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Assessing the suitability of SCoT markers for studying genetic variation and genetic structure of *Lepidium* species

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ABSTRACT

Lepidium spp. (Brassicaceae) are herbaceous plants grown worldwide and considered as vegetable, phytofood or medicinal plants. The genetic variation and genetic structure of 22 *Lepidium* (L.) accessions representing 3 species (*L. sativum*, *L. draba* and *L. latifolium*) from Iran were assessed using 14 Start Codon Targeted (SCoT) markers. A high polymorphism (98.4%), polymorphic information content (0.35) and polymorphic bands (4.5) indicated that SCoT markers are reliable for genetic variation analysis in *Lepidium* spp. Mean values of resolving power (Rp), marker index (MI) and effective multiplex ratio (EMR) were 5.0, 1.6 and 4.4, respectively. The highest percentage of polymorphic loci (92.2%), Nei's gene diversity (0.35) and Shannon index (0.51) were observed in *L. sativum*. According to analysis of molecular variance (AMOVA), genetic variation within species was higher than between species. The highest similarity was found between *L. draba* and *L. latifolium* (r=0.94). A high level of gene flow was estimated in accessions of *Lepidium* species (Nm=2.65), which is further confirmed by neighbor-joining (NJ) cluster analysis, principal coordinates analysis (PCoA) and STRUCTURE analysis, that could reveal a poor separation between *Lepidium* species. NJ cluster analysis divided the *Lepidium* accessions into three groups, and the grouping of accessions was generally consistent with their origins. This study is the first to explore and prove SCoT markers suitability in genetic diversity of *Lepidium* spp. The genetic analysis information provided here would be helpful for breeding programs and germplasm conservation in *Lepidium* species.

Key words: Accession, Brassicaceae, Cluster analysis, Genetic diversity, Molecular markers.

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INTRODUCTION

Lepidium is one of the largest genera in the mustard family (Brassicaceae), which is distributed in temperate and subtropical regions of the world (Bona, 2014). The genus *Lepidium* comprises about 150 annual, perennial herb or undershrub species (Ma *et al.,* 2020; Ramadan and Oraby, 2020). Iran, having nine types of ecosystems out of 11 ecosystems defined in the world, has a rich biodiversity of plant species (Azizi Jalilian *et al.,* 2020; Fallahi *et al.,* 2020; Shamsolshoara *et al.,* 2020), such that 18 species of this genus have been reported in Iran (Nasseh and Joharchi, 2019). Some *Lepidium* species are used as medicinal plants, phytofoods or vegetables. In addition, species of this genus are important for their therapeutic properties such as antibacterial, antifungal, antioxidant, cytotoxic, diuretic, antihypertensive, hepatoprotective, hypoglycemic, antiosteoporotic, antiasthmatic, cardiotonic, and hemagglutinating activities (Kaur *et al.,* 2013; Mahomoodally *et al.,* 2018; Roughani and Miri, 2018; Baregama and Goyal, 2019).

Plant genetic diversity is one of the critical factors contributing to food security and agricultural production, because plant germplasm carry genes that can improve quantity and quality of the crops, and their adaptation to biotic and abiotic stresses (Govindaraj *et al.,* 2015; Pathirana and Carimi, 2022). Therefore, estimating genetic diversity is very critical for the efficacious use of germplasm for the development of new varieties by plant breeders (Zhang *et al*., 2016; Roughani *et al*., 2018a and 2021). Genetic diversity is generally evaluated using molecular markers, which can identify the variation at the DNA level to differentiate genotypes or species (Velasco-Ramírez *et al.,* 2014). They are very important and effective tools in plant breeding programs, such as genome mapping, genetic diversity and phylogenetic analysis, due to their stability, cost-effectiveness and ease of use. Since the 1980s, a large number of molecular markers have been developed and successfully applied for the improvement of agricultural crops (Grover and Sharma, 2016; Nadeem *et al.,* 2018; Jelvehgar *et al.,* 2021). Start codon targeted (SCoT) markers are one of the novel, simple, reliable and reproducible markers based on short conserved region surrounding the ATG start codons. SCoT markers employ long primers (18 bp) and require no sequence information. The short flanking regions of the ATG start codon are also highly conserved across plant species. These have led to its widespread use in quantitative trait locus (QTL) mapping, marker-assisted breeding, bulked segregate analysis, phylogenetic analysis and genetic variation (Collard and Mackill, 2009; Sankhla *et al.,* 2015).

Several studies have been carried out on determining the genetic diversity of *Lepidium* species using different systems of molecular markers such as RAPD (Kumar *et al*., 2012; Bansal *et al*., 2013; Singh *et al.,* 2020; Mortazavi Moghadam *et al.,* 2021), SSR (Tadesse *et al*., 2018; Jelvehgar *et al.,* 2021), ISSR (Mohammed and Tesfaye, 2015; Kumar and Yadav, 2019; Singh *et al.,* 2020; Jelvehgar *et al.,* 2021) or AFLP (Robin *et al*., 2014). However, to the best of authors' knowledge, genetic diversity of *Lepidium sp.* by SCoT markers has not been studied previously. The present study aims to determine the efficiency of SCoT markers in studying *Lepidium* genetic diversity, which can be useful for managing the conservation of *Lepidium* germplasm or breeding programs.

MATERIALS AND METHODS

Plant materials

Twenty-two *Lepidium* spp. accessions were analyzed in this study using 14 SCoT markers. The seeds of *L. draba* and *L. latifolium* accessions were received from the Research Institute of Forests and Rangelands (RIFR), and *L. sativum* accessions from Iranian Biological Resource Center (IBRC), Tehran, Iran. Two accessions of *L. sativum* were also provided from local farmers (Table 1). Seedlings were grown in pots in a greenhouse, and leaves of 10-day old seedlings (10 plants for each accession) were harvested.

DNA extraction

The DNA was extracted from 0.1 g fresh young leaves by the DNA–easy Plant Mini kit (Qiagen, USA, Cat. no. 69104), and its purity and concentration were estimated by running on 0.7% agarose gel electrophoresis analysis and NanoDrop, respectively.

SCoT markers

Fourteen primers, developed by Collard and Mackill (2009), were employed (Table 2). PCR reaction mixtures contained 1 µl template DNA (20 ng/µl), 5 µl master mix 2×PCR buffer, and 1 µl of primers (10 pmol/µl) in a total volume of 10 µl. PCR amplification was carried out using a Thermal cycler (Corbett CGI-96 Palm-Cycler, USA) using the following thermal profile: 94 °C for 5 min, 35 cycles of 95 °C for 60 s, 47.0-54.7 °C (depends on the primer) for 60 s, and 72 °C for 120 s, and a final extension at 72 °C for 5 min. PCR products were fractionated on 1.5% agarose gels in 1×TAE buffer and stained with 0.8 µg/ml ethidium bromide for 10 min.

Table 1. Geographical coordinates of the 22 accessions belonging to three *Lepidium* species used in this study.

Code number	Species	Country, City	Latitude	Longitude	Accession no./Herbarium code
1	*L. latifolium*	Yazd, Khatam	30°07′35′′N	53°59′64′′E	33845
2		Yazd, Tabas	33°21′35′′N	57°20′32′′E	33828
3		Yazd, Tabas	33°49′22′′N	56°52′34′′E	33760
4		Yazd, Tabas	33°37′57′′N	57°09′46′′E	33780
5		Yazd, Mehriz	31°30′23′′N	54°18′28′′E	33813
6		Isfahan, Shahreza	31°40′67′′N	51°47′25′′E	27262
7		Yazd, Taft	31°34′43′′N	53°54′41′′E	33678
8	*L. draba*	Kohgiluyeh and Boyer–Ahmad, Yasuj	30°58′19′′N	51°13′11′′E	31214
9		Hamedan, Hamedan	34°48′10′′N	48°28′54′′E	43699
10		South Khorasan, Sarbisheh	36°08′90′′N	72°06′00′′E	41618
11		Ardabil, Nir	38°01′25′′N	47°57′67′′E	36834
12		Hamedan, Asadabad	34°50′15′′N	48°10′18′′E	33169
13		South Khorasan, Khusf	36°39′06′′N	68°72′45′′E	44485
14		Markazi, Saveh	35°06′30′′N	49°40′52′′E	35418
15	*L. sativum*	Isfahan, Isfahan	32°40′22′′N	51°39′55′′E	P1012484
16		Tehran, Tehran	35°35′56′′N	51°26′09′′E	P1013473
17		East Azarbaijan, Tabriz	38°04′18′′N	46°17′32′′E	–
18		Razavi Khorasan, Mashhad	36°14′55′′N	59°39′16′′E	P1012230
19		Markazi, Arak	34°05′15′′N	49°42′10′′E	P1012440
20		Guilan, Rasht	32°01′28′′N	49°53′07′′E	P1012276
21		Qazvin, Qazvin	36°16′40′′N	50°00′26′′E	P1012324
22		West Azarbaijan, Urmia	37°32′43′′N	45°03′29′′E	–

Table 2. The primer sequences, annealing temperatures, and attributes of banding pattern recognized by SCoT primers in the 22 accessions of *Lepidium* spp.

Primer	Sequence (5'→3')	Ta (°C)	TB	PB	P (%)	PIC	Rp	MI	EMR
SCoT-02	CAACAATGGCTACCACCC	48.9	3	3	100	0.29	2.72	0.87	3
SCoT-03	CAACAATGGCTACCACCG	49	5	5	100	0.34	4.10	1.69	5
SCoT-05	CAACAATGGCTACCACGA	47	3	3	100	0.40	3.30	1.20	3
SCoT-06	CAACAATGGCTACCACGC	49	8	8	100	0.37	7.10	2.96	8
SCoT-12	ACGACATGGCGACCAACG	49	6	6	100	0.43	5.90	2.58	6
SCoT-14	ACGACATGGCGACCACGC	54.7	4	4	100	0.38	2.40	1.52	4
SCoT-15	ACGACGTGGCGACCGCGA	54.7	5	5	100	0.29	5.00	1.47	5
SCoT-16	CCATGGCTACCACCGGCC	54.7	4	4	100	0.33	5.00	1.33	4
SCoT-17	CATGGCTACCACCGGCCC	54.7	4	4	100	0.37	5.90	1.49	4
SCoT-18	ACCATGGCTACCACCGCC	54.7	6	5	83.3	0.32	7.70	1.38	4.31
SCoT-19	GCAACAATGGCTACCACC	49	5	5	100	0.41	7.80	2.05	5
SCoT-20	AACCATGGCTACCAACGC	49	4	4	100	0.38	4.50	1.52	4
SCoT-21	CACCATGGCTACCACCAT	49	2	2	100	0.26	5.20	0.52	2
SCoT-39	CAATGGCTACCACTAGCG	47	5	5	100	0.36	3.60	1.80	5
Mean			4.57	4.50	98.4	0.35	5.07	1.59	4.47

Ta: Temperature of annealing, TB: Total bands, PP: Polymorphic bands, P (%): Polymorphic percentage, PIC: Polymorphism information content, Rp: Resolving power, MI: Marker index, EMR: Effective multiplex ratio.
A: Adenine, T: Thymine C: Cytosine, G: Guanine.

Statistical analysis

The amplified DNA fragments were scored as present (1) or absent (0) of bands to form a binary matrix. The attributes of banding pattern, genetic indices, Nei's genetic distance, the coefficient of genetic differentiation among populations (PhiPT) and principal coordinate analysis (PCoA) were determined using GenAlEx 6.5 software. Analysis of molecular variance (AMOVA) was carried out to analyze the genetic differentiation (Gst) and gene flow (Nm)

among species in POPGENE 1.32 software.

The neighbor-joining (NJ) clustering dendrogram based on Jaccard's similarity coefficient was constructed using DARwin 5 software. NTSYS software was used to check the goodness fit of each NJ clustering to the genetic similarity matrix by measuring cophenetic correlation coefficient.

The population structure was estimated in the STRUCTURE 2.3.4 software using admixture model, correlated allele frequencies, and a burn-in time of 100,000 iterations, followed by 100,000 Markov Chain Monte Carlo (MCMC) replications (Evanno *et al.,* 2005). The value of K ranged from 1 to 5, with 10 independent runs. The log probability of the data (LnP(K)) and delta K (ΔK) were used to identify the optimum number of subpopulations. The structure results were analyzed by Structure Harvester 6.0 program.

RESULTS

Analysis of SCoT markers

In 22 *Lepidium* spp. accessions, 14 SCoT primers amplified 65 amplicons. The number of amplicons varied from 2 (SCoT-21) to 8 (SCoT-6) per primer with an average of 4.57 amplicons per primer. All markers except for SCoT-18 (83.3%) were 100% polymorphic. Among different SCoT markers, SCoT-12 showed the maximum PIC value (0.43) and SCoT-19 produced the maximum value of Rp (7.80). Higher MI (2.96) and EMR (8) values were detected for SCoT-06 (Table 2).

Genetic diversity analysis

The SCoT-based AMOVA analysis revealed that 12% of the genetic variance attributed to among species (PhiPT=0.12; $p<0.001$), whereas 88% of the variation occurred within species (Figure 1). The Gst/Nm among species was 0.16/2.65.

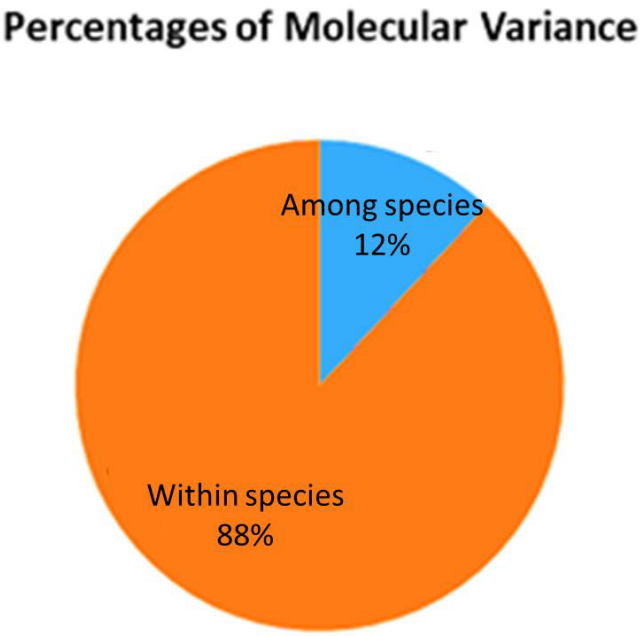

Figure 1. Analysis of molecular variance based on the SCoT markers for 22 accessions of three *Lepidium* species.

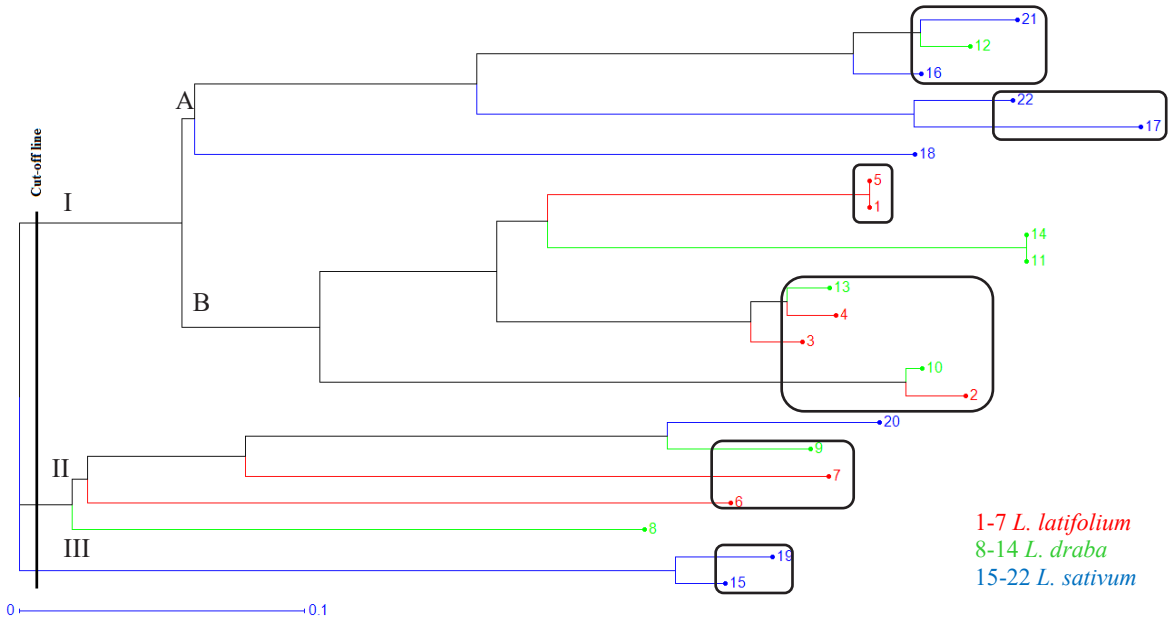

Figure 2. The NJ tree grouping of the 22 accessions of *Lepidium* spp., based on the SCoT markers. Numbers correspond to accessions details given in Table 1. The accessions within the box are located in the same geographical or ecological location.

SCoT-based AMOVA analysis showed that 12% of the genetic variation was accounted for among species variation (PhiPT=0.12; p<0.001), while 88% of variation occurred within species.

High diversity indices (Na, Ne, I, He and PPL) were observed in *L. sativum* with the values of 1.91, 1.61, 0.51, 0.35, and 92.2, respectively, while, the lowest values were found in *L. latifolium*, with the values of 1.58, 1.40, 0.35, 0.23 and 70.31, respectively (Table 3).

Pairwise population matrix of Nei's unbiased genetic identity ranged from 0.82 (*L. sativum* versus *L. latifolium*) to 0.94 (*L. draba* versus *L. latifolium*) (Table 4), which was later supported by NJ clustering, PCoA and population structure. In addition, Nei's unbiased genetic identity among accessions of *Lepidium* species ranged from 0.56 (between *L. latifolium* (Tabas-33828) and *L. sativum* (Arak)) to 1.00 (between *L. latifolium* (Khatam) and *L. latifolium* (Mehriz), and *L. draba* (Nir) with *L. draba* (Saveh) (Table 5).

Cluster analysis

Results of the cluster analysis revealed that accessions could be separated into three main groups (Figure 2). In group I, fifteen accessions of each *Lepidium* species were presented. Five accessions of *L. sativum* and one accession of *L. draba* were placed in the subgroup I–A and, the other nine accessions (four accessions of *L. draba* and five accessions of *L. latifolium*) were placed in subgroup I–B. One accession of *L. sativum* and two accessions of each *L. latifolium* and *L. draba* species were placed in group II. The third group only contained two accessions of *L. sativum*. The cophenetic correlation coefficient between the NJ dendrogram and the genetic similarity matrix showed a good fit for SCoT markers (r=0.92).

Table 3. Genetic diversity indices for each *Lepidium* species identified by SCoT markers.

Species	Na	Ne	H	I	PPL
L. sativum	1.91	1.61	0.35	0.51	92.19
L. draba	1.75	1.49	0.29	0.43	82.81
L. latifolium	1.58	1.40	0.23	0.35	70.31
Mean	1.74	1.50	0.29	0.43	81.77

Na: Number of observed alleles, Ne: Number of effective alleles, H: Nei's gene diversity index, I: Shannon's information index, PPL: Percentage of polymorphic loci.

Table 4. Nei's genetic identity between *Lepidium* species.

Species	*L. latifolium*	*L. draba*
L. draba	0.94	
L. sativum	0.82	0.88

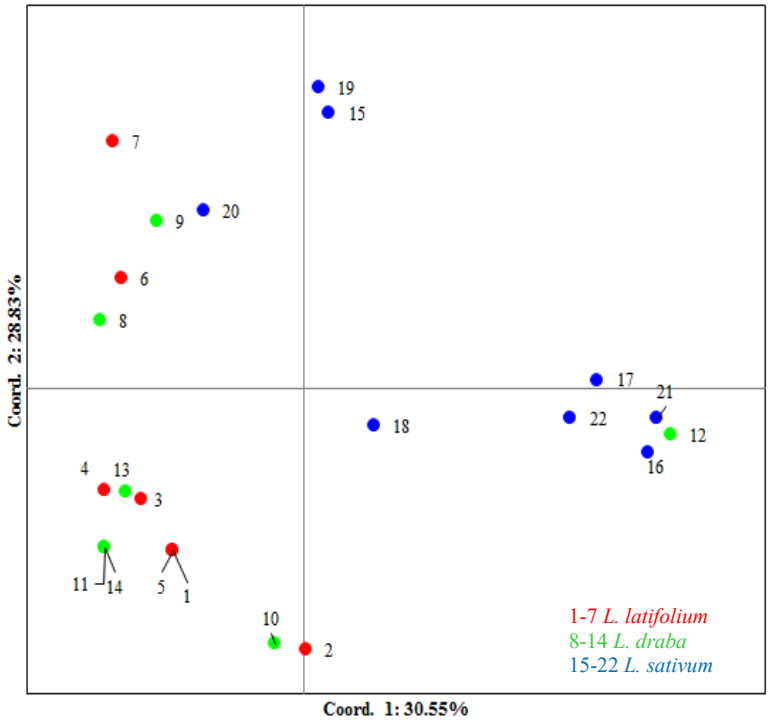

Figure 3. PCoA of the 22 accessions of *Lepidium* spp., based on the SCoT markers. Numbers correspond to accessions details given in Table 1.

Table 5. Nei's genetic identity among the accessions of *Lepidium* species.

Accession	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
2	0.87																				
3	0.94	0.84																			
4	0.94	0.84	0.98																		
5	1.00	0.87	0.94	0.94																	
6	0.75	0.73	0.73	0.76	0.75																
7	0.72	0.65	0.76	0.76	0.72	0.84															
8	0.81	0.73	0.87	0.85	0.81	0.81	0.84														
9	0.78	0.69	0.81	0.79	0.78	0.76	0.87	0.80													
10	0.88	0.99	0.85	0.85	0.88	0.75	0.67	0.75	0.68												
11	0.92	0.81	0.90	0.92	0.92	0.78	0.71	0.83	0.75	0.83											
12	0.75	0.78	0.73	0.71	0.75	0.64	0.64	0.69	0.68	0.77	0.68										
13	0.93	0.83	0.99	0.99	0.93	0.75	0.75	0.86	0.80	0.84	0.91	0.72									
14	0.92	0.81	0.90	0.92	0.92	0.78	0.71	0.83	0.75	0.83	1.00	0.68	0.91								
15	0.72	0.59	0.73	0.76	0.72	0.75	0.77	0.79	0.76	0.61	0.68	0.72	0.75	0.68							
16	0.75	0.80	0.76	0.73	0.75	0.64	0.64	0.69	0.68	0.79	0.68	0.98	0.75	0.68	0.72						
17	0.73	0.72	0.72	0.69	0.73	0.65	0.62	0.68	0.72	0.71	0.75	0.85	0.71	0.75	0.71	0.83					
18	0.77	0.73	0.76	0.78	0.77	0.72	0.64	0.67	0.65	0.75	0.73	0.77	0.77	0.73	0.75	0.77	0.62				
19	0.72	0.56	0.73	0.76	0.72	0.75	0.77	0.77	0.76	0.58	0.68	0.69	0.75	0.68	0.98	0.69	0.73	0.72			
20	0.73	0.72	0.79	0.77	0.73	0.73	0.85	0.78	0.97	0.71	0.69	0.68	0.78	0.69	0.73	0.68	0.72	0.65	0.76		
21	0.75	0.76	0.73	0.71	0.75	0.67	0.67	0.69	0.68	0.75	0.68	0.98	0.72	0.68	0.69	0.97	0.85	0.75	0.69	0.68	
22	0.78	0.75	0.77	0.75	0.78	0.65	0.65	0.71	0.72	0.73	0.75	0.87	0.76	0.75	0.73	0.85	0.97	0.68	0.73	0.69	0.87

Numbers correspond to accessions details given in Table 1.

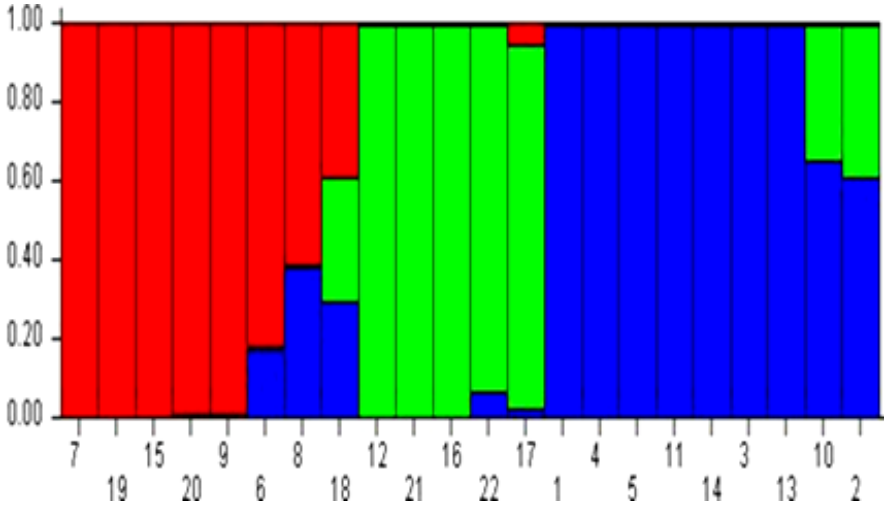

Figure 4. Population structure of the 22 accessions of *Lepidium* spp. based on the SCoT markers. Numbers correspond to accessions details given in Table 1.

Principal coordinate analysis

Similar to NJ clustering, all *L. draba* (except No. 12) and *L. latifloium* accessions were clustered together in two groups, while most *L. sativum* accessions (except No. 15, 18, 19, 20) were clustered into a distinct group (Figure 3). PCoA indicated that 59.38% of the total variation accounted for the first two coordinates, with PCo1 accounting for 30.55% and PCo2 for 28.83%.

Genetic structure analysis

The most suitable subpopulations based on the highest ΔK and L(K) values were found to be K=3. The subpopulation 1 (red), 2 (green) and 3 (blue) included 8, 5 and 9 accessions, respectively (Figure 4).

DISCUSSION

SCoT polymorphisms

Evaluation of variability among *Lepidium* species provide opportunities for understanding the value of germplasm resources to develop breeding programs (Kumar and Yadav, 2019; Roughani *et al.,* 2018b). SCoT marker is a novel gene targeted marker system that have potential in plant genotyping (Sankhla *et al.,* 2015). To our knowledge, this study is the first successful effort on the use of SCoT markers to characterize the genetic variation and population structure of the *Lepidium* spp. In the present investigation, 14 SCoT primers were screened in studying the genetic variation of *Lepidium* species. The mean values for polymorphism percentage (98.80%) and PIC (0.35) determined by SCoT markers were much higher than the values of previous studies employing RAPD, SSR or ISSR markers (Bhalala *et al.,* 2016; Kumar and Yadav, 2019; Singh *et al.,* 2020). These findings indicate that it is possible to identify a high level of polymorphism among *Lepidium* species using SCoT markers. Plant species at the center of their origin show high levels of genetic variation (Ramanatha Rao and Hodgkin, 2002). The *Lepidium* species are mostly distributed in Central, Middle and Southwest Asia, making them one of the centers of biodiversity and complexity of *Lepidium* L. (German, 2014). This could be a possible reason for the high rates of genetic variation of Iranian *Lepidium* accessions in our study.

To assess the discriminatory power of markers for genetic relationships, the polymorphism parameters such as PIC, Rp, MI and EMR are usually employed. The PIC value is commonly used to estimate the discriminating ability of a genetic marker, while Rp indicates the characterizing potential of a primer to detect differences between large numbers of genotypes. MI measures the total efficiency of a molecular marker in assessing polymorphism and the higher EMR value indicates that the primer–marker system is more efficient (Chesnokov and Artemyeva, 2015; Sandeep *et al.,* 2020). According to the classification of molecular markers based on PIC values mentioned by Botstein *et al.* (1980), the markers with PIC values of 0.25<PIC<0.5 are moderately informative. In our study, the PIC value ranged from 0.26 to 0.43, with an average value of 0.35, indicating that these primers are suitable to evaluate genetic diversity of *Lepidium* spp.

Genetic differentiation and gene flow

AMOVA analysis exhibited a higher genetic variation within species than among species. This may be related to the out-crossing nature of these species. Kumar and

Yadav (2019) found a higher genetic variation (67%) within population than among population across 94 Indian accessions of *L. sativum*. Several factors like mating system, gene flow, population size, selection, and reproduction method influence the extent and distribution of the genetic diversity within populations (Kumar and Yadav, 2019; Jelvehgar *et al.,* 2021).

The Gst value is considered as a very useful indicator for the proportion of gene diversity that is distributed among populations. According to Wright (1978), populations with Gst values of >0.15 has high levels of genetic differentiation. In our results, the Gst values was 0.16, indicating a high proportion of genetic differentiation among species. On the other hand, if Nm>1, the gene flow could prevent the differentiation among populations due to the genetic drift (Hutchison and Templeton, 1999). In this study, the Nm value was 2.65, implying the possibility of gene flow among the studied *Lepidium* species. The rate of gene flow depends on the population size, spatial isolation, mating system, and distribution of seeds or pollens between populations (Frye and Neel, 2017).

The genetic diversity indices such as PPL, Ne, H and I have been reported to be important parameters in assessing genetic diversity (Yang *et al.,* 2019). In the present study, higher values of these parameters were obtained in *L. sativum* followed by *L. draba* and *L. latifolium*. These results are in contrary to Roughani *et al.* (2018b) in which the highest variation was reported in *L. latifolium* accessions compared to *L. sativum* and *L. draba* using agro–morphological traits. Agro-morphological characters are influenced by the genetic and environmental factors, while DNA-based molecular markers detect variations in specific regions of DNA (Nadeem *et al.,* 2018). Therefore, these two independent sets of data reflect a different pattern of genetic diversity (Beyene *et al.,* 2005), and could be the reason why these results are contradictory. *L. sativum* is an annual plant while *L. draba* and *L. latifolium* are perennial herbs (Bona, 2014). In addition, *L. draba* has a sporophytic self-incompatibility system whereas *L. sativum* and *L. latifolium* are both self- and cross-pollinated plants (Roughani *et al.,* 2018b). The higher genetic variation in *L. sativum* or *L. draba* compared to *L. latifolium* might be attributed to the annual nature of *L. sativum* and the self-incompatibility of *L. draba*. These reproductive characteristics may have resulted in the rapid appearance of several new gene recombinations and increasing heterozygosity. Moreover, *L. sativum* is widely cultivated by farmers and has a relatively high distribution. Generally, the larger the distribution region, the higher the genetic variation (Yang *et al.,* 2019).

The values of Nei's unbiased genetic identity among *Lepidium* species or accessions indicated that *L. sativum* and *L. latifolium* have the greatest distance from each other, which was previously confirmed by morphological traits (Roughani *et al.,* 2018b), amount of nuclear DNA (Roughani *et al.,* 2021), and SSR and ISSR markers (Jelvehgar *et al.,* 2021). It is considered that crosses between distantly related accessions could lead to an increase in heterozygosity and the production of better offspring (Mohammed and Tesfaye, 2015; Kumar and Yadav, 2019).

Genetic relationships among accessions

According to the NJ clustering, the three *Lepidium* species were divided into three groups, which did not reveal any specific taxonomic grouping. However, the clustering patterns were associated with the geographical distribution of the accessions in most subclusters. To further distinguish the distributions of these species, we used PCoA and STRUCTURE analysis, resulting again in classification into three groups. The clustering pattern of 22 *Lepidium* accessions in NJ dendrogram was highly in agreement with the results of PCoA analysis. The STRUCTURE analysis also indicated the absence of a distinct genetic structure among the studied *Lepidium* species and accessions. These results indicate that there are certain variations in the short flanking regions of the ATG start codon of the accessions, which can be explained by a long-term adaptation process to edaphic and climate factors (Velasco-Ramírez *et al.,* 2014; Jelvehgar *et al.,* 2021). However, the placement of some accessions with different geographical origin or taxonomic classification in a group could be a result of sharing the allelic pool among them. The results are in line with the results of gene flow. The relationship between clustering patterns and geographical distribution in *L. latifolium* (Roughani *et al.,* 2018c) and *Lepidium* spp. (Jelvehgar *et al.,* 2021) has also been previously reported.

CONCLUSION

This study is the first attempt to demonstrate the capability of SCoT markers in determining the genetic variation in several accessions of three *Lepidium* species. Our results illustrated a high rate of genetic variation and gene flow in the accessions of *Lepidium* spp., and it was found that genetic variance mainly existed within the species. By screening the studied accessions with superior traits and high genetic variation, it is expected that some individuals will be

utilized in the strategies for breeding and conservation programs to develop or improved new varieties of *Lepidium* spp. to meet the market demand as vegetable or medicinal plant.

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