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ORIGINAL RESEARCH

Analysis of genetic diversity and population structure in a mini core collection of flax (*Linum usitatissimum* L.) based on URP and SCoT markers

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Abstract
The importance of plant genetic resources has received increasing attention in crop improvement and plant variation in breeding procedures. Here, plant variation and the structures of plant populations were examined among 93 flax accessions using SCoT and URP molecular markers. Polymorphic-fragments by URP and SCoT primers were, on average, 9.3 and 10.9, respectively, and the mean of polymorphism information content for primers revealed a good efficiency of both marker techniques (0.34 and 0.35 for URP and SCoT, respectively). The neighbor-joining (NJ)-based clustering, via combined data, classified all investigated accessions into four main groups that were further confirmed by principal coordinate analysis (PCoA). Moreover, molecular diversity (88%) was mostly observed inside the populations. STRUCTURE-analysis confirmed the cluster analysis and all samples were separated into four subpopulations ($\Delta K = 4$). The results implied that the sub-populations consisted of diverse accessions, suggesting that this gene pool has the potential for flax breeding programs. Furthermore, our findings indicated that URP and SCoT markers are reliable techniques in assessing plant variation, especially in elucidating the structures of flax populations. However, gene-targeting markers like SCoT are preferable because of their roots in functional genome-based regions.

Keywords Flax · Genetic diversity · DNA markers · Population structure · AMOVA

Introduction

Flax (*Linum usitatissimum* L.), the common type of linseed, belongs to the Linaceae family and is grown for different purposes. Its high amount of linolenic acid (45–60%) has made linseed oil popular with several applications in the industry, such as the manufacture of paints, printing inks, varnishes, and stains (Kurt and Bozkurt 2006; Patial et al. 2019). Linseed has high linolenic acid (ALA, omega 3 fatty acid) and high lignans, making it nutritious with anti-cancer properties. The essential polyunsaturated fatty acids in the seed oil of linseed also make it very effective to reduce coronary heart diseases and add to the health benefits of this valuable herb. In addition, this plant is primarily grown for the extraction of fiber (Bassett et al. 2009; Goyal et al. 2014; Patial et al. 2019; Thakur et al. 2021).

Since plants play a key role in agriculture, their variation seems essential in plant breeding. Having genetic diversity in crop germplasm can guarantee a successful selection of parents in providing populations with superior plant varieties. In fact, enough variation is a precursor to new, enhanced plant cultivars (Govindaraj et al. 2015). The commercialization of optimal cultivars has put less competitive variations at risk of permanent loss. The evaluation of plant variation not only assists in generating new varieties but also helps with the efficient management and conservation of germplasms (Vinu et al. 2013). Genetic diversity is also substantially crucial when maximizing breeding for efficiency. Genetic mapping with an evaluation of plant diversity helps with the maintenance of the genetic well-being of plant populations. It assists in discovering new valuable alleles

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for breeding programs (Diederichsen and Richards 2003; Kumari et al. 2017; Patial et al. 2019). Although the investigation of a genetic construct, with a focus on variation, among plant accessions can be carried out via molecular, morphological, and biochemical markers, the use of new, molecular-based methods can be more efficient than traditional phenotypic and biochemical markers. Molecular markers, in particular, DNA markers, are more reliable, and reproducible, with a larger capacity to be detected among the plant tissue. Moreover, molecular-based markers can be less influenced by environmental factors and offer higher polymorphism than the other types of markers (Mohammadi and Prasanna 2003; Collard and Mackill 2009; Govindaraj et al. 2015).

Many applications are known for molecular-based markers in plant breeding, such as assessment of genetic diversity, analysis of population structure, marker-assisted selection, cultivar identification and fingerprinting, Association mapping, and QTL mapping (Govindaraj et al. 2015). More recently, many different DNA-based marker(s) are introduced because of developments in the area of genomics. Among different types of DNA markers, gene-targeted markers are a preferable choice to random markers for the assessment of genetic diversity because of their concentration on gene loci, rendering them efficiently operative in QTL-mapping, gene-targeting, and marker-assisted breeding. Furthermore, gene-targeted markers work with lengthier primers that have hotter annealing temperatures, causing a greater level of reproducibility in them, more stability and reliability, compared to other types of non-efficient constructs (Collard and Mackill 2009; de Lucena et al. 2013; Ding et al. 2015).

Collard and Mackill (2009) reported a simple and novel DNA marker technique Start Codon Targeted (SCoT) Polymorphism. SCoT can be regarded as a reliable, new gene-based marker technique, with a wide range of applications in plants. The markers can be made with single-primer constructs, while relying on short-conserved regions in genes in the vicinity of the ATGs translation initiation codon, occurring as a conserved format in the majority of genes (Collard and Mackill 2009; Feng et al. 2018). This marker system does not require any sequence information for primer designing and SCoT markers usually correspond with functional modes of genetic material, while correlating with traits as well (Mulpuri et al. 2013). SCoT markers have been successfully employed in revealing plant diversity and population structures among plant species, e.g. safflower (Talebi et al. 2018), Cactaceae (Abouseadaa et al. 2020), grape (Guo et al. 2012), Sugarcane (Que et al. 2014), wild relatives of wheat (Ghobadi et al. 2021; Pour-Aboughadareh et al. 2017, 2018, 2022; Khodaee et al. 2021), Cicer (Amirmoradi et al. 2012), peanut (Xiong et al. 2011), Damask Rose (Asadi and Shooshtari 2021), and durum-wheat (Etminan et al. 2016; Heidari et al. 2017).

Universal rice primer (URP) technique is another type of the DNA markers, designed based on repetitive sequences of the rice-species genetic materials (Kang et al. 2002). The URP primers are basically random, but their length and higher annealing temperature cause them to be more reproducible than many other PCR-based markers, e.g. ISSR and RAPD (Mehrparvar et al. 2012). The URP marker system is simple, powerful, and reliable in a wide range of genomes, especially in evaluating plant variations (Kang et al. 2002; Jana et al. 2005; Mehrparvar et al. 2012; Amirmoradi et al. 2012; Kandan et al. 2014; Kumar et al. 2018; Salahlou et al. 2019). Several different markers rely on plant variations at a genetic level, representing differences among flax populations. Molecular evaluation has been successfully undertaken on flax germplasm using different DNA marker-assisted approaches, e.g. ISSR (Uysal et al. 2010; Kumari et al. 2018; El Sayed et al. 2018; Mhiret and Heslop-Harrison 2018), AFLP (Everaert et al. 2001), SSR (Soto-Cerda et al. 2013; Yadav et al. 2018), IRAP (Mhiret and Heslop-Harrison 2018), and RAPD (Fu et al. 2003; Kumari et al. 2018).

The current research aimed at evaluating plant variations among 93 flax genotypes belonging to five *Linum usitatissimum* subspecies based on SCoT and URP markers.

Materials and methods

DNA extractions and plant materials

Ninety-three flax genotypes of four populations (35 accessions of *usitatissimum* subspecies, 27 accessions of *elongatum* subspecies, 12 accessions of *mediterranian* subspecies, and 19 accessions of an unknown subspecies) were considered for evaluation of plant variation via SCoT as well as URP molecular-based markers. A detailed list of accessions with gene bank codes is provided in Table 1. The seeds of 93 accessions were grown under greenhouse conditions. DNA of genomes was obtained through the CTAB protocol (Doyle and Doyle 1987) from fresh leaf tissue of a single plant per accession. DNA was extracted in high-quality form and checked through 0.8% (w/v) agarose-gel electrophoresing.

SCoT and URP assays

Based on sequences, suggested by Kang et al. (2002), 10 URP-primers of sequence-based materials were considered. Also, the SCoT primers were selected based on the primer developed by Collard and Mackill (2009) and Luo et al. (2010). In previous study on the genetic variation in Damask rose (Mostafavi et al. 2021), the primers of the two markers

Table 1 List of the 93 investigated Flax accessions

Code	Gen bank code	Sub-population	Origin	Country
1	TN-97–2	Unknown	Kerman	Iran
2	TN-97–167.2	Unknown	Rabor	Iran
3	TN-369	Unknown	Tajikistan	Iran
4	TN-97–33	Unknown	Zabol	Iran
5	TN-368	Unknown	Czech	Iran
6	TN-97–316	Unknown	Amazon	Iran
7	TN-97–167.1	Unknown	Rabor	Iran
8	TN-97–301	Unknown	Dehgolan	Iran
9	TN-97–90	Unknown	Shahin Dezh	Iran
10	TN-97–321	Unknown	Khomein	Iran
11	TN-97–289	Unknown	Samian	Iran
12	TN-97–10	Unknown	Sarab	Iran
13	TN-97–322	Unknown	Urmia	Iran
14	TN-97–310	Unknown	ORDC	Iran
15	TN-97–249	Unknown	Khodabandeh	Iran
16	TN-97–95	Unknown	Miandoab	Iran
17	TN-97–178	Unknown	Bardsir	Iran
18	TN-97–209	Unknown	Osku	Iran
19	TN-97–100	Unknown	Bandar Imam Khomeini	Iran
20	LIN-939	Elongatum	Bulgarian (BGR)	IPK, Germany
21	TN-382	Elongatum	CSFR (CSK)	IPK, Germany
22	TN-2344	Elongatum	Niederlande (NLD)	IPK, Germany
23	TN-1514	Elongatum	Poland	IPK, Germany
24	TN-1101	Elongatum	Poland	IPK, Germany
25	TN-1983	Elongatum	Sowjetunion (SUN)	IPK, Germany
26	TN-1954	Elongatum	Sowjetunion (SUN)	IPK, Germany
27	TN-1943	Elongatum	Deutschland (DEU)	IPK, Germany
28	TN-941	Elongatum	Bulgarien (BGR)	IPK, Germany
29	1044	Elongatum	Frankreich (FRA)	IPK, Germany
30	1119	Elongatum	Spanien (ESP)	IPK, Germany
31	1497	Elongatum	–	IPK, Germany
32	1879	Elongatum	Rumänien (ROU)	IPK, Germany
33	1942	Elongatum	Deutschland (DEU)	IPK, Germany
34	2035	Elongatum	Schweden (SWE)	IPK, Germany
35	22	Usitatissimum	Iran (IRN)	IPK, Germany
36	30	Usitatissimum	Iran (IRN)	IPK, Germany
37	58	Usitatissimum	Iran (IRN)	IPK, Germany
38	60	Usitatissimum	Iran (IRN)	IPK, Germany
39	69	Usitatissimum	Iran (IRN)	IPK, Germany
40	70	Usitatissimum	Iran (IRN)	IPK, Germany
41	286	Usitatissimum	Ungarn (HUN)	IPK, Germany
42	338	Usitatissimum	Italien (ITA)	IPK, Germany
43	1086	Usitatissimum	Estland (EST)	IPK, Germany
44	1091	Usitatissimum	Irland (IRL)	IPK, Germany
45	1109	Usitatissimum	Poland	IPK, Germany
46	1116	Usitatissimum	Spanien (ESP)	IPK, Germany
47	1118	Usitatissimum	Spanien (ESP)	IPK, Germany
48	1484	Usitatissimum	–	IPK, Germany
49	1565	Usitatissimum	China (CHN)	IPK, Germany
50	1570	Usitatissimum	USA	IPK, Germany
51	1577	Usitatissimum	Niederlande (NLD)	IPK, Germany

Table 1 (continued)

Code	Gen bank code	Sub-population	Origin	Country
52	1732	Usitatissimum	Äthiopien (ETH)	IPK, Germany
53	2121	Usitatissimum	–	IPK, Germany
54	75	Usitatissimum	Iran (IRN)	IPK, Germany
55	134	Usitatissimum	Argentinien (ARG)	IPK, Germany
56	391	Usitatissimum	China (CHN)	IPK, Germany
57	1032	Usitatissimum	Sowjetunion (SUN)	IPK, Germany
58	1149	Usitatissimum	Poland	IPK, Germany
59	1172	Usitatissimum	Brasilien (BRA)	IPK, Germany
60	1182	Usitatissimum	Uruguay (URY)	IPK, Germany
61	1505	Usitatissimum	Balkan ()	IPK, Germany
62	1506	Usitatissimum	Balkan ()	IPK, Germany
63	1568	Usitatissimum	Argentinien (ARG)	IPK, Germany
64	1600	Usitatissimum	–	IPK, Germany
65	330	Mediterranian	Italien (ITA)	IPK, Germany
66	332	Mediterranian	Italien (ITA)	IPK, Germany
67	333	Mediterranian	Italien (ITA)	IPK, Germany
68	388	Mediterranian	Italien (ITA)	IPK, Germany
69	1509	Mediterranian	Balkan ()	IPK, Germany
70	1510	Mediterranian	Balkan ()	IPK, Germany
71	1567	Mediterranian	Argentinien (ARG)	IPK, Germany
72	1573	Mediterranian	Pakistan (PAK)	IPK, Germany
73	1591	Mediterranian	–	IPK, Germany
74	1834	Mediterranian	–	IPK, Germany
75	1545	Elongatum	–	IPK, Germany
76	1428	Elongatum	Sowjetunion (SUN)	IPK, Germany
77	1724	Elongatum	Äthiopien (ETH)	IPK, Germany
78	1590	Elongatum	Poland	IPK, Germany
79	2093	Elongatum	Schweden (SWE)	IPK, Germany
80	2092	Elongatum	–	IPK, Germany
81	1070	Elongatum	Litauen (LTU)	IPK, Germany
82	2394	Elongatum	–	IPK, Germany
83	1945	Elongatum	Deutschland (DEU)	IPK, Germany
84	1081	Elongatum	Frankreich (FRA)	IPK, Germany
85	1535	Elongatum	–	IPK, Germany
86	1586	Elongatum	Ungarn (HUN)	IPK, Germany
87	769	Usitatissimum	Großbritannien (GBR)	IPK, Germany
88	877	Usitatissimum	Belorußland (BLR)	IPK, Germany
89	2288	Usitatissimum	Kolumbien (COL)	IPK, Germany
90	2358	Usitatissimum	–	IPK, Germany
91	1563	Usitatissimum	Neuseeland (NZL)	IPK, Germany
92	1504	Mediterranian	Balkan	IPK, Germany
93	1511	Mediterranian	Balkan	IPK, Germany

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were used. Based on the results of the previous studies, the primers had a good ability to reveal genetic variation among the plants, therefore, they were used in the present study.

PCR reactions were carried out with a 20 µL solution of 10 µL master-mix PCR, 2 µL primer, 6 µL ddH_2O (double distilled water), and 2µL DNA template (approximately 50 ng of template DNA). Amplifications of PCR were carried out in a Biorad thermal Cycler (Model: T-100) by denaturing (94 °C) for 5 min, and then 45 times denaturing (94 °C) within a timeframe of 45 s, primer-annealing (48–56 °C) (which varied depending on SCoT and URP primers) for 45 s, and extension at 72 °C within 90 s, leading

to an ultimate extension-step (72 °C, 10 min), and then remaining at 10 °C. The products of PCR were separated on 1.5% agarose-based gel, marked with Safestaine-II, and digitally photographed using a gel documentation device.

Analyses of data

Amplified fragments were described as 1 or 0, if bands were present or absent, respectively, across the 93 genotypes. To compare the efficiency of markers, polymorphism information content (PIC) was measured according to Anderson et al. (1993). The marker index (MI) was designated based on a method proposed by Powell et al. (1996). Measurement of genetic distances relied on Jaccard's dissimilarity coefficient matrix and cluster analysis was based on the neighbor joining (NJ) algorithm, using DARwin software ver. 6.0.13 (Perrier and Jacquemoud-Collet 2006). These were applied for principal-coordinate analysis (PCoA) in visualizing genetic correlations between 93 flax accessions. A fan dendrogram was constructed with MEGA software ver. 5.1 (Tamura et al. 2011) by combined (SCoT + URP) data. Molecular variation was assessed by GenAlEx v6.5 (Peakall and Smouse 2006) (AMOVA) to calculate genetic variations, observed alleles (Na), number of effective alleles (Ne), Nei's gene diversity (He), Shannon's information index (I), and polymorphic loci percentage. Population structures were analyzed via STRUCTURE (Pritchard et al. 2000). Clusters were counted through ten independently-running modes. Structural analyses were determined in an estimation of subpopulations (ΔK) through STRUCTURE HARVESTER (Earl and vonHoldt 2012).

Results

Markers diversity pattern

Twelve SCoT primers were tested, among which only 10 primers generated distinct, scorable band patterns. Ten SCoT primers generated 109 distinct banding patterns with a polymorphic appearance. Maximal and minimal counts of the polymorphic banding appeared through SCoT-5 (14 bands) as well as SCoT-10 (8 bands). SCoT primers had PIC values that occurred between 0.22 (SCoT-2) and 0.43 (SCoT-7 and SCoT-8) while averaging 0.35 for each primer. Moreover, the mean values of MI for each SCoT primer were 3.8 and ranged from 2.4 to 5.5 for primers SCoT-2 and SCoT-8, respectively (Table 2). Ten URP primers generated a total of 93 bands of polymorphic appearance. Amplified fragments were counted as 7 (by URP-1, URP-7, and URP-10) to 16 (by URP-4), averaging 9.3 bands for each primer. The minimal and maximal values of PIC occurred in URP-5 (0.29) and URP-7 (0.43), averaging 0.34 for each primer. MI values for these primers spanned between 2.2 (by URP-10) to 5.4 (by URP-4). The MI average for all URP primers was 3.2 (Table 2). In summarizing the pattern of bands, each population was described differently through combined data (URP + SCoT) (Fig. 1).

Genetic distances and grouping of the accessions

According to the dendrogram, from the cluster analysis of SCoT data (Fig. S1) and URP data (Fig. S2), the 93 flax accessions were classified into three and four clusters, respectively. Neither Scot nor URP marker data grouped the flax accessions exactly according to their sub-species. There were levels of admixture between the clusters. Based on the SCoT data, pairwise genetic distances between 93 flax genotypes had a range between 0.23 and 0.89, averaging at 0.65. Maximal genetic distance occurred among an accession of unknown origin (TN-97–95) and accession 1504, belonging to *mediterranian* population. Meanwhile, two accessions of the *elongatum* (LIN-939 and TN-382) showed minimal genetic distance. According to the URP data, pairwise genetic dissimilarity had a range between 0.12 and 0.91, averaging 0.58. Maximal genetic dissimilarity (0.91) happened among accession 2358 (of the *usitatissimum* population) and accession TN-97–10 (from an unknown population). Minimal genetic dissimilarity (0.12) occurred among two accessions from *usitatissimum* population (1172 and 1182). Moreover, for more reliable classification and a better explanation of genetic relationships, cluster analysis was performed using combined (SCoT + URP) data. A fan dendrogram resulted from cluster analysis on both markers (SCoT + URP) data, and classified all 93 accessions into four groups (Fig. 2). A clearer classification for accessions was made according to their genetic construct in a complimentary dendrogram, where the accessions of each population are shown in different colors (Fig. 3). The principal-coordinate-analysis made use of combined data in accordance with cluster analysis (Fig. 4).

Genetic diversity analysis

According to molecular variance (AMOVA), based on SCoT data, the variance between populations covered 11% of the total diversity, but intra-population diversity stood at 89%. Nonetheless, based on URP data, intra- and interspecies genetic diversity stood at 14% and 86%, respectively, which was almost similar to the results of SCoT data. Moreover, the AMOVA analysis according to the combined (SCoT + URP) data, revealed a major intra-population diversity (88%), whereas a mere 12% was attributed to inter-population diversity (Fig. 5). To compare the genetic diversity among populations, several attributes were considered for plant variation per population. Based on combined (SCoT + URP) data,

Table 2 SCoT and URP primers and their amplification results generated in the 93 flax accessions

No	Primer code	Sequence	TB	PB	PIC	MI
1	SCoT 1	CAACAATGGCTACCACCA	10	10	0.33	3.3
2	SCoT 2	CAACAATGGCTACCACCC	11	11	0.22	2.4
3	SCoT 3	CAACAATGGCTACCACCG	11	11	0.34	3.7
4	SCoT 4	CAACAATGGCTACCACCT	12	12	0.32	3.9
5	SCoT 5	CAACAATGGCTACCACGA	14	14	0.30	4.1
6	SCoT 6	CAACAATGGCTACCACGC	10	10	0.38	3.8
7	SCoT 7	CAACAATGGCTACCACGG	11	11	0.43	4.7
8	SCoT 8	CAACAATGGCTACCACGT	13	13	0.43	5.5
9	SCoT 9	CAACAATGGCTACCAGCA	9	9	0.39	3.5
10	SCoT 10	CAACAATGGCTACCAGCC	8	8	0.40	3.2
		Mean	10.9	10.9	0.35	3.8
11	URP 1	ATCCAAGGTCCGAGACAACC	7	7	0.33	2.3
12	URP 2	CCCAGCAACTGATCGCACAC	10	10	0.35	3.5
13	URP 3	AGGACTCGATAACAGGCTCC	8	8	0.36	2.8
14	URP 4	ATGTGTGCGATCAGTTGCTG	16	16	0.34	5.4
15	URP 5	GATGTGTTCTTGGAGCCTGT	9	9	0.29	2.6
16	URP 6	GGACAAGAAGAGGATGTGGA	10	10	0.30	3.0
17	URP 7	AAGAGGCATTCTACCACCAC	7	7	0.43	3.0
18	URP 8	AATGTGGGCAAGCTGGTGGT	8	8	0.34	2.7
19	URP 9	TACACGTCTCGATCTACAGG	11	11	0.40	4.4
20	URP 10	GGCAAGCTGGTGGGAGGTAC	7	7	0.31	2.2
		Mean	9.3	9.3	0.34	3.2

TB, PB, PIC and MI indicate total amplified bands, polymorphic amplified bands, polymorphism information content and marker index parameters, respectively

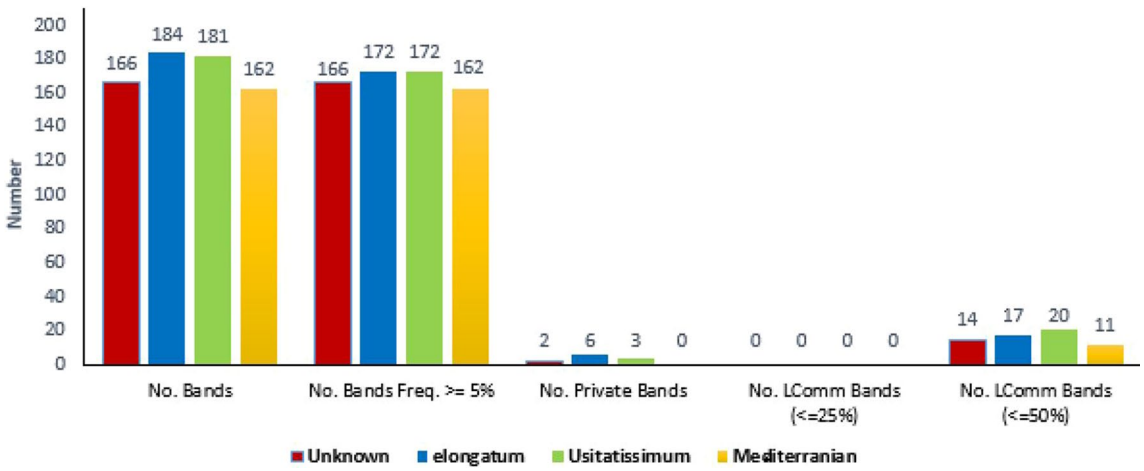

Fig. 1 Banding patterns in sub-populations of Flax, resulted from analysis of combined (SCoT + URP) data

effective alleles (Ne) were between 1.46 (for the unknown population) and 1.51 (for *elongatum*), averaging at 1.49. According to Shannon's index (I), there was a variation between 0.40 and 0.44, with 0.42 as the average. Meanwhile, *elongatum* and unknown populations showed maximal and minimal (I) values, respectively. Nei's genetic diversity (He) had an average (0.28) but differed between 0.26 (for the unknown population) and 0.30 (for *elongatum*). Besides, polymorphism loci (PPL) on average stood at 84%, whereas the highest and lowest values of PPL belonged to *elongatum* (90%) and *mediterranian* (75%) populations, respectively (Table 3).

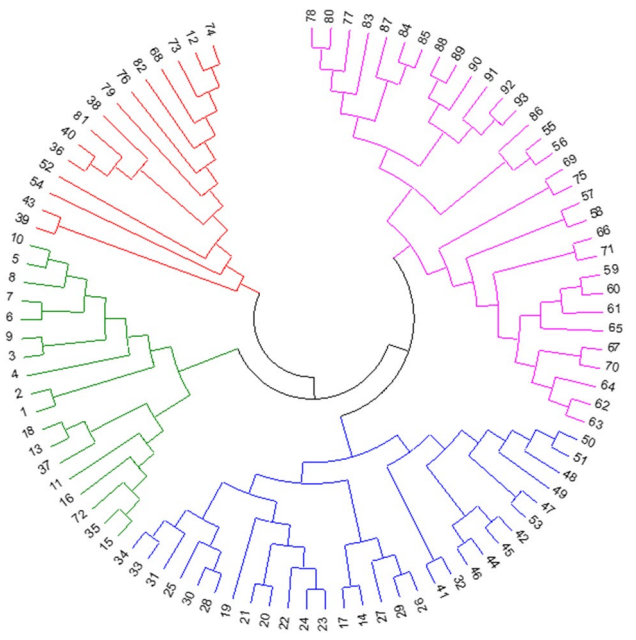

Fig. 2 Fan-dendrogram generated by neighbor joining based clustering of 93 Flax accessions using Jaccard's similarity coefficients according to the combined (SCoT + URP) data. The accession codes, are presented in Table 1

Population structure and pattern of classification

In analyzing population structures, through SCoT data, maximal ΔK value occurred at $K = 3$, indicating that the 93 accessions should be categorized into 3 sub-populations (Fig. 6). Accordingly, membership by each sample had a threshold of ≥ 0.5 in each subpopulation. According to SCoT values, 22, 31, and 25 accessions formed the first, second, and third subpopulations, respectively, and fifteen accessions (16%) were not included in any of the subpopulations. Population structures were analyzed through URP values, showing a maximal peak of $K = 4$ and the occurrence of four subpopulations (Fig. 7). An optimal subpopulation count was revealed by maximal ΔK values via a structure harvester. Thresholds of membership per sample in each subpopulation were described as ≥ 0.5, along with the analysis of SCoT data. Bar plotting the models were clustered upon 28 genotypes (30.1%) and attributed to sub-population 1. Subpopulation 2 comprised 14 accessions (15.1%), including *elongatum* subspecies. Subpopulation 3 had 15 accessions (16.1%). Twenty-seven accessions (29%) were clustered into the fourth subpopulation. Nine accessions (9.7%) were not included in any of the subpopulations.

Discussion

The knowledge of genetic variation in flax and analysis of plant variation as intra- and inter-populations of flax is essential for sampling genetic resources when adopting suitable programs for the development of unprecedented varieties and for continuing germplasm conservation. In the case of linseed, different studies have been reported on evaluating plant variations and the relationships that exist between using different molecular markers and morphological characteristics (Fu et al. 2002; Cloutier et al. 2010; Uysal et al. 2010; Patial et al. 2019; Hoque et al. 2020; Ghobadi-Namin et al. 2021; Thakur et al. 2021). However, the current study failed to discover applications of gene-specific marker molecules for the evaluation of flax diversity. Here, twenty SCoT and URP primers were applied when analyzing plant variation to recognize different populations, comprising 93 flax accessions. SCoT marker as a gene-targeted marker is a reliable marker to assess genetic diversity in plant germplasms. These markers are diagnostic markers with many important advantages such as more reproducibility compared to arbitrary markers, low cost and convenient applications in lab settings, direct link with loci and alleles that are important, with acceptable polymorphism across plants (Collard and Mackill 2009; Talebi et al. 2018).

The number of polymorphic fragments generated by these 20 primers span between 7 and 16, averaging 10.1 bands for each primer (10.9 bands per SCoT primer and 9.3 bands per URP primer). This mean value in polymorphic fragments for each primer happened to be higher than those reported in other evaluations of plant variations within flax populations (Fu et al. 2002; Bhawna et al. 2017; Chandrawati et al. 2017). The higher number of polymorphic fragments could have resulted from largely different genotypes and suitable genome coverage of the markers used in this study. The average values of PIC for SCoT primers (0.35) and URP primers (0.34) were found to be higher than some other applications of SCoT and URP molecular markers to investigate the genetic diversity in different species including durum wheat (Heidari et al. 2017), *Trigonella foenum-graecum* (Daneshmand et al. 2017), *Papaver bracteatum* (Qaderi et al. 2019), and chickpea (Pakseresht et al. 2013). The PIC index depends on the frequency of alleles and reveals the discriminatory ability of the used primers to produce informative fragments. Regarding the maximum amount of PIC for dominant markers, (0.5), the average values of PIC in the present study confirmed the efficiency of SCoT and URP markers for further genetic diversity analyses in Flax and other plants.

In both SCoT and URP analysis, the investigated accessions were clustered relatively based on the sub-species

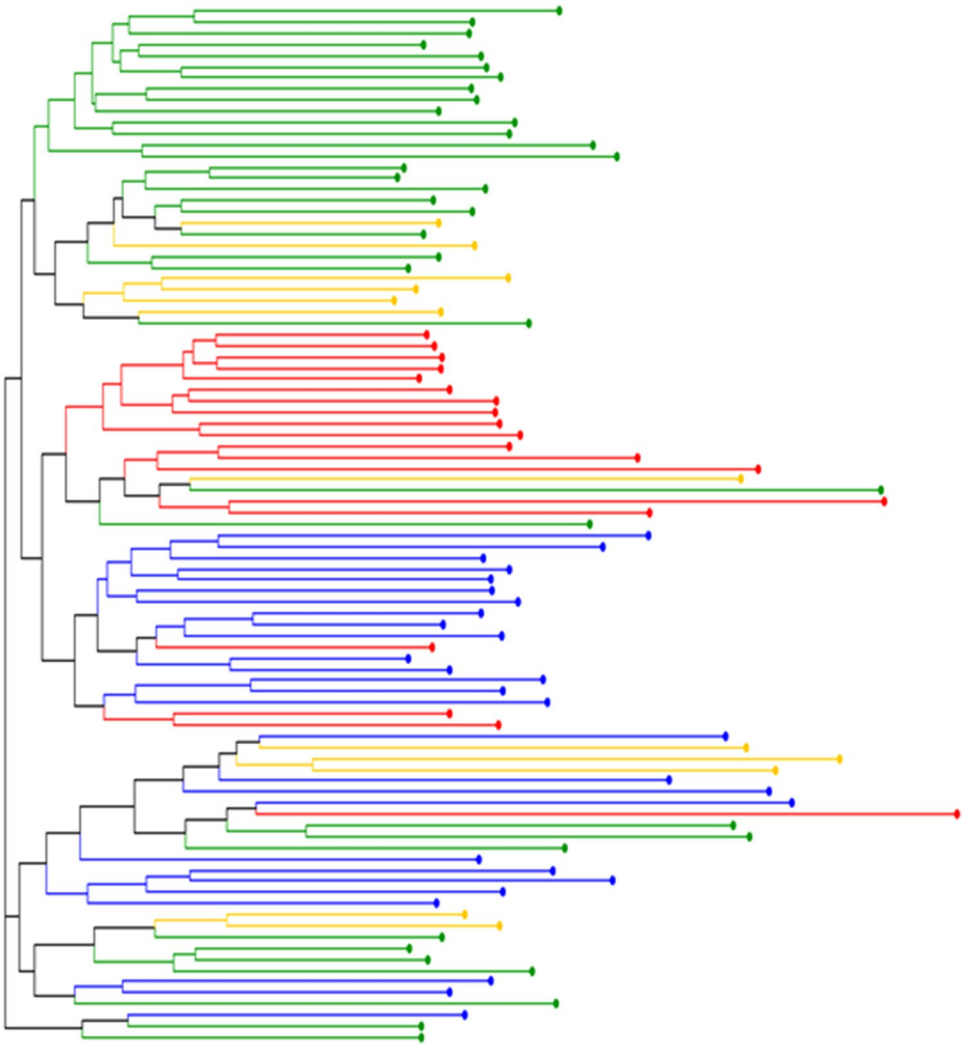

Fig. 3 Classification of accessions based on their genomic constitution (The accessions of unknown, elongatum, usitatissimum and mediterranian subpopulations are shown in red, blue, green and yellow colors, respectively)

with amounts of admixtures. The flax accessions based on SCoT and URP analyses were classified into three and four clusters, respectively. The URP marker data grouped the flax accessions exactly according to the four sub-species. This indicated that the URP marker was more useful to classify the flax accessions than the SCoT marker. In addition, cluster analysis using combined data revealed a clearer classification of accessions not only based on their sub-species but also approximately according to the place where they were collected. For example, as shown in Fig. 2, about 70% of the accessions collected from Iran were clustered together in a distinct group and this group was consisting of 18 accessions of which 17 accessions were collected from Iran. These results suggested significant associations between genetic and geographical distances. Also, these findings might be due to the presence of local adaptation. Similar results were reported in the study of Uysal et al. (2010) who reported significant associations not only between genetic and geographical distances but also between genetic distances and elevation differences in the assessment of genetic diversity of different species of flax using ISSR markers.

In this study, 93 Flax accessions showed a high level of genetic diversity (the average of dissimilarity calculated based on Jaccard's coefficient were 0.65 and 0.58 using SCoT and URP markers data respectively). The wide range of genetic distances between accessions (0.12 to 0.91 and 0.23 to 0.89 based on URP and SCoT data, respectively) suggested diverse accessions of Flax. However, further complementary genetic analysis on these accessions is required to verify our findings. However, this result was not in agreement with Fu et al. (2002), so these authors in the assessment of genetic diversity in a set of Flax genotypes using RAPD markers, reported a low level of genetic diversity in evaluated cultivars and landraces of Flax. These contradictions could be due to different reasons such as the type of DNA marker system, genome coverage of selected primers, origins of genetic materials, and the number of investigated genotypes. The result of the genetic diversity analysis of Flax accessions collected from different regions of Turkey

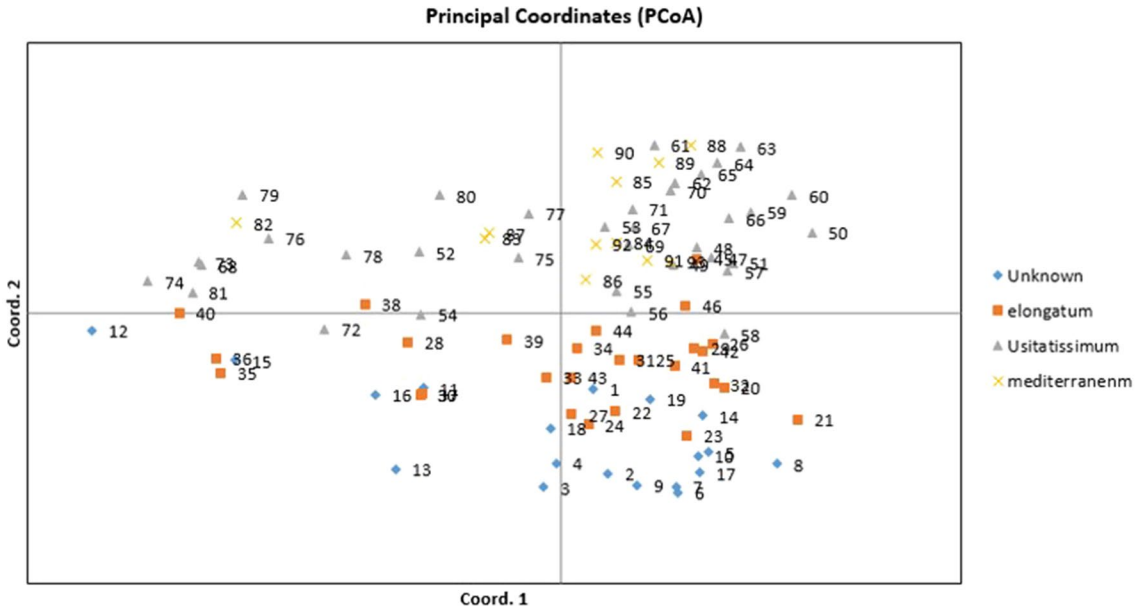

Fig. 4 Principal coordinate analysis (PCoA) plot for 93 Flax accessions based on Nei's genetic distances using combined data (SCoT + URP). Coord. 1 and Coord. 2 are the first and second principal coordinate

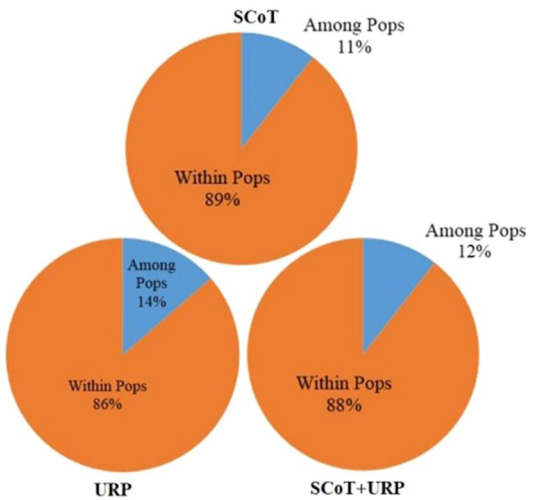

Fig. 5 Distribution of molecular variance as per AMOVA of SCoT, URP and combined (SCoT + URP) data from 93 Flax accessions

using ISSR markers (Uysal et al. 2010) was in agreement with our results. Besides, in the other study, the genetic variation in a set of 220 Flax (*Linum usitatissimum* L.) accessions was evaluated based on agro-morphological traits and revealed high genetic variability among Flax accessions (Saroha et al. 2022). It seems that the high level of genetic variation in this core collection was connected to the frequency of allelic variation which is affected by different climatic conditions (Nowak et al. 2020). The direct impacts of climate change on a decrease in diversity of those alleles controlling plant responses to climate changes have been reported in earlier studies (Jump and Penuelas 2005). From the genetic structure point of view, the high levels of genetic diversity provide ample opportunity to create new favorite varieties. Owing to the lack of genetic information on some crop plants like Flax, in comparison with other crops such as wheat, barley, and maize, the characterization and utilization of diverse germplasms of Flax is needed to enhance the genetic resources for breeding programs.

Furthermore, the results of the analysis of molecular variance (AMOVA) based on combined data, indicated 88% of the genetic variation resided within the populations and 12% was due to differences among the four populations, suggesting the presence of specific alleles within the populations (Shaygan et al. 2021). This finding represents that the studied populations consist of unique individuals and the accessions in each population have a wide genetic differentiation. In an assessment of genetic diversity in linseed (*Linum usitatissimum* L.) using SSR markers it was noticed that 79% of the total genetic variation was within the population and 21% was among the population (Chandrawati et al. 2017). These findings not only allow for the partitioning of the total molecular variation into within- and between populations components but also revealed good efficiency of URP and SCoT markers regarding genome coverage to detect the genetic diversity among and within populations. Higher distribution of genetic diversity within populations in comparison to among populations has been reported based on the AMOVA in different evaluations of genetic diversity using molecular markers (Feng et al. 2015, 2016; Chandrawati et al. 2017; Etminan et al. 2019; Ghobadi et al.

Table 3 Estimated genetic parameters in four populations of flax based on SCoT and URP data

Marker	Population	Sample size	Ne	I	He	PPL%
SCoT	Unknown	19	1.40 ± 0.03	0.36 ± 0.03	0.24 ± 0.01	78.9 ± 4
	Elongatum	27	1.48 ± 0.03	0.44 ± 0.03	0.29 ± 0.01	90.8 ± 4
	Usitatissimum	35	1.49 ± 0.03	0.44 ± 0.03	0.29 ± 0.01	92.6 ± 4
	Mediterranian	12	1.45 ± 0.03	0.39 ± 0.03	0.26 ± 0.01	77.1 ± 4
URP	Unknown	19	1.54 ± 0.03	0.45 ± 0.02	0.30 ± 0.02	84.2 ± 3
	Elongatum	27	1.53 ± 0.03	0.45 ± 0.02	0.30 ± 0.02	89.4 ± 3
	Usitatissimum	35	1.52 ± 0.03	0.43 ± 0.02	0.29 ± 0.02	84.2 ± 3
	Mediterranian	12	1.51 ± 0.03	0.42 ± 0.02	0.28 ± 0.02	74.7 ± 3
Combined (SCoT + URP)	Unknown	19	1.47 ± 0.02	0.40 ± 0.01	0.27 ± 0.01	81.3 ± 3
	elongatum	27	1.51 ± 0.02	0.44 ± 0.01	0.30 ± 0.01	90.2 ± 3
	Usitatissimum	35	1.50 ± 0.02	0.43 ± 0.01	0.29 ± 0.01	88.7 ± 3
	Mediterranian	12	1.48 ± 0.02	0.41 ± 0.01	0.27 ± 0.01	75.9 ± 3

Ne, I, He and PPL indicate the number of effective alleles, Shannon's information index, Nei's genetic diversity and percentage polymorphism loci, respectively

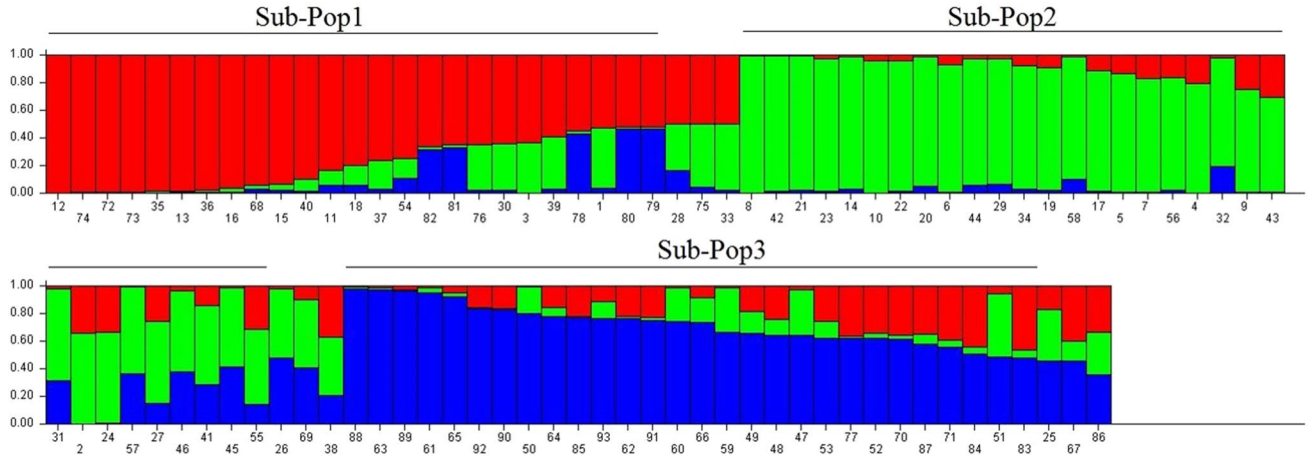

Fig. 6 Population structure of 93 Flax accessions based on SCoT markers performed in STRUCTURE for $K = 3$

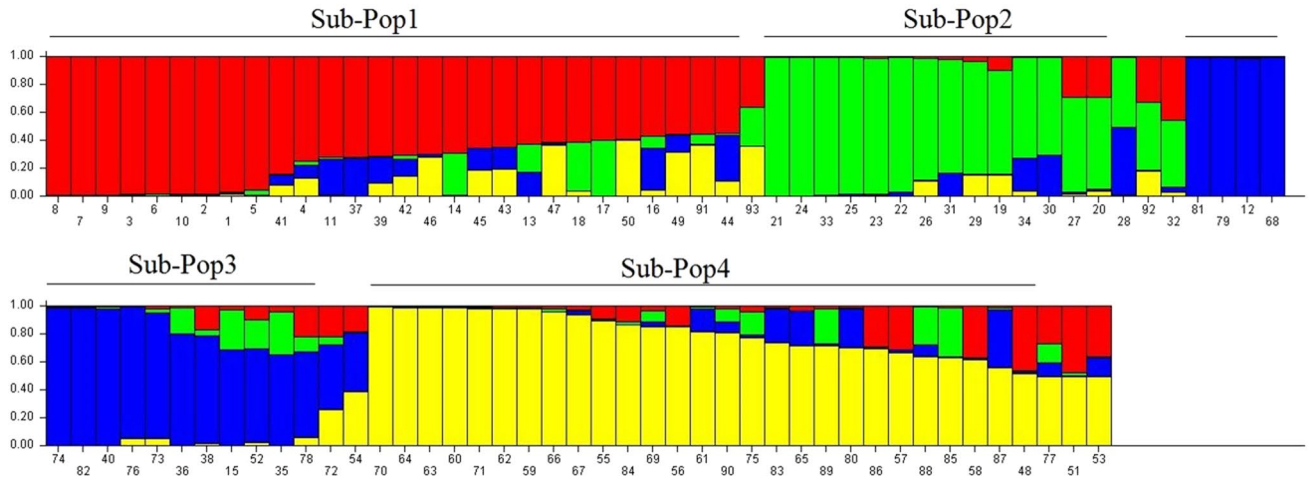

Fig. 7 Population structure of 93 Flax accessions based on URP markers performed in STRUCTURE for $K = 4$

2021; Khodaee et al. 2021; Shaygan et al. 2021). The results obtained from the STRUCTURE analysis were in agreement with those obtained from the NJ clustering and PCoA. In a comparison of SCoT and URP markers, both molecular markers showed an acceptable efficiency to detect polymorphism and genetic relationships in Flax accessions, indicating these DNA markers can be used as reliable techniques for analyzing genetic diversity. SCoT primers showed good amplification and polymorphism with an acceptable average of PIC and MI values. This finding is in accordance with those of the previous studies that reported good efficiency of SCoT markers to study the genetic diversity in different plant species (Collard and Mackill 2009; Pakseresht et al. 2013; Etminan et al. 2016; Bhawna et al. 2017; Pour-Aboughadareh et al. 2018; Feng et al. 2018; Mishra et al. 2019; Gogoi et al. 2020; Zhao et al. 2020; Mostafavi et al. 2021). Although SCoT primers generated more polymorphic bands than URP primers, URP markers provided a better clustering pattern which was more similar to clustering based on combined data and in agreement with STRUCTURE analysis for evaluated samples. The efficiency of URP primers for fingerprinting and estimating genetic diversity in different species were reported in earlier studies (Jana et al. 2005; Amirmoradi et al. 2012; Salahlou et al. 2019).

Conclusion

It is believed that climate change has affected agricultural products and treats food security in the world. Renewing the crop varieties is one of the effective strategies to adapt to these changes. Providing diverse genetic resources is crucial to improve new varieties with desirable characteristics. Moreover, maintaining genetic variation is important for adaptation to various biotic and abiotic environmental stresses. The study of genetic diversity in plant germplasms is an essential requirement for plant breeding programs and the efficient maintenance of genetic reservoirs and gene pools. Flax is a multipurpose crop grown in many regions for several different uses. The present study was conducted to evaluate the genetic diversity of a set of 93 Flax accessions belonging to different subspecies. The results revealed a high level of genetic diversity in the collected Flax germplasm and clustering the accessions based on SCoT and URP markers, showed a significant association between genetic and geographical distances. Besides, different populations were consisting of diverse accessions with specific alleles, suggesting the potential of these genetic materials for future Flax genetic improvement programs. Our findings also confirmed that both URP and SCoT techniques are useful and reliable markers for the detection of genetic diversity and relationships in flax germplasm. Whereas molecular markers may or may not show a correlation with our desirable traits, the use of gene-targeting markers like SCoTs which are generated from the functional regions of the genomes would be more useful for the evaluation of genetic diversity and elucidation of population structure in breeding programs.

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Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate This article does not contain any studies with human participants or animals performed.

Consent for publication Not applicable.

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