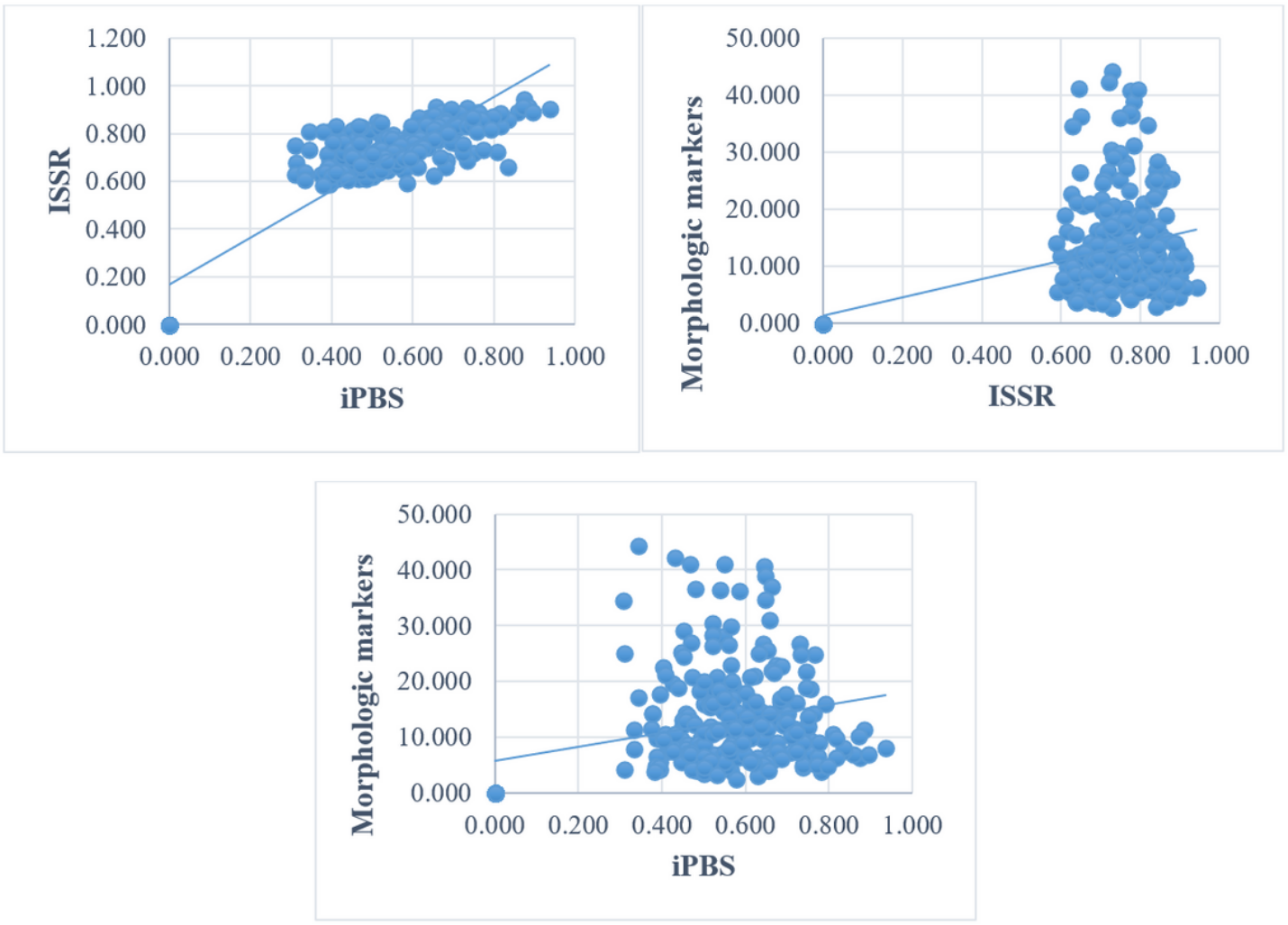

Figure 12

Correlation of mantel test between ISSR and iPBS (r=0.78, p=0.01), ISSR and morphologic markers (r=0.15, p=0.04), iPBS and morphologic markers (r=0.07, p=0.94)

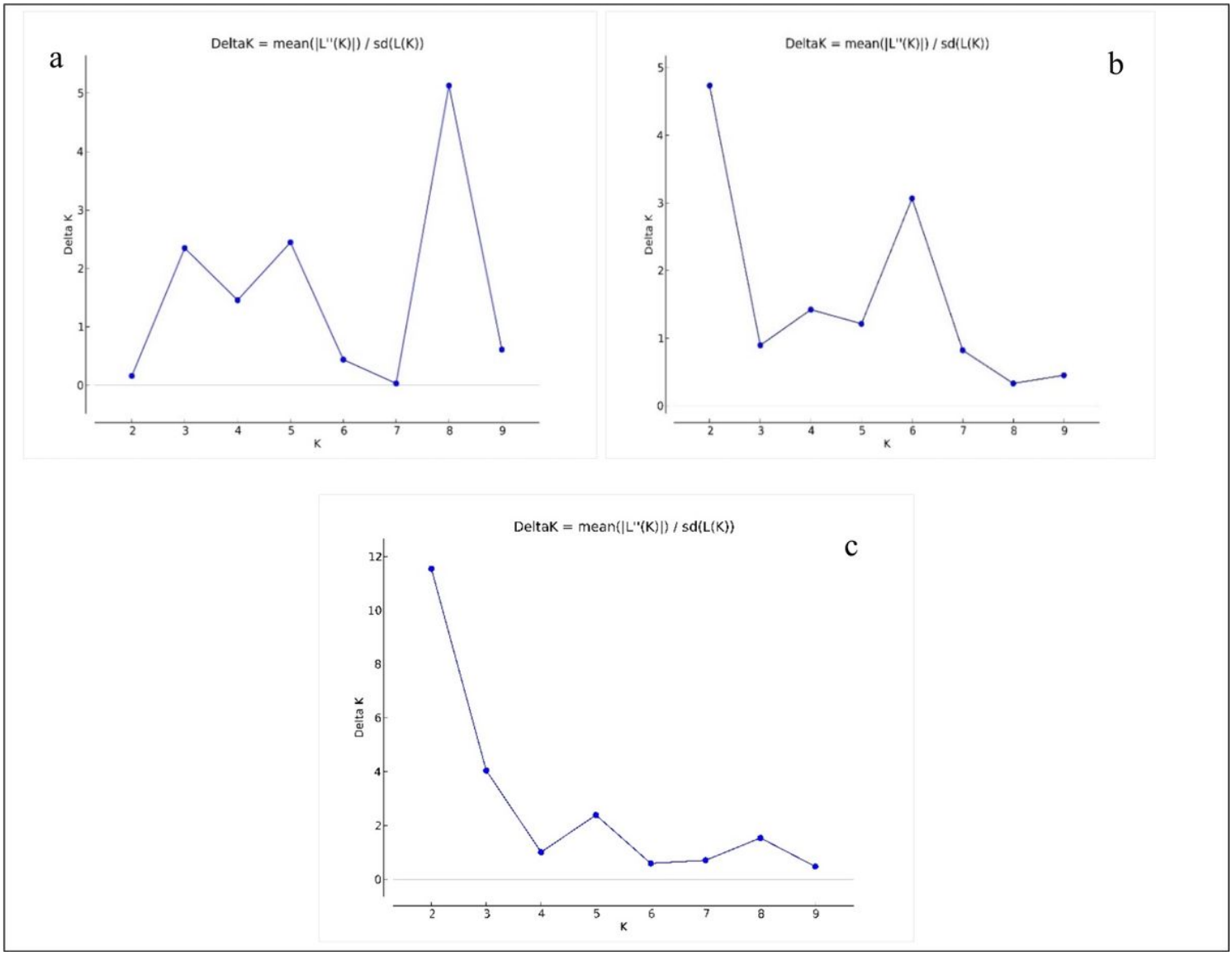

Figure 13

Delta K values for different numbers of populations expected (K) in the STRUCTURE analysis for iPBS-retrotransposon markers (a) and ISSR markers (b) and combined molecular data (iPBS+ISSR).

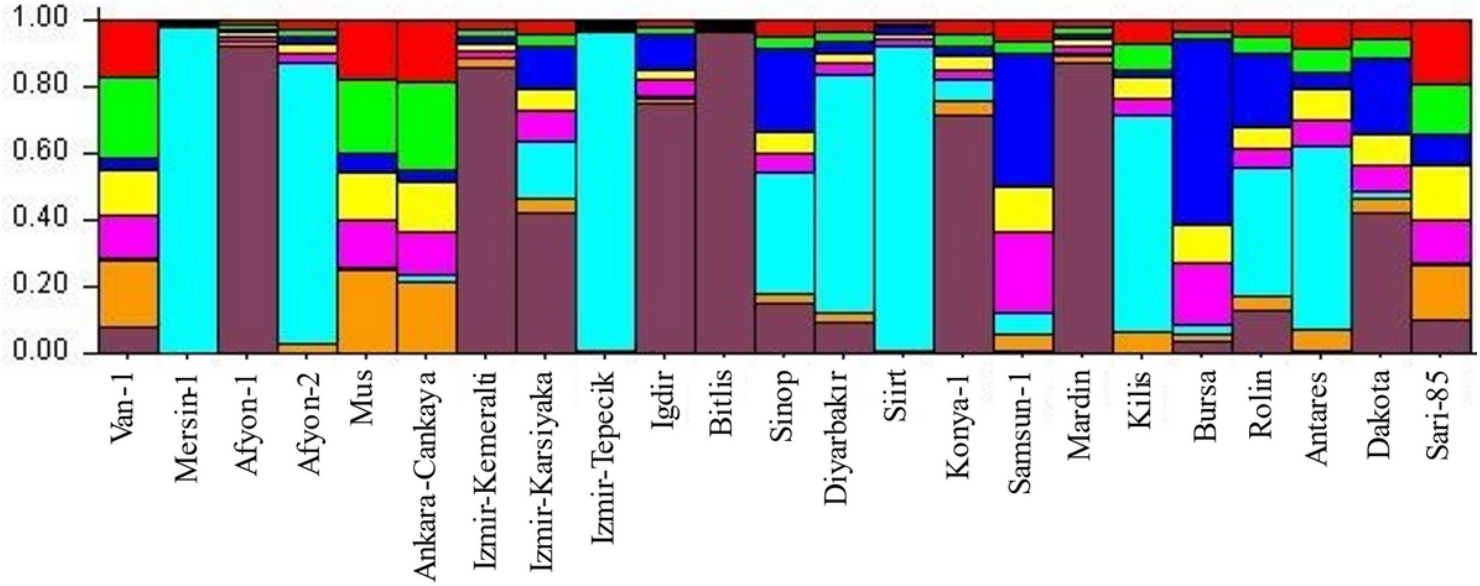

Figure 14

Population structure analysis of flax (*Linum usitatissimum* L) genotypes using iPBS-retrotransposon markers

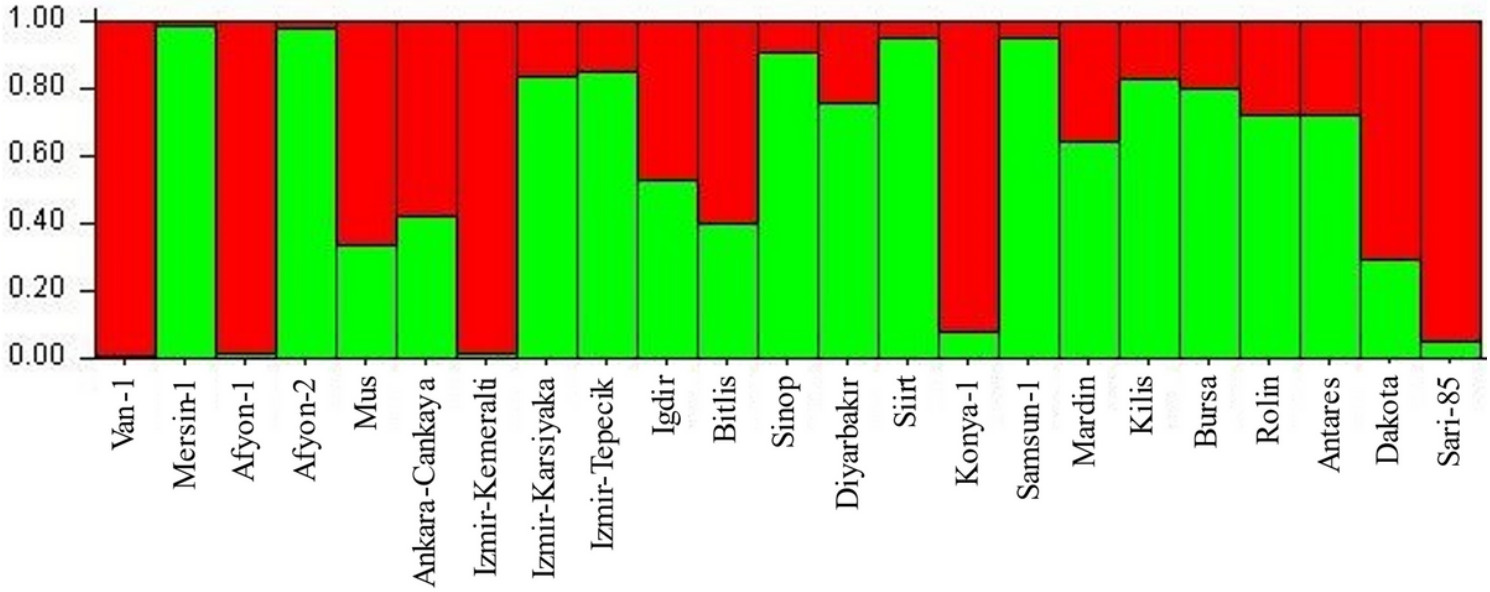

Figure 15

Population structure analysis of flax (*Linum usitatissimum* L) genotypes using ISSR markers

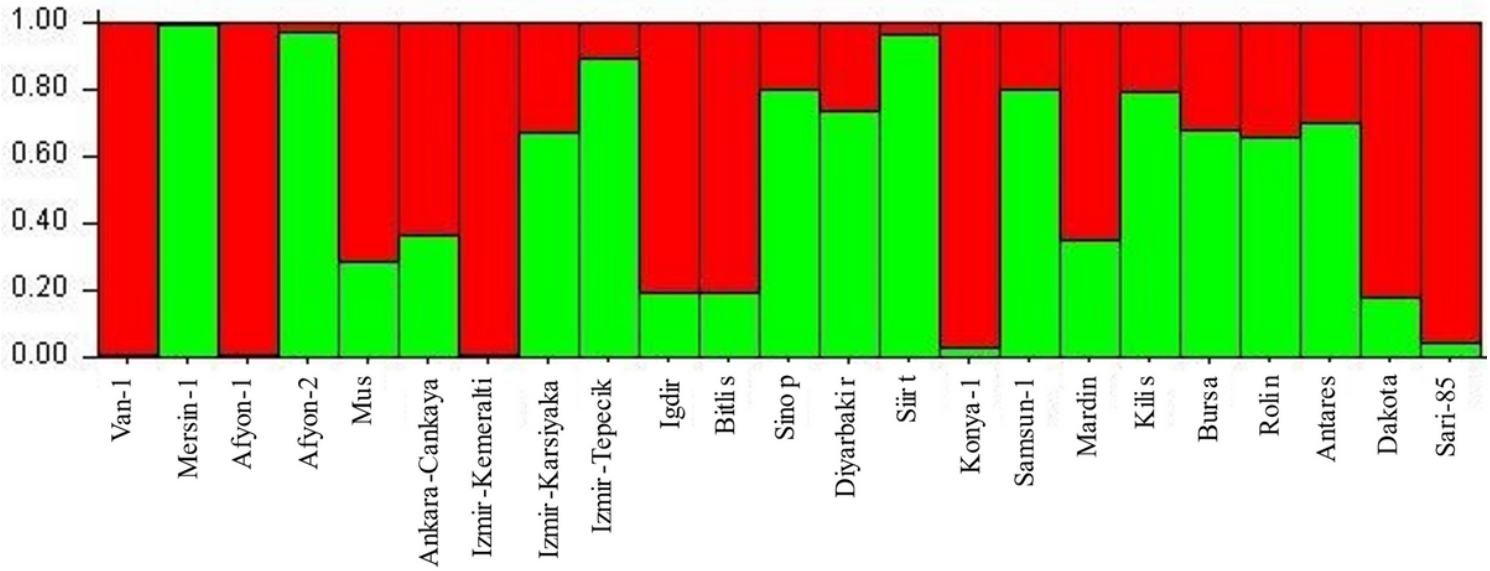

Figure 16

Population structure analysis of flax (*Linum usitatissimum* L) genotypes using combined molecular data