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GENETIC DIVERSITY ANALYSIS OF *Camelina sativa* LINES USING IPBS MARKERS: INSIGHTS INTO DIVERSITY, PRIMERS EFFICACY, AND CLUSTER ANALYSIS

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ABSTRACT

Camelina (*Camelina sativa* L.) is an oilseed plant valued for its low water and fertilizer needs, environmental adaptability, and rich fatty acid contents. In this study, genetic diversity among 16 camelina doubled haploid lines and the Soheil and Sepehr varieties was assessed using 15 inter-primer binding site (IPBS) retrotransposon primers to investigate their molecular characteristics. Fresh leaf samples were collected for DNA extraction using Dellaporta's method, followed by quantification and quality assessment using a NanoDrop and agarose gel electrophoresis. Polymerase chain reaction (PCR) was performed with IPBS retrotransposon primers. Amplified DNA fragments were separated via electrophoresis on an agarose gel and visualized under UV light. The study evaluated genetic variation among 18 doubled haploid lines of camelina using 15 IPBS retrotransposon primers, of which 14 produced scorable bands ranging from 200 to 5000 bp. A total of 325 bands were generated, with 83 showing polymorphism, resulting in an average polymorphism percentage of 25.41%. Primers IPBS (2076) and IPBS (2237) were the most effective, each yielding 10 polymorphic bands and high polymorphic information content (PIC) values of 0.30. The Jaccard genetic similarity matrix indicated moderate genetic diversity among the lines, with similarity values ranging from 0.4 to 0.9. Cluster analysis categorized the lines into four distinct groups, while principal coordinates analysis revealed that the first two components explained 78.99% of the total variation, corroborating the clustering results. Overall, the findings highlight the utility of IPBS markers in assessing genetic diversity in camelina lines, demonstrating their potential for future breeding programs. The scatter diagram generated from the principal coordinate analysis depicted the lines grouped into five clusters, showing some consistency with the cluster analysis results. The IPBS marker seems to be a suitable tool for assessing genetic diversity in camelina. The observed genetic diversity provides valuable insights for camelina breeding programs focused on developing cultivars with desirable traits.

Keywords: Camelina, doubled haploid, IPBS marker, molecular characteristic, PCR

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INTRODUCTION

Camelina (*Camelina sativa* L.), belonging to the Brassicaceae family, is known for its dual purpose as an oil-medicinal plant and an adaptable crop to divergent growing conditions. It is capable of flourishing in diverse soil types and climates, making it suitable for cultivation during both summer and winter, particularly in temperate regions (Kahrizi *et al*, 2015; Eskandarzadeh *et al*, 2024). Camelina is cost-effective, requiring less water, fertilizer, and pesticide than crops like sunflower, soybean, or canola (Fereidooni *et al*., 2024; Heidari and Hosseini, 2024)). Indeed, camelina demonstrates the lowest sensitivity among cruciferous plants to temporary soil water scarcity. Its cultivation is considered relatively straightforward and environmentally friendly compared to other crops (Zubr, 2003). Oil plants are significant due to their rich content of biologically active compounds, such as essential fatty acids, phytosterols, and tocopherols, which offer diverse nutritional and health benefits. Camelina seeds typically contain around 30% to 40% oil, with the oil being rich in polyunsaturated fatty acids. Notably, linolenic acid and alpha-linolenic acid are prominent in camelina oil, serving as valuable sources of omega-3 fatty acids. Overall, camelina oil is recognized for its beneficial effects on human health attributed to its diverse array of compounds (Kanclerz *et al*., 2019).

Simple Sequence Repeat (SSR) and Inter-Simple Sequence Repeat (ISSR) markers provide valuable insights into genetic diversity, they often suffer from issues such as low polymorphism in certain populations and high costs associated with their

development. RFLP (Restriction Fragment Length Polymorphism) markers, although informative, require extensive genomic knowledge and can be labor-intensive (Sadeghikian *et al*., 2022). In contrast, IPBS (Inter-primer binding site) retrotransposon markers offer a more universal approach, enabling the amplification of conserved regions across diverse cultivars, thereby overcoming these limitations. This study aims to fill the research gap by applying IPBS markers to assess genetic diversity in *Camelina sativa* lines. Recent advancements in molecular marker technology have underscored the importance of utilizing diverse marker systems for effective breeding strategies (Ghasemi *et al*., 2019; Eren *et al*., 2023). Studies have shown that retrotransposon-based markers like IPBS not only enhance genetic resolution but also facilitate a deeper understanding of genetic relationships among cultivars (Haliloğlu *et al*., 2022). By integrating these findings, we aim to provide a comprehensive assessment of genetic diversity in camelina.

Inter-primer binding site (IPBS) retrotransposons have emerged as a universal marker for characterizing animal and plant species (Arystanbekkyzy *et al*., 2019). They have become a dominant and preferred marker in assessing genetic diversity due to their universality (Öztürk *et al*., 2020). IPBS is a retrotransposon marker system based on the amplification of the conserved region of PBS sequences (initiator binding site) (Kalendar *et al*., 2010). IPBS serves as a method to identify diverse LTR sequences and directly detect their polymorphism among cultivars. This method targets the PBS region next to the 5′ LTR, which is highly conserved across LTR retrotransposon families (Kalendar *et al*., 2010). The PBS sequence, which binds to tRNA to initiate reverse transcription, is complementary to the 3′ terminal sequence of tRNA and is highly conserved across nearly all LTR retrotransposon families (Monden *et al*, 2014).

Designing PCR primers in the PBS region enables the amplification of DNA fragments with diverse LTR sequences, encompassing non-autonomous elements like TRIMs and LARDs that are devoid of protein-coding regions. Traditional cloning methods for LTR sequences typically focused on conserved protein-coding domains, necessitating genome-wide walks to LTRs, thereby constraining the exploration of distinct elements. In summary, the IPBS method offers advantages in screening diverse LTR sequences and conducting DNA fingerprinting (Kalendar *et al*., 2010). Furthermore, advancements in next-generation sequencing technologies have expedited genetic and genomic research by enabling the analysis of larger sequence datasets in shorter timeframes and at reduced costs (Monden *et al*., 2014).

This study was conducted to seek answers to the following questions: (1) What is the level of genetic diversity among *C. sativa* doubled haploid lines using IPBS markers? (2) How do the polymorphic characteristics of IPBS markers compare to those of traditional markers? Therefore, this study was conducted with the hypothesis that the application of IPBS markers will reveal higher levels of genetic diversity than traditional methods due to their ability to capture a broader range of polymorphic sites. Given camelina's agricultural and health benefits, understanding its genetic diversity is essential for optimizing cultivation and breeding practices. So, the genetic variability of camelina doubled haploid lines was explored using the IPBS molecular marker.

MATERIALS AND METHODS

Plant materials: In this study, 16 doubled haploid lines of Camelina (*C. sativa*), along with the Soheil and Sepehr cultivars as a control, were cultivated in a randomized complete block design with three replications on a farm at Razi University, Kermanshah, Iran, grown during the 2020-2021 cropping season. On November 19, the lines were planted in three rows, spaced 30 cm apart, each 2 meters long, with a planting density of 400 plants per square meter (Table 1).

Table 1. Parental Cultivars and Corresponding Doubled Haploid Lines of Camelina.

Cross No.	paternal parent (♂)		maternal parent (♀)			
	Cultivar	Origin	Cultivar	origin	Doubled haploid lines code	Number assigned to each code
1	Czestochowska	Poland	Voronezh349	Russia	201, 202, 203, 204, 205, 207, 208, 209, 210, 211, 212, 213, 214, 216, 219, 220, 222	1 to 16 (in order)
2	Blaine Greek	Greece	Calena	France	Soheil & Sepehr	18 and 19

DNA extraction: The DNA extraction process was streamlined to focus on essential steps. Fresh leaf samples were collected from field-grown plants and immediately immersed in liquid nitrogen to preserve nucleic acids and prevent degradation. The samples were then stored at -80°C before extraction using Dellaporta's method (Dellaporta, 1993), which is known for its efficiency in isolating high-quality genomic DNA. This approach minimizes the risk of contamination and ensures that the extracted DNA is suitable for downstream applications such as PCR amplification.

Determining the quantity and quality of DNA samples: The quantification of the extracted DNA samples was performed using a Thermo 2000 NanoDrop device. Two µL of each sample were applied to the device's sensor. The concentration of the DNA solution was measured in nanograms per µL, and the absorbance levels at wavelengths 230, 260, and 280 nm (OD) were recorded and displayed on the device's monitor. Absorbance ratios between 260/280 nm and 260/230 nm within the range of 1.7 to 2.1 indicated the quantity and quality of the DNA samples. To further assess the quality of the DNA, 0.8% agarose gel electrophoresis was conducted in TAE 1X buffer at 80 volts for 50 minutes.

Polymerase chain reaction (PCR): The PCR reaction was conducted utilizing 15 IPBS retrotransposon primers (Table 2). The selection of primers for this study was based on several key criteria aimed at ensuring their effectiveness in amplifying diverse genetic regions within camelina sativa lines. Primarily, the IPBS markers were chosen due to their ability to target conserved regions of retrotransposons, which are known to exhibit high levels of polymorphism.

The PCR reaction mixture consisted of the following components:

- 2 µL of template DNA (20 ng/µL)
- 2.5 µL of primer (10 µM)
- 7 µL of 2X Master Mix (CinaClon, Cat. No.: MM2062)
- 3 µL of double-distilled water

The PCR was performed in a Thermocycler, specifically the FlexCycler Block Model 96G, using a program that involved an initial denaturation step at 94°C for two minutes, followed by 30 cycles consisting of denaturation at 94°C for 45 seconds, annealing at the primer-specific temperature for 45 seconds (determined via the PCR temperature gradient), and extension at 72°C for two minutes. A final extension step was carried out at 72°C for 10 minutes. Additionally, a negative control (PCR reaction mixture without DNA) was included for each primer to monitor contamination and ensure the absence of contaminants in the PCR materials.

Agarose gel electrophoresis and staining: To separate and observe the amplified DNA fragments, electrophoresis was performed on a 1.2% agarose gel in TAE 1X buffer at 130 V. The gel was stained with ethidium bromide for 45 minutes, and the bands were visualized under UV light using a Vilber Lourmat gel imaging system.

Table 2. Characteristics of IPBS primers used in investigating the diversity of camelina doubled haploid lines.

Row	Primer name	Sequence (5′to 3′)	Anneling temperature ℃
1	IPBS 2074	5′-GCTCTGATACCA-3′	46.6
2	IPBS 2076	5′-GCTCCGATGCCA-3′	46.6
3	IPBS 2078	5′-GCGGAGTCGCCA-3′	42.3
4	IPBS 2079	5′-AGGTGGGCGCCA-3′	45
5	IPBS 2226	5′-CGGTGACCTTTGATACCA-3′	29.2
6	IPBS 2228	5′-GGCTCATGATACCA-3′	45
7	IPBS 2237	5′-CCCCTACCTGGCGTGCCA-3′	36
8	IPBS 2239	5′-ACCTAGGCTCGGATGCCA-3′	42.3
9	IPBS 2242	5′-GCCCCATGGTGGGCGCCA-3′	38.4
10	IPBS 2271	5′-GGCTCGGATGCCA-3′	47
11	IPBS 2376	5′-TAGATGGCACCA-3′	47
12	IPBS 2378	5′-GGTCCTCATCCA-3′	41.2
13	IPBS 2395	5′-TCCCCAGCGGAGTCGCCA-3′	38.4
14	IPBS 2399	5′-AAACTGGCAACGGCGCCA-3′	47
15	IPBS 2400	5′-CCCCTCCTTCTAGCGCCA-3′	41.2

Statistical analyses: In assessing polymorphism among the targeted camelina lines, a raw data matrix was generated in the Excel 2016 environment by scoring the bands from electrophoresis. The presence of band one and the absence of band zero were considered for analysis. To evaluate primer efficiency, marker parameters including percentage polymorphism (PP) according to the method of Mohammadi and Prasanna (2003) and polymorphic information content (PIC) were determined (Mousapour *et al*., 2011). The effective

multiplex ratio (EMR) and marker index (MI) were calculated using the method described by Powell *et al.* (1996), while resolution power (RP) was calculated using the method outlined by Altintas *et al.* (2008), employing the following formulas:

$$P = \frac{\text{The number of people who have bands}}{\text{Number of persons}}$$

q=1-p

$$PP = \frac{\text{The number of polymorphic bands}}{\text{Total number of replicated bands}} \times 100$$

$$PIC = 1 - p^2 - q^2$$

MI=PIC× EMR

$$EMR = \frac{\text{The number of polymorphic bands}}{\text{Total number of replicated bands}} \times \text{The number of polymorphic bands}$$

Ib=1-[2× (0.5-p)] RP $= \sum Ib$

In short, P = The proportion of individuals exhibiting a band for a given marker, where q = 1 – P.

We utilized several key formulas to assess polymorphism among the camelina lines. The variable "P" represents the proportion of individuals exhibiting a specific band, calculated as the number of individuals with that band divided by the total number of individuals assessed. Conversely, "q" is defined as 1−P, representing the proportion of individuals not exhibiting that band. These definitions are crucial for understanding the subsequent calculations of PP, PIC, and other marker parameters used in our analysis.

PP measures the proportion of polymorphic bands among the total bands produced in a genetic analysis. It indicates the level of genetic diversity within a population, reflecting how many variations exist among individuals. PIC quantifies the informativeness of a genetic marker based on its level of polymorphism. A higher PIC value signifies greater discrimination power, suggesting that the marker can effectively differentiate between different genotypes within a population. MI assesses the effectiveness of a genetic marker in detecting polymorphism by considering both the number of polymorphic bands and their genomic coverage. A higher MI indicates a greater capacity to capture genetic diversity, making it a valuable parameter for genetic analyses. RP evaluates a primer's ability to distinguish between different alleles based on their frequency and number. Higher RP values indicate better resolution in identifying genetic variations, which is crucial for effective primer selection in genetic studies.

The genetic similarity matrix for the camelina lines was computed using Jaccard's similarity coefficient with NTSYSPC 2.02 software. Cluster analysis was performed based on this coefficient, and the optimal cutting point for the dendrogram was determined using the pseudo-F value in SYSTAT 13.0 software. Additionally, principal coordinate analysis (PCoA) was conducted using NTSYSPC 2.02 software.

RESULTS

Out of the 15 IPBS retrotransposon primers used, 14 generated scorable bands (Figure 1). The sizes of the produced bands ranged from 200 to 5000 bp. These 14 primers collectively produced a total of 325 bands, with 83 showing polymorphism, accounting for 25.41% of the total bands. The average number of amplified fragments was 23.21, with IPBS (2076) and IPBS (2271) yielding the most bands at 31 each, while IPBS (2226) generated the fewest, with only 13 bands. The average number of polymorphic bands per primer was 5.93, with IPBS (2076) and IPBS (2239) exhibiting the highest number of polymorphic fragments, each producing 10 polymorphic bands. Conversely, IPBS (2226) and IPBS (2400) had the lowest number of polymorphic fragments, generating two polymorphic bands each.

The average polymorphism percentage (PP) across all primers was 25.41%. Among the primers, IPBS (2237) exhibited the highest polymorphism percentage at 41.17%, while IPBS (2400) had the lowest at 9.09%. The primers IPBS (2078) and IPBS (2271) showed the highest levels of polymorphic information content (PIC), with values of 0.30 and 0.30, respectively, while IPBS (2376) had a PIC value of 0.28. PIC is a crucial parameter for assessing the discrimination power of genetic markers by quantifying their level of polymorphism.

Primers such as IPBS (2076), IPBS (2237), and IPBS (2239) demonstrated high levels of PIC, indicating their superior ability to discern genetic distances among camelina lines compared to other primers. In contrast, primers IPBS (2228) and IPBS (2076) exhibited the lowest levels of PIC, with values of 0.15 and 0.17, respectively.

The average MI for the primers was 0.39. Notably, primers such as IPBS (2239), IPBS (2242), IPBS (2237), and IPBS (2376) exhibited the highest MI values at 0.82, 0.63, 0.62, and 0.59, respectively. Conversely, the lowest MI values were found for primers IPBS (2400) at 0.03 and IPBS (2226) at 0.08, indicating their lower efficiency in capturing genetic diversity.

The Effective Multiplex Ratio (EMR) values were highest for IPBS (2239) at 3.57 and IPBS (2076) at 3.23, while the lowest EMR values were found for IPBS (2400) at 0.18 and IPBS (2226) at 0.31. The primers IPBS (2078) and IPBS (2271) exhibited the highest resolution values at 2.84, followed by IPBS (2239) and IPBS (2242) at 2.74, and IPBS (2276) at 2.63. Conversely, the lowest resolution values were observed for IPBS (2400) at 0.42, IPBS (2226) at 0.63, and IPBS (2228) at 0.84.

Primers IPBS (2076), IPBS (2237), and IPBS (2239) are highlighted for their efficiency in line differentiation due to their high values across various parameters such as the number of polymorphic bands,

polymorphism percentage, marker index, effective multiplex ratio, resolution power, and polymorphic information content. These primers have been identified as informative initiators in distinguishing genetic variations in camelina plant lines.

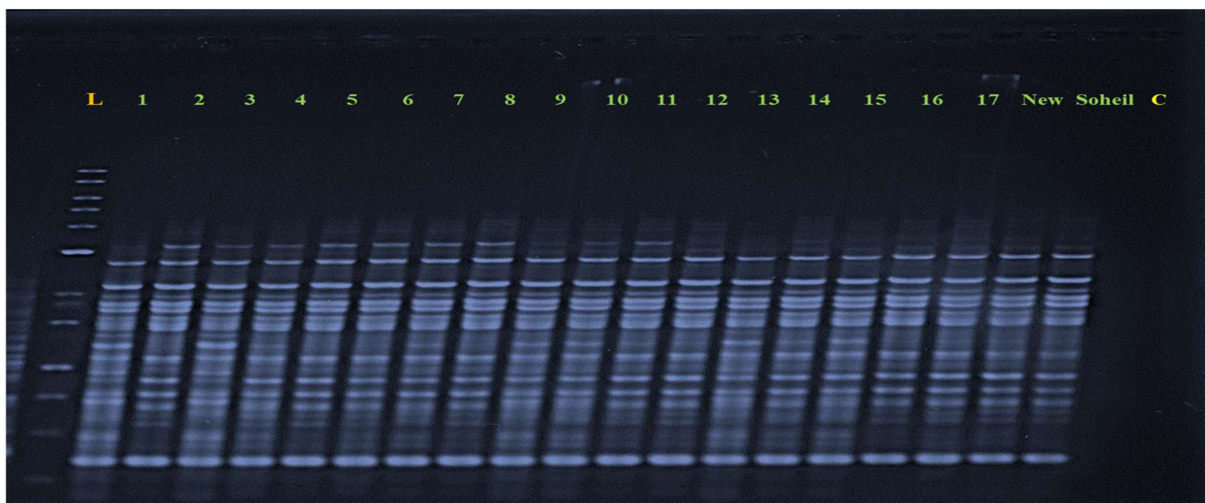

Figure 1. Banding patterns of IPBS primers used in doubled haploid lines of camelina. L: 1 kb standard marker; C: control (negative); wells labeled 1 to 17: investigated camelina lines; New: Sepehr cultivar; Soheil: Soheil cultivar.

Table 3. Properties of IPBS primers used in camelina lines.

Row	Primer name	The number of duplicated parts	The number of polymorphic parts	polymorphic percentage (PP)	Indicator Index (MI)	effective polymorphic ratio (EMR)	resolving power (RP)	Polymorphic Information Content (PIC)
1	IPBS 2074	23	6	26.08	0.38	1.57	2.11	0.24
2	IPBS 2076	31	10	32.25	0.55	3.23	2.11	0.17
3	IPBS 2078	29	7	24.13	0.51	1.69	2.84	0.30
4	IPBS 2079	23	4	17.39	0.18	0.70	1.26	0.25
5	IPBS 2226	13	2	15.38	0.08	0.31	0.63	0.27
6	IPBS 2228	19	5	26.31	0.19	1.32	0.84	0.15
7	IPBS 2237	17	7	41.17	0.62	2.88	1.79	0.22
8	IPBS 2239	28	10	35.71	0.82	3.57	2.74	0.23
9	IPBS 2242	20	7	35	0.63	2.45	2.74	0.26
10	IPBS 2271	31	6	19.35	0.35	1.16	2.84	0.30
11	IPBS 2376	23	7	30.34	0.59	2.12	2.63	0.28
12	IPBS 2378	23	6	26.08	0.38	1.56	2.11	0.24
13	IPBS 2395	23	4	17.39	0.18	0.70	1.26	0.26
14	IPBS 2400	22	2	9.09	0.03	0.18	0.42	0.18
Average	_	23.21	5.93	25.41	0.39	1.67	1.88	0.24
Total	_	325	83	_	_	_	_	_

Examination of the Jaccard genetic similarity matrix based on the IPBS marker data: The Jaccard genetic similarity matrix showed a range of genetic similarity values between lines, from 0.4 to 0.9, indicating moderate levels of diversity in this species. The lowest genetic similarity value of 0.4 was observed between the Soheil cultivar (line number 19) and line number 1 (201), which had non-common parents. In contrast, the highest genetic similarity value of 0.9 was found between lines 17 (222) and 18 (NEW), as well as between lines 13 (214) and 9 (210), where the latter two shared common parents

(Czestochowska from Poland and Voronezh349 from Russia).

Cluster analysis: The camelina lines were clustered using the Complete method based on Jaccard's similarity coefficient. To determine the optimal number of clusters, the pseudo-F index was utilized, resulting in the classification of the studied lines into four clusters at a distance of 0.4 (Figure 3). The clustering results are as follows:

- **First Cluster:** Lines 1 and 3.
- **Second Cluster:** Lines 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, and 18.
- **Third Cluster:** Lines 2, 5, and 6, which share common parents (Czestochowska from Poland and Voronezh349 from Russia), except for line 18.
- **Fourth Cluster:** The Soheil variety.

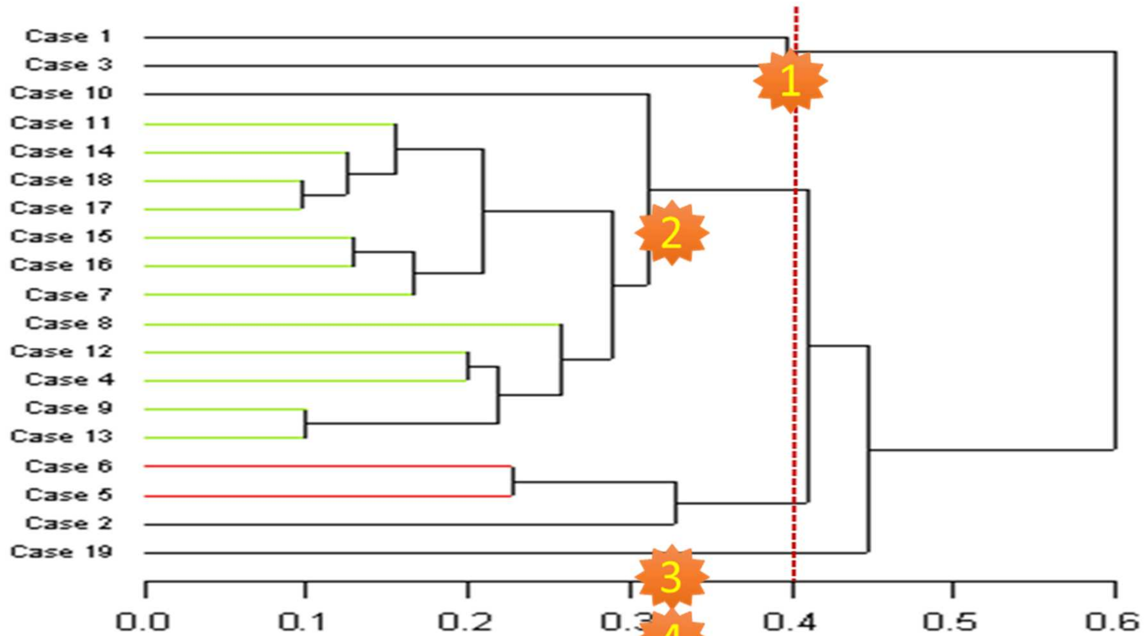

Figure 2. The dendrogram was obtained from the cluster analysis of the examined camelina lines based on Jaccard's similarity coefficient using the Complete method.

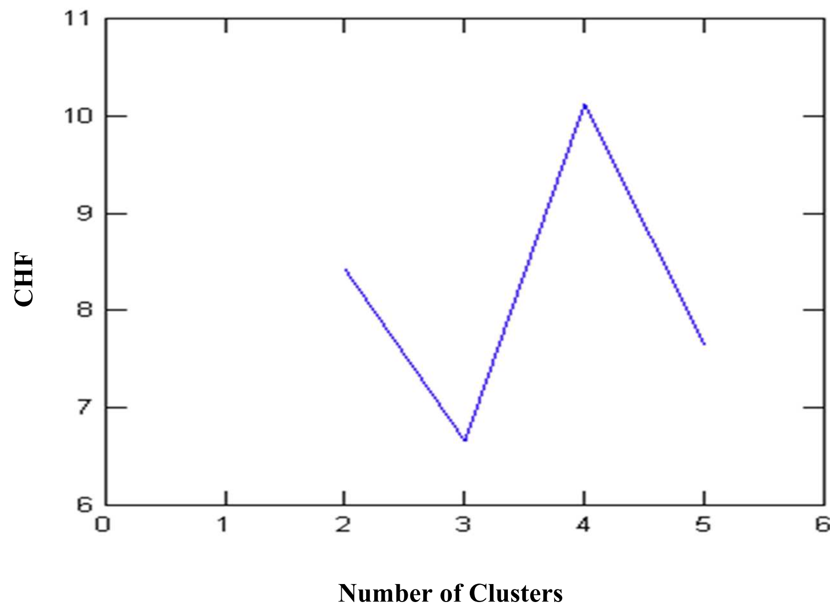

Figure 3. Pseudo-f index diagram to determine the number of groups in the cluster analysis of camelina plant lines in terms of molecular traits.

Principal coordinates analysis: The first two components explained 78.99% of the total variation, highlighting their significant contribution to the observed variability in the camelina lines (Table 4). The two-dimensional scatter diagram based on these components closely aligned with the results of the cluster analysis. The lines were grouped into five clusters, revealing some discrepancies compared to the groupings derived from the cluster analysis. This visualization provided additional insights into the genetic relationships among the camelina plant lines (Figure 4).

Table 4. Results of the Principal Coordinates Analysis for Doubled Haploid Lines of Camelina Based on IPBS Markers.

Components	special amount	The percentage of justified variation	Cumulative variance percentage
1	14.03	73.82	73.82
2	0.99	5.17	78.99
3	0.59	3.1	82.1
4	0.48	2.5	84.6
5	0.43	2.2	86.86
6	0.39	2.04	88.9
7	0.34	1.8	90.7
8	0.28	1.45	92.15
9	0.23	1.22	93.37
10	0.22	1.17	94.54
11	0.19	1.01	95.55
12	0.16	0.87	96.41
13	0.15	0.77	97.18
14	0.12	0.64	97.82
15	0.11	0.61	98.43
16	0.10	0.52	98.95
17	0.08	0.43	99.38
18	0.07	0.37	99.75
19	0.05	0.25	100

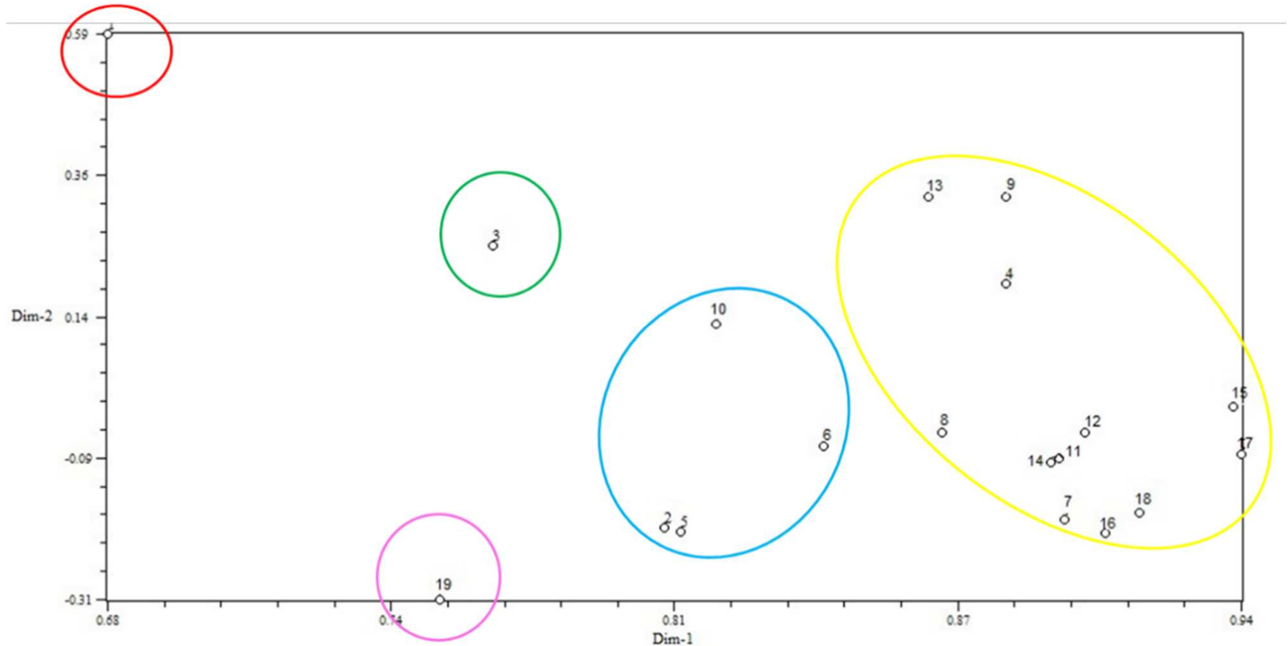

Figure 4. Two-dimensional distribution diagram of the main coordinates of the investigated lines of camelina plant using the data obtained from the IPBS indicator.

DISCUSSION

The study examined the genetic diversity of *C. sativa*, an oilseed plant valued for its adaptability and nutrition. Using 15 IPBS retrotransposon primers, researchers analyzed 16 doubled haploid lines and two varieties, revealing significant genetic variation. The findings highlighted the effectiveness of IPBS markers in identifying key polymorphisms for breeding programs aimed at desirable traits. This research underscores the importance of genetic diversity for optimizing cultivation and improving oil production amid declining domestic supplies. Overall, it demonstrated the potential of IPBS markers as reliable tools for genetic analysis in camelina and similar crops (Ghasemi *et al.,* 2019). A higher PIC value signifies increased polymorphism and reflects the strong discrimination power of the genetic marker under investigation (Carvalho *et al*., 2009).

Recent studies have demonstrated the effectiveness of Inter-Primer Binding Site (IPBS) markers in assessing genetic diversity across various plant species. Eren *et al*. (2023) revealed significant polymorphism in alfalfa genotypes, with an average polymorphism percentage of 93.71% from 280 bands generated using 10 IPBS primers. Similarly, Haliloğlu *et al*. (2022) employed 26 IPBS primers to investigate the genetic diversity and population structure of bean germplasm in Turkey's Erzurum Isper region, confirming the markers' suitability for both local and commercial varieties. These findings collectively highlight the reliability of IPBS markers for genetic analysis, supporting breeding programs and germplasm conservation efforts in agriculture. Kantar *et al*. (2021) reported only 24.1% polymorphism in 26 okra accessions using 14 IPBS primers, while Androsiuk *et al*. (2023) found a polymorphism rate of 20.5% among 13 oat species assessed with seven markers. These results indicate that genetic diversity detected by IPBS markers can vary significantly across different species, often showing limited levels of polymorphism.

The genetic analysis of *C. sativa* revealed significant divergence between the Soheil cultivar and line number 1, with a low similarity value of 0.4, indicating potential for breeding new cultivars with enhanced traits. Conversely, lines 17 and 18, as well as lines 13 and 9, showed a high similarity value of 0.9 due to shared parentage, which may restrict the introduction of novel traits. These findings emphasize the importance of understanding genetic diversity to optimize breeding strategies for improving yield, disease resistance, and adaptability. Utilizing effective markers like IPBS can enhance breeding efficiency and support the development of superior cultivars to meet agricultural needs.

Jaccard's similarity coefficient is particularly valuable for assessing genetic diversity using dominant markers, as it categorizes similarities based on the presence or absence of bands (Yang *et al.,* 2020). The similarity matrix reflects pairwise similarities between individuals, indicating that greater similarity in various genetic indicators between two individuals corresponds to higher genetic similarity, while lower similarity results in reduced genetic similarity (Arjmand Qahestani *et al.,* 2015). In another study, a similarity matrix was constructed for 32 duplicated camelina haploid lines using data from 11 REMAP (ISSR+IRAP) combined markers. The analysis, employing Jaccard's similarity coefficient, revealed a range of genetic distance variations between the lines, spanning from 0. 13 to 0. 85 (Minaei Chenar *et al.,* 2022). Rahaei *et al*. (2002) conducted a study on rapeseed cultivars using 20 AFLP primers, which revealed Jaccard similarity coefficient variations among cultivars ranging from 0. 38 to 0. 84, with an average coefficient value of 0. 61.

The marker index (MI), which assesses a marker's effectiveness in detecting polymorphism by considering factors such as the number of polymorphic bands generated and the marker's genomic coverage, provides insights into its utility in genetic analyses. A high MI indicates a greater capacity to extract genetic information from the genome through a larger number of polymorphic bands, reflecting the marker's efficacy in capturing genetic diversity (Sagbas *et al*., 2023).

Resolution Power (RP), influenced by allele number and band frequency, is crucial for primer selection as it aids in distinguishing between different primers effectively (Kayis *et al*., 2010). The clustering analysis of *C. sativa* lines, using the Complete method based on Jaccard’s similarity coefficient, identified four distinct clusters at a distance of 0.4. The first cluster included lines 1 and 3, indicating a close genetic relationship. The second cluster comprised a larger group of 13 lines, which share greater genetic similarities. The third cluster contained lines 2, 5, and 6, sharing common parents, while line 18 exhibited distinct characteristics. The fourth cluster represented the Soheil variety, highlighting its unique genetic makeup. These results provide insights into genetic diversity and can guide targeted breeding strategies for developing cultivars with desirable traits.

In studies by Sadeghikian *et al*. (2022) and Minaei Chenar *et al*. (2022), genetic diversity among doubled haploid lines of *Camelina sativa* was assessed using ISSR markers. Sadeghikian *et al*. analyzed 81 lines, clustering them into five groups based on Jaccard's similarity coefficient, which reflected the geographic distribution of their parental origins. Minaei Chenar *et al*. categorized 32 doubled haploid lines into six main groups using the same method. Both studies highlight the effectiveness of ISSR markers in revealing genetic relationships and diversity within camelina, aiding in breeding strategies for this oil crop.

The PCA of camelina lines showed that the first two components accounted for 78.99% of the total variation, effectively capturing the majority of genetic diversity. The PCA results closely aligned with those from cluster analysis, although PCA identified five clusters, slightly differing from the cluster analysis groupings. This suggests that while both methods provide insights into genetic relationships, they emphasize different aspects of the data. Overall, the findings highlight PCA's value as a complementary tool to cluster analysis, enhancing understanding of genetic diversity among camelina lines and informing breeding strategies for improved cultivars. In a study by Bayat *et al*. (2016) on six saffron crop ecotypes, genetic diversity was assessed using 28 IPBS primers and 22 SSR primers. Cluster analysis based on both marker types classified the ecotypes into three groups, a classification further supported by principal component analysis. The research indicated that IPBS markers were more effective in delineating genetic diversity among saffron ecotypes compared to SSR markers. In another study, Sadeghikian *et al*. (2022) explored the genetic diversity of 81 doubled haploid lines of camelina using 15 ISSR primers, with principal coordinates analysis grouping the lines into six distinct clusters.

Several points can be mentioned regarding the strengths of the study. The study successfully utilized 15 IPBS retrotransposon primers, demonstrating their effectiveness in assessing genetic diversity among camelina sativa lines. The ability of these markers to generate a significant number of polymorphic bands enhances the resolution of genetic analysis. The research provided a thorough evaluation of genetic variation, utilizing multiple analytical methods including cluster analysis and principal coordinates analysis. This multifaceted approach allowed for a robust interpretation of genetic relationships among the lines. The findings revealed moderate levels of genetic diversity within the studied lines, which is crucial for breeding programs aiming to develop cultivars with desirable traits. This insight can guide future breeding strategies and selection processes. By focusing on camelina sativa, an oilseed crop with significant agricultural and health benefits, the study addresses important issues related to oil production and food security, making its findings highly relevant to current agricultural challenges.

There are some limitations in this study. The study assessed only 16 doubled haploid lines and two varieties, which may not fully represent the genetic diversity present in the broader *C. sativa* population. A larger sample size could provide more comprehensive insights into genetic variability. The research was conducted in a specific geographic location (Kermanshah, Iran), which may limit the generalizability of the findings to other regions with different environmental conditions or genetic backgrounds. Although IPBS markers were effective in this study, reliance on a single marker system may overlook other potentially informative markers that could provide additional insights into genetic diversity. The study reflects a snapshot of genetic diversity at a specific time point. Longitudinal studies are needed to assess how genetic diversity may change over time due to environmental pressures or breeding practices.

Conclusion: The study reveals significant findings on the genetic diversity of *C. sativa* using 15 IPBS retrotransposon markers. Analysis of 16 doubled haploid lines and two varieties showed a moderate level of diversity, with 325 bands generated and 83 exhibiting polymorphism. This underscores the effectiveness of IPBS markers in capturing genetic variation, which is essential for breeding programs aimed at developing cultivars with desirable traits. The study identified key primers, such as IPBS (2076) and IPBS (2237), which demonstrated high polymorphic information content and marker index values, indicating their superior ability to differentiate between genetic variations among camelina lines. The study's analyses improved understanding of genetic relationships among *C. sativa* lines, aiding in the development of high-yielding and stress-resistant cultivars to meet agricultural demands. It highlights the potential of IPBS markers for assessing genetic diversity and suggests that future research could explore additional markers or a wider range of varieties to enhance breeding efforts.

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