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Genetic diversity of some thornless blackberry genotypes using ISSR molecular markers

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Abstract: Genetic diversity in blackberries is crucial for improving quality and yield. This study evaluates thornless blackberry genotypes using 26 morphological traits and 19 inter simple sequence repeats (ISSR) markers to assess diversity and genetic relationships. A total of 28 blackberry genotypes, including both thorny and thornless types, were analyzed. Fourteen ISSR primers were selected from a pool of 19 based on their capacity to generate polymorphic bands. The findings highlight the efficiency of ISSR markers in distinguishing thorny and thornless blackberry genotypes at the subgenus level, effectively differentiating chimera-derived thornless samples. A total of 406 bands were produced, of which 402 were polymorphic. The average percentage of polymorphic bands for each primer in this experiment was 98.98%, and the highest polymorphic information content (PIC) was associated with ISSR 16, which had a value of 0.35. The findings highlight the efficiency of ISSR markers in distinguishing thorny and thornless blackberry genotypes at the main genotype groups: the first linked to initial thornless generations of *Rubus laciniatus*, and the second comprising crosses related to the Merton cultivar. Overall, significant genetic diversity among blackberry cultivars suggests valuable applications in breeding and improvement programs.

Keywords: *Rubus laciniatus*, merton thornless, genetic diversity, molecular marker.

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Introduction

Thornless blackberry (*Rubus* L. subgenus *Rubus* Watson) belongs to the Rosaceae family. This perennial plant typically exhibits a semi-erect growth habit, which distinguishes it from erect and trailing blackberry species. The Rosaceae family includes pome fruits, stone fruits, and aggregate fruits, with blackberries classified among the latter (Swanson et al., 2011). The domestication of blackberries began with the selection of wild samples, and by the early 20th century, genetic improvement was pursued through selected cultivars. The first thornless cultivar, "Evergreen," was derived from wild germplasm (*Rubus laciniantus*), following a mutation that led to the introduction of 'Thornless Evergreen (Abdi et al., 2021). Issues related to chimerism which easily return to thorny traits. In subsequent step through tissue culture the introduction of the 'Everthornless' cultivar. As reported by the thornless Merton from the John Innes Institute in the UK Swanson et al. (2011) developed and resulted in the creation of the Navaho, Arapaho, Apache, Ouachita, and Natchez cultivars. The trend of improving thornless continues with the introduction of new cultivars and the use of molecular markers to identify diverse sources (Coyner et al., 2005). PCR-based markers (RAPD, AFLP, SSR, ISSR, etc.) are widely used for the molecular classification of plant species in breeding programs (Adje et al., 2023; Agbo et al., 2023; Hosseinpour Azad, 2023; Kour et al., 2023). Coyner et al. (2008) investigated four sources of thornless using molecular markers (RAPD), differentiating cultivars of various origins and identifying common backgrounds among them. They also introduced the molecular markers used as tools for identifying valuable cultivars. The latest thornless cultivar, Prime-Ark Traveler, emphasizes early fruiting in the first year and superior quality, making it a suitable option for commercial transport (Clark and Salgado, 2016).

Hadadinejad and Moradi (2016) have been assessing wild blackberries with high antioxidant properties, and have established of a collection of various cultivars of blackberries, including both thorny and thornless. Initial studies indicated differences among the thornless cultivars in this collection, and these differences were confirmed by analyzing five thornless genotypes using ISSR molecular markers (Abdi et al., 2018). ISSR is recognized as an effective technique in studies of genetic diversity and phylogenetics, with advantages including low cost, easy preparation, and high accuracy in identifying cultivars, although its main drawback is the initial cost associated with designing markers (Barandalla et al., 2006; Selkoe and Toonen, 2006).

Debnath (2008) assessed genetic diversity and similarity within blackberry genotypes using ISSR markers and pedigree information. This study included nine North American blackberry cultivars (*Rubus*) and four Canadian breeding lines. Using 18 primers, the analysis produced 306 polymorphic bands. Cluster analysis results indicated a similarity range of 24% to 49% among 13 genotypes and 3% to 25% for nine genotypes.

Coyner et al. (2008) utilized RAPD markers to examine the relationship among thornless blackberries. RAPD markers have been used in several blackberry-related programs. Coyner et al. used molecular markers to investigate the genetic relationships among 11 blackberry cultivars derived from four thornless backgrounds. They used 140 random primers, and their analysis placed the cultivars into three distinct categories. They also reported that 98 primers produced 113 specific bands that could be useful for cultivar identification. Several primers, capable of distinguishing a cultivar, produced between one and 24 bands. A study estimated the genetic diversity and relationships among 74 blackberry accessions from five different cultivars using interspersed simple sequence repeat (ISSR) marker analysis and morphological characterization. Sixteen characteristics were examined, including phenological, vegetative, and reproductive traits in 57 accessions, alongside 10 ISSR primers. Findings revealed that yield had the highest genetic diversity (diversity index = 62.57), while leaf width showed the lowest (13.74), with a strong correlation (r = 0.98) between flowering and ripening dates. The primers generated 161 amplified fragments, of which 113 were polymorphic. Principal component analysis (PCA) and principal coordinate analysis (PCoA) explained 84.9% and 67.06% of the total variation, respectively. Cluster analysis categorized the populations into two to three groups based on

morphological traits and ISSR data, reflecting the influence of species and geographical origin on genotype distribution (Garazhian et al., 2022).

Sedighi and Rahimmalek (2015) examined the genetic diversity of wild blackberry species scattered around the Caspian sea using both morphological traits and ISSR molecular markers. Based on the results, they differentiated two groups of blackberries: those from the western and eastern regions of the northern strip of the country and those related to the central region. Given the high genetic diversity observed in the central region, it appears that samples from the eastern and western regions may have originated from the central region, leading to a report of relatively narrow genetic base for Caspian blackberries. Their results also indicated that of the 20 primers used, only 10 were polymorphic, and out of 204 bands produced, 157 were polymorphic. Ataei-e et al. (2015) investigated the genetic diversity of blackberries in northern Iran. A total of 60 genotypes from Gilan and Mazandaran provinces, representing seven species, were studied using SSR markers. Among the populations, high genetic diversity was observed within the species, while there was low genetic differentiation among the studied populations. Out of the 10 primers used by these investigators, six were polymorphic, yielding 32 polymorphic alleles. The average number of alleles per locus and the polymorphism information content (PIC) were 2.8 and 0.593, respectively. The highest PIC value obtained was 0.71, while the lowest was 0.50. The number of alleles at each gene locus ranged from one to five, with an average of 3.15. The species *R. sanctus* had the highest effective number of alleles at 1.7, while *R. hirtus* had the lowest at 0.1. The average expected heterozygosity across populations was 0.121, indicating low genetic differentiation within the species. Geographic separation, natural barriers, pollen transfer by insects, cross-pollination, and polyploidy were significant factors in creating and maintaining genetic diversity among blackberry species. Abdi et al. (2021) investigated the genetic diversity of thorny blackberries in the collection at the University of Agricultural Sciences and Natural Resources in Sari using ISSR molecular markers. All 10 primers used exhibited a desirable level of polymorphism in the studied population. A total of 345 bands were generated, of which 344 were polymorphic. The blackberry genotypes in the collection examined in this study are considered a genetic reservoir, which includes both an initial genetic reserve comprising thorny cultivated and thornless cultivars, as well as a secondary genetic reserve consisting of wild thorny blackberries. According to the results and the resulting dendrogram, the primers employed successfully divided the samples into three distinct groups. Based on the Dice similarity coefficient, the highest similarity was found between the thornless samples from Sari and Qaem Shahr at 73%, while the lowest similarity was between the wild samples from Qaem Shahr and Kermanshah at 7%. The imported thorny cultivars, which were completely distinguishable by ripening time and included early, mid-season, and late-maturing cultivars, were grouped separately. This study demonstrated that ISSR markers effectively distinguish genotypes and identify the existing genetic diversity.

In another study screening and evaluating blackberry cultivars and strains, 17 plant growth indices and orchard characteristics, as well as fruit nutritional traits, were measured. Twenty simple sequence repeat (SSR) markers were analyzed, and a fingerprinting of 23 blackberry cultivars and variety was developed, along with processing characteristics evaluated for 10 of the cultivars and strains assessed. The results indicated that 'Chester' and 'Shuofeng' had the highest yield per plant (6.5 kg per plant), with 'Chester' also showing the highest fruit firmness (2.78 kg/cm^2). 'Kiowa' had the highest individual fruit weight (10.43 grams). The cultivar "10-5n-2" had the highest total anthocyanin content (225.4 mg per 100 g FW) and total phenolic content (3.24 mg per gram FW), although it exhibited low plant yield. These results suggest that 'Shuofeng' and 'Chester' are the top-performing blackberry cultivars cultivated in Nanjing, exhibiting the best growth and overall quality. Additionally, a total of 119 alleles were identified, with an average of 6 alleles per locus. The polymorphism information content (PIC) ranged from 0.374 to 0.844, with an average of 0.739, indicating high genetic diversity among the 23 blackberry cultivars and strains (Zhao et al., 2023).

This study aimed to examine the genetic diversity of thornless blackberry genotypes collected from

various locations of south of the Caspian sea using ISSR molecular markers to identify the genetic relationships among the genotypes.

Materials and Methods

Investigating morphological diversity

Most of the desired morphological traits were selected based on the blackberry UPOV descriptor (Button, 2006) and measured as the average of the available repetitions. The examined traits and their measurement units were listed in Table 1. The coded data related to the color of leaves, fruits, canes, etc., were recorded according to the coding provided in the international descriptor of blackberry. For data without clear geographical origin, such as leaf blooming time, flowering time, and ripening time, we aimed to establish the lowest common range among the recorded data for a trait by selecting the appropriate origin. Continuous traits were measured using a ruler and caliper. A digital refractometer (ATAGO PR-32) was employed to measure total dissolved solids. Anthocyanin was quantified using the pH difference method (Wrolstad, 1993).

Samples from a collection of blackberries were selected, which included 28 genotypes from various geographical regions (Table 2). This collection comprises both thorny and thornless genotypes. Genomic DNA was extracted from young leaves using a modified CTAB method (Murray and Thompson, 1980). The DNA of the samples was diluted after assessing quality and quantity using a 0.8% agarose gel and a 260/280 nm absorbance ratio with a spectrophotometer (Biochrome Ltd, Cambridge, UK). Out of 19 primers, 14 ISSR primers were selected that produced the most polymorphic bands (Table 2). PCR reactions were conducted in a BioRad system. The thermal cycle included an initial denaturation step for 5 minutes at 94 °C, followed by 34 cycles of 50 seconds at 94 °C, 60 seconds at the annealing temperature (54-55 °C), and 80 seconds at 72 °C with a final extension step for 7 minutes at 72 °C. The amplified products were separated by electrophoresis in a 1.8% (w/v) agarose gel at 80 volts for 2 hours and 30 minutes in 0.5X TBE buffer, and after staining with ethidium bromide, the gel image was recorded using a Gel Doc Analyzer. The amplified bands were scored based on the presence (1) or absence (0) of bands. The Jaccard similarity coefficient, suitable for binary data and effectively indicating genetic similarity based on shared traits, along with the arithmetic mean algorithm (UPGMA), was employed to construct a cluster diagram using the weighted pair group method, as it visually displays these relationships in a clear and interpretable manner. Cluster analysis and principal coordinate analysis (PCA) were performed using NTSYSpc software, version 2.02. The polymorphic information content (PIC) was calculated using a formula for dominant markers: PICi = 2pi (1–pi), where pi is the frequency of the amplified alleles.

Table 1. Investigated traits and their measurement unit.

Raw	Traits	Unit	Raw	Traits	Unit
1	Growth habit	code	14	Flower anthesis	day
2	Spin number	number	15	Unripeand ripe friut color	code
3	Spin size	ml	16	Ripening	day
4	Spin direction	code	17	Fruit diameter	cm
5	Shoot cross sec	code	18	Fruit lenght	cm
6	Shoot diameter	cm	19	Fruit size	cm
7	Shoot number	number	20	Fresh weight	gr
8	Leaflet width	cm	21	TSS	Brix
9	Leaflet number	number	22	TA	%
10	Flower length	cm	23	TSS/TA	-
11	Flower diameter	cm	24	Seed number	number
12	Flower size	cm	25	Empty seed	number
13	Leaf anthesis	day	26	Anthocyanin	mg/100ml

Table 2. The list of genotypes were used in this study.

No.	Genotype ID	Loccation of collection	No.	Genotype ID	Loccation of collection
1	Marion	Sari University	15	cvTLNurnazari53	Mazandaran, Nur
2	Silvan	Sari University	16	cvTLSafzalian81	Mazandaran, Sari
3	cvTLSshirazi13	Mazandaran, Sari	17	cvTLAjafarzadeh35	Mazandaran,Amol
4	cvTLAdavudi18	Mazandaran,Amol	18	cvTLBaghajanzadeh49	Mazandaran,Babol
5	cvTLBizadkhast23	Mazandaran,Babol	19	cvTLQrastkhiz77	Mazandaran, Qaemshahr
6	cvTLmohammadi25	Mazandaran,Amol	20	cvTLAhoseinirad80	Mazandaran,Amol
7	cvTLSDrSadeghi6	Mazandaran, Sari	21	cvTLQrastkhiz175	Mazandaran, Qaemshahr
8	cvTLAbakhtiari224	Mazandaran,Amol	22	cvTLSmoafei69	Mazandaran, Sari
9	cvTLBrazinejad17	Mazandaran,Babol	23	cvTLBmiladi85	Mazandaran,Babol
10	cvTLSasadi26	Mazandaran, Sari	24	cvTLAjafarzadeh63	Mazandaran,Amol
11	cvTLBaghajanzadeh22	Mazandaran,Babol	25	cvTLSnurmohammadi68	Mazandaran, Sari
12	cvTLMshojaei51	Mazandaran, Miandorud	26	cvTLSDrMoradi2	Mazandaran, Sari
13	cvTLRahmadi54	Mazandaran, Ramsar	27	cvTL.mashaal66	Mazandaran, Sari
14	cvTLStaherpour38	Mazandaran, Sari	28	cvTLBkalantari92	Mazandaran,Babol

The order of blackberry genotypes on the wells of agarose gel was as follows and was also analyzed in NTSYS 2.02 software in the same way.

The marker index (MI), which indicates marker efficiency, was calculated for each primer using the following formula: PIC × percentage of polymorphism. The Shannon index (I), which indicates diversity for each primer, was determined using expected and observed heterozygosity, the number of alleles, and the number of effective alleles with GeneAlex software version 6.5. Correlation and factor analysis calculations were also conducted using SPSS software version 27.

Results

Correlation between morphological traits

Examining the correlation coefficient between the morphological traits revealed that the growth habit is inversely correlated with the number, size, and direction of spines, as well as the TSS/TA ratio, at a significance level of 1%. This shows that thornless genotypes have better growth habit compared to thorny cultivars. The growth habit also has an inverse correlation with the number of thorns (-0.54**), the size of the thorns (-0.49*), and the direction of the thorns (-0.47**) as well as with the TSS/TA ratio (-0.51**) (P≤ 0.01 level), which indicates that thornless genotypes are more vigorous than thorny cultivars. The number of thorns is also inversely related to traits such as cane diameter, flower length, flower diameter, ripening time, and TA.

In general, thornless genotypes are more late than thorny genotypes, which is consistent with the results of this study. Also, this trait, relative to the size and direction of the thorn, as well as the cross-sectional area of the cane, the fresh weight of the fruit, the TSS/TA ratio, and the seed pod, has a significant direct relationship at the probability level of one percent (Supplementary Table 1). The results of the correlation coefficient for the ripening time trait showed that this trait has a significant positive correlation with flower length (0.61**), flower diameter (0.75**), and acidity (0.55**) (P≤ 0.01 level). This means that the later the blackberry is, the higher its acidity is, and as a result, the taste index decreases. Therefore, in breeding programs, we should look for early thornless cultivars to have a better taste from a marketable point of view. Also, this trait has an inverse and significant relationship with fruit TSS, TSS/TA ratio, and soft seed, which indicates the lower quality of late cultivars compared to early cultivars.

Factor analysis

In this analysis, seven main and independent factors with eigenvalues greater than one accounted for a total of 83.12% of the overall variance (Supplementary Table 2). Based on the results, it can

be stated that the first factor includes traits such as the number, size, and orientation of thorns, cross-sectional area of the cane, the diameter of the cane in winter, flower length, flower diameter, maturity, fresh weight, acidity, and the ratio of TSS to acidity. The second factor comprised the number of leaflets, fruit color, fruit diameter, fruit length, fruit size, seed count, and growth habit. The third factor included traits such as primary canees on the plant, flower size, and fruit TSS content, while factors four, five, and six were characterized by flowering time, presence of float or hollow seeds, and bud blooming, respectively. Finally, the seventh factor included two traits: anthocyanin content and the width of the terminal leaflets (Supplementary Table 2). From the factor analysis, it can be concluded that the combination of the first and second factors explained 59.54% of the variance.

Cluster analysis based on morphological traits

The cluster analysis using the WARD method divided the genotypes into three groups. The thorny cultivars Marion and Silvan were placed in one group with a similarity of less than 20%, distinctly separated from other thornless genotypes by 100% difference. The thornless genotypes were further divided into two categories: genotypes cvTLRahmadi54, cvTLSafzalian81, cvTLSasadi26, and cvTLNurnazari53 from Ramsar and Noor in western Mazandaran and Sari formed the second group, which was distinguished from other thornless genotypes by a 40% difference. These genotypes were differentiated from others based on flower size, fruit color, flavor index, seed count, and dry seed weight. The third group comprised 22 genotypes that could not be distinguished through morphological markers due to a similarity of over 80% (Figure 1).

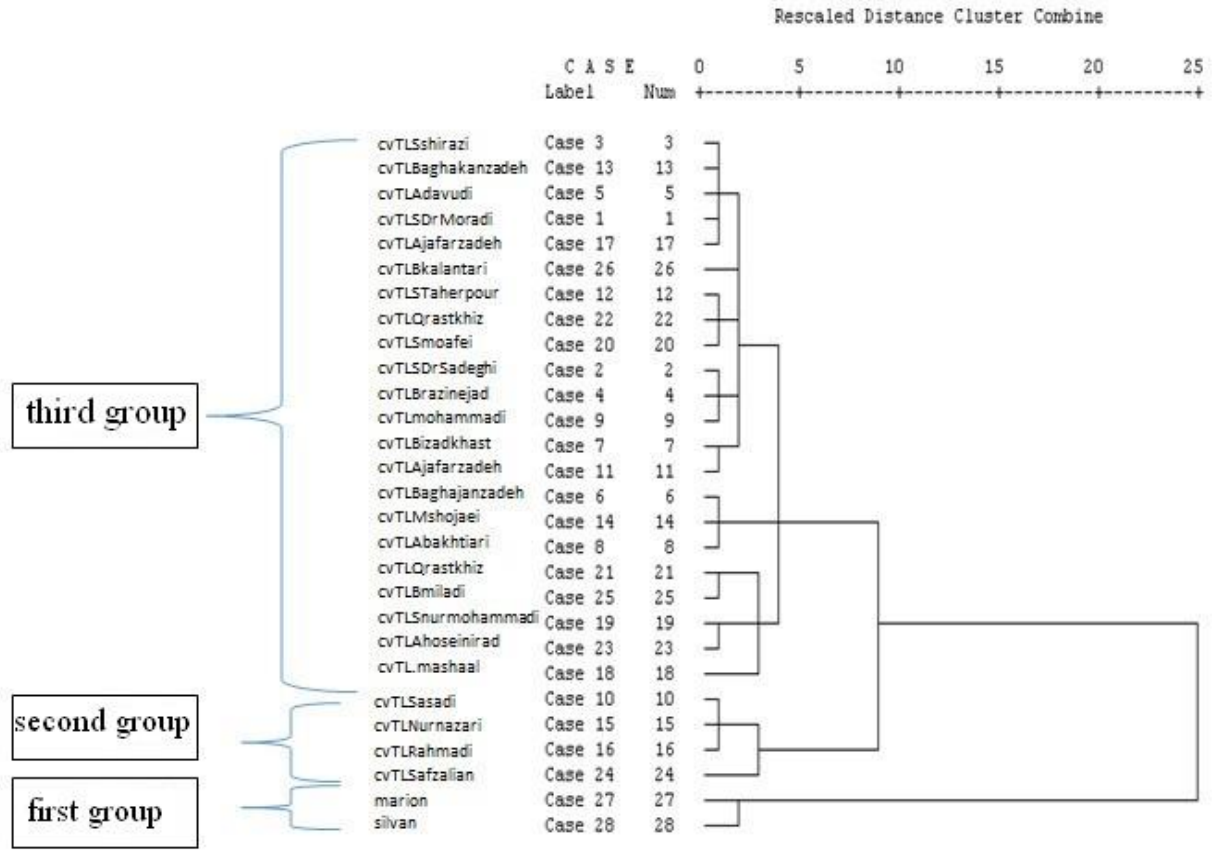

Figure 1. Cluster analysis by Ward method using morphological traits in blackberry genotypes.

Table 3. Results of statistical analyses for each ISSR primer in blackberry genotypes.

Primer Name	Total Bands	Polymorphic Bands	Polymorphic Percentage	Na[1]	Ne[2]	I[3]	Ho[4]	He[5]	PIC[6]	MI[7]
ISSR 1	25	25	100	2	1.2843	0.3315	0.19	0.19723	0.143	4.2188
ISSR 2	29	29	100	2	1.17	0.26	0.151	0.14	0.22	19.31
ISSR 3	39	39	100	2	1.23	0.29	0.23	0.16	0.26	26.21
ISSR 4	22	20	90.9	2	1.3458	0.3371	0.21	0.2115	0.108	4.4868
ISSR 5	18	18	100	2	1.2905	0.3025	0.197	0.1831	0.119	4.1546
ISSR 6	24	24	100	2	1.4783	0.4389	0.289	0.2860	0.144	6.7694
ISSR 7	36	35	97.22	2	1.2970	0.313	0.21	0.1902	0.114	3.9895
ISSR 10	40	40	100	2	1.3213	0.340	0.20	0.20788	0.132	4.5146
ISSR 11	13	13	100	2	1.35	0.38	0.24	0.23	0.30	29.32
ISSR 18	10	10	100	2	1.697	0.5760	0.277	0.3934	0.20	10.627
ISSR 15	53	53	100	2	1.22	0.28	0.17	0.16	0.25	25.11
ISSR 16	20	20	100	2	1.41	0.42	0.39	0.26	0.35	33.24
ISSR 18	34	34	100	2	1.37	0.36	0.242	0.23	0.29	28.38
ISSR 19	43	42	97.67	2	1.22	0.28	0.163	0.15	0.23	20.21
mean	29	28.71	98.98	2	1.33	0.350	0.225	0.214	0.204	16.48

1-number of different alleles, 2- number of effective alleles, 3- shannon's information index, 4- observed heterozygosity, 5- expected heterozygosity, 6- polymorphic information contents, 7- marker index

Molecular marker results

The thornless blackberry genotypes in Iran included imported cultivars which form a rich source of biodiversity, and it is essential to thoroughly identify their genetic structure for preservation and utilization. As shown in Supplementary Figure 1, the mentioned markers exhibited significant polymorphism and produced a clear and high-quality pattern, indicating the potential of these markers for further studies in blackberry genotypes. The results indicated that out of 20 primers used on five randomly selected genotypes, 14 primers generated distinct and clear bands, and the most suitable annealing temperatures for the primers during PCR reactions were 54 and 55°C. All 14 primers used exhibited desirable polymorphism in the studied population. A total of 406 bands were produced, of which 402 were polymorphic. The number of bands generated varied from 40 bands with primer number 10 to 10 with primer number 18. The average number of bands for each primer across the examined genotypes was 29. The average percentage of polymorphic bands in this experiment for each primer was 98.98%, with the lowest being 90.9% for primer number 4. Based on the obtained information, the highest polymorphic information content (PIC) was related to primer number 16, with a value of 0.35 Therefore, this primer also had the highest marker index (MI) at 33.24. Primer number 7 had the lowest PIC and MI, with values of 0.114 and 3.9, respectively (Table 3).

Cluster analysis of molecular data

The results of the comparison between the cophenetic coefficients obtained from three algorithms—UPGMA, complete linkage, and single linkage—with the Jaccard, Dice, and Simple similarity coefficients showed that the Jaccard similarity coefficient, based on the UPGMA algorithm, had the highest cophenetic correlation for grouping the blackberry genotypes (Supplementary Table 3). The value of this coefficient was 0.84, indicating a good fit; therefore, the Jaccard similarity coefficient was used for constructing the cluster diagram.

Similarity matrix

According to the results obtained from the Jaccard similarity matrix derived from the UPGMA algorithm, the highest similarity was observed between two samples collected from Sari (Sadeghi and Taherpour), which was approximately 70%. The lowest similarity was between the thorny genotype (control) Marion and cvTLBkalantari92, with a value of 0.21. Given that more distant cultivars have greater genetic differences,

performing crosses between more distant cultivars, provided they have complementary traits, can enhance hybrid productivity and the potential for heterosis. However, since only 14 markers cover a small portion of the blackberry genome, it cannot be expected that this clustering will fully differentiate the genotypes based on their origin and genetic traits (Dossett et al., 2012). At the same time, it is evident that the differentiation of genotypes based on the mentioned markers has resulted in a fairly acceptable grouping.

Principal component analysis (PCA)

The principal component analysis revealed that the first component, which is the largest, explained 24% of the total diversity. In total, the first three components accounted for 30.8% of the total variance. While this percentage may not be sufficient for drawing strong conclusions, it still encompasses meaningful patterns of genetic diversity among the blackberry genotypes studied. It is important to note that the relatively low percentage of explained variance may be attributed to the uneven distribution of markers across the genome. Therefore, future analyses might benefit from utilizing a greater variety of markers to achieve a more comprehensive understanding of the genetic diversity present in these populations (Supplementary Table 4, Figure 2).

The gathering of people in one point of the plot shows the genetic similarity of those people. In fact, the purpose of displaying two-dimensional and three-dimensional visualizations of indicator data is to reduce the amount of data in order to clarify the relationships between two or more variables and justify the changes in the original and primary data by means of a limited number of new independent variables. The name is the main coordinate.

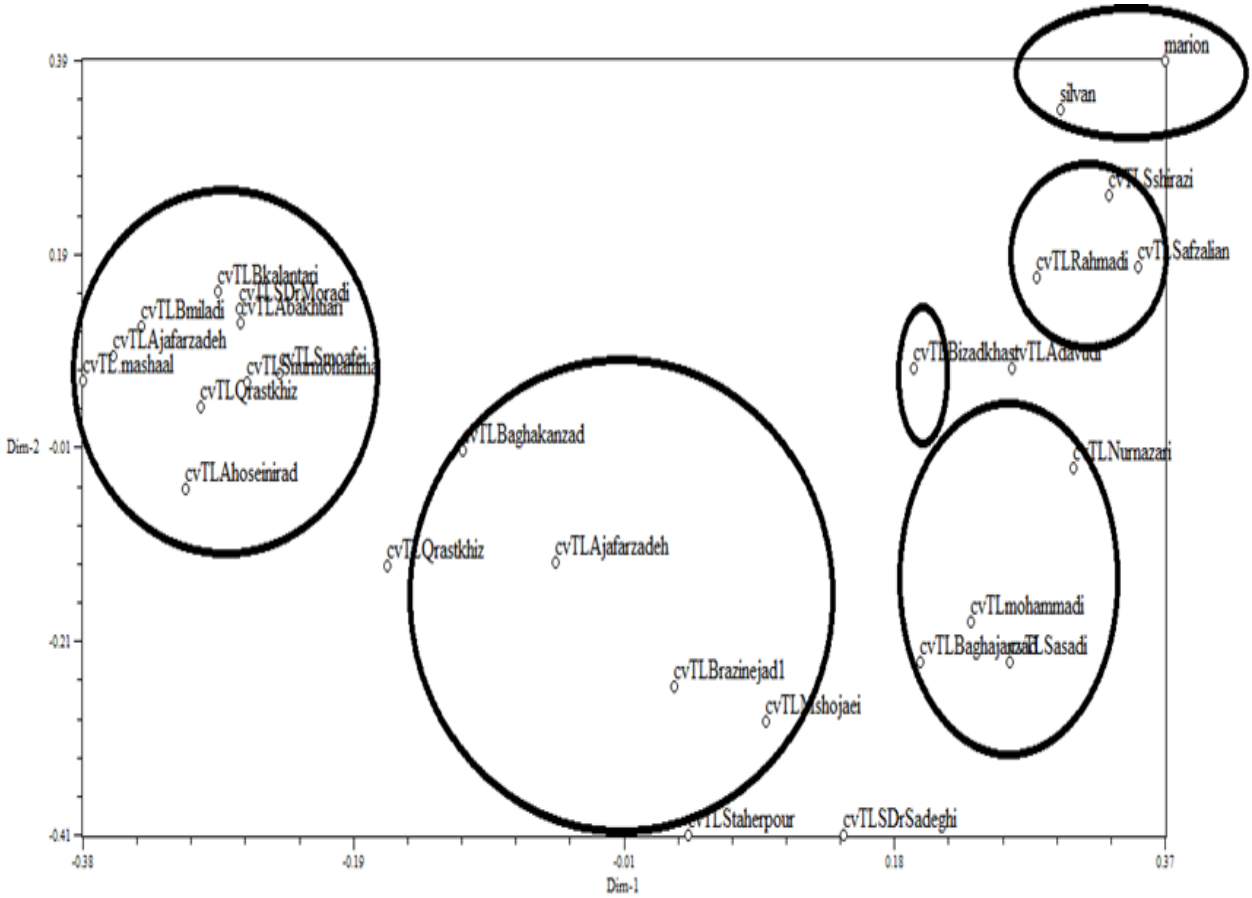

Figure 2. The two-dimensional diagram of decomposition into principal coordinates based on the Jaccard similarity matrix.

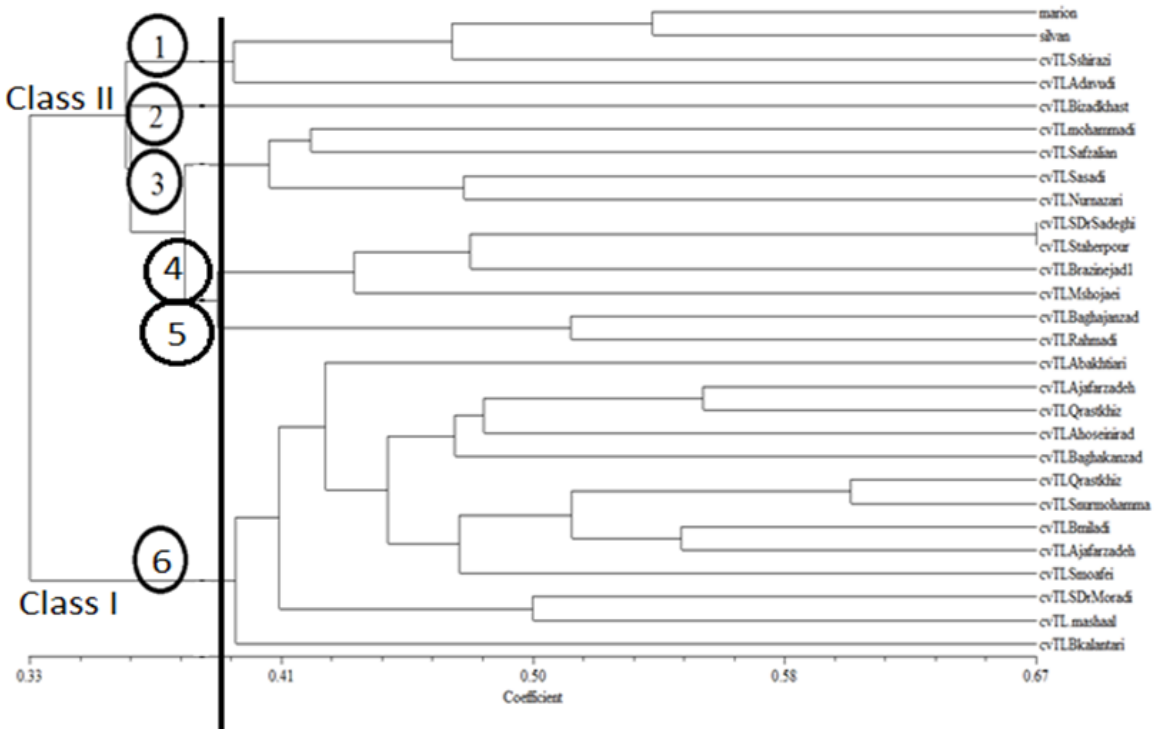

Figure 3. Dendrogram drawn based on UPGMA method and Jaccard similarity coefficient for 28 blackberry genotypes.

Cluster analysis

In the first group, the prickly cultivars Marion and Silvan along with cvTLSshirazi13 and cvTLADavudi18 were placed, and the genotype Izadkhast (cvTLBizadkhast23) alone was placed in the second group. Samples from Noor and Sari In the data related to molecular markers, the justification of the small amount of variation between the markers by the first few principal components is proof of the independence of the places and markers studied and their distribution in different regions of the genome. Therefore, if the markers are selected from different parts of the genome, the connection between them will be low, and as a result, more components are needed to justify their total changes. In examining the diversity, the best case is that the markers have a distribution of genetic DNA using data related to uniform and suitable markers in the genome and can be sampled from the entire genome. Therefore, if the markers are selected from different parts of the genome and their correlation is low, more components are needed to justify the total changes (Idrees and Irshad, 2014). The results of principal component analysis showed that the markers used in this research are scattered in different regions of the genome, and the information obtained from principal component analysis confirms that these starting compounds have a relatively good distribution at the genome level. Analysis of principal components was done in order to compare it with the results of cluster analysis. According to the two-dimensional diagram of the analysis of the main coordinates based on the Jaccard similarity matrix, the genotypes can be divided into five groups (Figure 2). formed the third group. The fourth group included Sadeghi and Taghipur cultivars (70% similarity) and Shojaei from Sari and Aghajanzadeh cultivar from Babylon. The fifth group included cultivars from Ramsar and Babylon. In the sixth group, cultivars from Amal and Babylon, Qaimshahr and Sari were also included (Figure 3).

Discussion

The results of the morphological markers indicated that the thornless genotypes were more vigorous

than the thorny cultivars, which was attributed to the differences in their semi-upright growth habit compared to the trailing growth habit of the thorny cultivars. However, this growth habit also resulted in the thornless genotypes being later-maturing than the thorny genotypes. The correlation coefficient (0.55+) suggested that as the blackberry becomes later-maturing, its acidity increases, which in turn reduces the flavor index. The results of the factor analysis indicated that all measured traits (26 traits) could be summarized into 7 factors, with the first two factors (thorn, flower, and fruit ripening) accounting for 54.59% of the variance.

Cluster analysis using the WARD method divided the genotypes into three groups. The thorny cultivars Marion and Sivan grouped with less than 20% similarity, distinctly separated from other thornless genotypes by 100% difference. The thornless genotypes were further divided into two categories. The genotypes cvTLRahmadi54, cvTLSafzalian81, cvTLSasadi26, and cvTLNurnazari53, which originated from Ramsar, Noor in western Mazandaran, and Sari, formed the second group, separated from other thornless genotypes by 40% difference. This group of genotypes differed from others in terms of flower size, fruit color, flavor index, seed number, and seed dry weight. The third group included 22 genotypes that could not be distinguished due to over 80% similarity based on the morphological markers.

The results showed that the average percentage of polymorphic bands in this experiment for each primer was 98.98%. In this context, Abdi et al. (2021) reported that the average percentage of polymorphism across various markers was 99.76%. the range of polymorphic information content (PIC) for dominant markers, such as ISSR, was from zero to 0.5, while for co-dominant markers, this range was from zero to 1. The average PIC obtained for all primers was 0.2, indicating that they contained relatively satisfactory polymorphic information. The highest PIC was associated with primer number 16, which had a value of 0.35. The level of polymorphism is one of the important indicators for comparing different markers in terms of their discriminatory power. A high value of this criterion indicates significant polymorphism at a marker locus, which plays a crucial role in differentiation and discrimination. Therefore, markers with high PIC are beneficial for distinguishing closely related genotypes (Santhosh et al., 2009). Abdi et al. (2021) reported. The Polymorphic Information Content (PIC) range for dominant markers, such as the ISSR, is 0 to 0.5, and for co-dominant markers, this range is between zero and one in blackberry. The average PIC is 0.29 and indicates adequate polymorphism. Primer 7 obtained the highest PIC (0.39). Considering that the MI marker index is a general criterion for determining the efficiency of a marker in estimating polymorphism, primer number 16, with the highest rate (33%), indicates a high level of effectiveness and better discriminatory power of this primer in determining genetic distance. This suggests that the aforementioned primer was able to better account for the genetic diversity within the population and can be recommended as a superior primer for this study.

Estimation of the cophenetic correlation coefficient is used to compare different clustering algorithms, and the method with the highest cophenetic correlation coefficient is considered the most suitable for analysis. The highest cophenetic correlation coefficient obtained in this study was 0.84, based on the Jaccard similarity coefficient and the UPGMA algorithm, indicating a good fit according to the defined range. According to the correlation coefficient, a value of r≥0.9r≥0.9 shows a very good fit, 0.9≥r≥0.80.9≥r≥0.8 indicates a good fit, 0.8≥r≥0.70.8≥r≥0.7 signifies a weak fit, and r≤0.7r≤0.7 represents a very weak fit. However, a low cophenetic correlation coefficient for molecular data does not imply inefficacy of the related algorithm; rather, it indicates the disruption caused by the presence of missing data. In fact, this coefficient reflects how much of the initial information or input matrix has been successfully transferred to the tree diagram. It essentially shows the correlation between the input and output matrices. In other words, this coefficient indicates the extent of similarity between the dendrogram and the similarity matrix. The larger the obtained rr value, the more closely aligned the dendrogram is with the similarity matrix, and they fit well with each other (Mohammadi and Prasanna, 2003).Mohammadi (2006) stated in a report analyzing molecular data from the perspective of genetic diversity that the UPGMA algorithm provides reliable results in

concordance with the phylogenetic relationships of genetic materials.

Mahjoob et al. (2015) utilized 36 genotypes of the Brassica genus and 13 ISSR markers to identify effective ISSR markers for distinguishing species within the genus. The results indicated that these markers could be used to identify inter-species and intra-species differences in phylogenetic studies.

The differences in classification resulting from cluster analysis and principal component analysis may arise from the nature of principal component analysis, in which missing data are simply replaced with the average of the corresponding variable when calculating the distance or similarity matrix. As a result, genotypes with a higher amount of missing data tend to cluster closer to the center of the relevant group. In the case of molecular data, the first two or three components account for approximately 10-20% of the variability related to the initial variability of the markers. Although these results may not be statistically suitable for PCA and graphical representation, they represent a desirable sampling of markers from the overall genome from a genetic perspective. This means that each of the markers used is derived from different parts of the genome, thereby exhibiting less correlation (Mohammadi and Prasanna, 2003). The first three components were able to account for a total of 30% of the overall variability, indicating a relatively adequate distribution of ISSR markers across the entire genome.

Cluster analysis of the blackberry genotypes divided them into six groups, with an approximate similarity of 0.38. In the first group, the thorny cultivars Marion and Silvan, along with cvTLSshirazi13 and cvTLAdavudi18, were included, suggesting that although morphological markers have less accuracy in distinguishing thorny from thornless cultivars, they are still sufficient. However, the use of molecular markers revealed the actual similarities between these two groups of blackberry cultivars, which relate to their subgenus similarity (*Rubus* sub *Rubus*). The highest similarity was found between the blackberry genotypes cvTLSDrSadeghi6 and cvTLStaherpour38, which had a similarity of 0.67, both collected from the Sari region. The lowest similarity was between the thorny genotype (control) Marion and cvTLBkalantari92, with a value of 0.21. The samples from Rashtakhiz and Dr. Moradi, which showed about 70% similarity with 10 markers in Abdiyan's work, revealed a difference of 60% in the current research using 14 markers. The similarity between a sample from Babol (cvTLBkalantari92) and a sample collected from Sari (cvTLSDrMoradi2) in Abdi et al. (2021) was 40%, which aligns with the findings of the present study. Among two samples collected from Babol, one from Amirkola (cvTLBizadkhast23) and the other from the western Bandpay area identified by Abdiyan, a 60% difference was recorded, whereas in this study, a 67% difference was observed.

Abdi et al. (2021) named the samples from Sari and Qaemshahr as Everthornless and noted the presence of genetic differences between the two samples collected from Babol due to genetic diversity in a geographic range. The results of Abdi et al. (2021) indicated that the Everthornless cultivar is approximately 60% similar to the Thornlessevergreen cultivar, while in the present study, using more markers, these two cultivars showed only 43% similarity. It seems that the good adaptability of the Everthornless cultivar to the climate of Amol has led to its highest frequency in that area and its presence in the sampling conducted. Additionally, the Thornless evergreen cultivar is also present in the same region. Amol has proven to be more suitable in terms of climate for older blackberry cultivars. Continuing the breeding efforts for thornless cultivars to overcome the dominance of the thornless trait, crosses and hybridizations between the used cultivars have been carried out to introduce the thornless Merton cultivar, which has a dominant gene for the thornless trait. Consequently, as observed in the second category, approximately 37% similarity was noted, which includes five groups. The first group contains two thorny cultivars, one being a parent and the other its offspring, which share over 50% similarity. The remaining thornless cultivars are grouped with a distance relative to these two cultivars in four additional groups. Two cultivars from Sari are the most similar. The Izadkhast cultivar from Babol (cvTLBizadkhast23) is placed alone in a separate group. Samples from Noor and Sari made up the third group, while samples from Mahmoudabad, Babol, and Sari were in the fourth group. Two samples collected from Ramsar and

Babol, which have a different fruit color from the others and are reported by farmers to be earlier and more sensitive to biotic and abiotic stresses, were placed alone in the fifth group. These two samples were called Ramezan-berry by the farmers after the worker who found them. The highest similarity, approximately 70%, was observed between the two samples collected from Sari (cvTLSDrSadeghi6 and cvTLStaherpour38).

Conclusion

The overall results of this study indicate that morphological markers perform well in distinguishing thornless cultivars from thorny ones and have some capability in classifying thornless cultivars. However, these markers were insufficient for differentiating species from one another. ISSR molecular markers successfully identified thorny and thornless genotypes at the subgenus level and were also able to separate the shimmer sample from the thornless samples that were obtained. Based on the information collected from the molecular markers, two categories of genotypes were identified. The first category includes the initial generation of thornless and belongs to the species *R. laciniatus*. The second category pertains to subsequent crosses aimed at producing the Merton cultivar. In this category, different groups were observed, either relating to various thornless Merton cultivars or reflecting the diversity that has arisen in the Mazandaran climate. Overall, these results indicate that the existing diversity in these cultivars is a result of various human and climatic factors, and they can be utilized in breeding program planning.

Supplementary Materials

The supplementary material for this article can be found online at: https://www.jpmb-gabit.ir/article_720852.html.

Supplementary Figure 1. ISSR banding pattern resulting from the multiplication of thornless blackberry genotypes using primers.

Supplementary Table 1. Correlation coefficient for studied traits.

Supplementary Table 2. Decomposition into factors.

Supplementary Table 3. Cophenetic coefficient resulting from algorithms with similarity coefficients.

Supplementary Table 4. Decomposition into PCA principal components.

Author Contributions

Conceptualization, M.H. and H.M.; methodology, K.Sh.; software K.Sh.; validation, M.H., H.M. and K.Sh.; formal analysis, K.Sh.; investigation, K.Sh.R; resources, H.M.; data curation, K.Sh.R; writing—original draft preparation, K.Sh.R; writing—review and editing, M.H. and H.M.; visualization, K.Sh.; supervision, M.H. and H.M.; project administration, K.Sh.; funding acquisition, H.M., H.M. and K.Sh.R (thesis funding).

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Conflict of Interest Statement

The author declares no conflict of interest.

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مقاله پژوهشی

بررسی تنوع ژنتیکی برخی از ژنوتیپ های تمشک سیاه بدون خار با استفاده از نشانگرهای مولکولی ISSR

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چکیده: تنوع ژنتیکی در تمشک سیاه برای بهبود کیفیت و عملکرد بسیار مهم است. این مطالعه ژنوتیپ های تمشک سیاه بدون خار را با استفاده ۲۶ صفت مورفولوژیکی و ۱۹ نشانگر تکراری بین توالی ساده (ISSR)برای ارزیابی تنوع و روابط ژنتیکی ارزیابی می‌کند. در مجموع ۲۸ ژنوتیپ تمشک سیاه شامل هر دو نوع خاردار و بدون خار مورد تجزیه و تحلیل قرار گرفت. ۱۴ آغازگر ISSR از بین ۱۹ پرایمر بر اساس ظرفیت آنها برای تولید باندهای چندشکلی انتخاب شدند. یافته‌ها کارایی نشانگرهای ISSR را در تشخیص ژنوتیپ‌های تمشک سیاه خاردار و بدون خار در سطح زیرجنس نشان داده و به طور موثر نمونه‌های بدون خار مشتق شده از ژنوتیپ‌های شیمری را متمایز می‌کنند. در مجموع ۴۰۶ باند تولید شد که ۴۰۲ باند چند شکلی بودند. میانگین درصد باندهای چندشکلی برای هر آغازگر در این آزمایش ۹۸.۹۸ درصد بود و بیشترین مقدار اطلاعات چندشکلی (PIC) مربوط به پرایمر شماره ۱۶ بود که دارای مقدار ۰/۳۵ بود. آنالیزها دو مورد را نشان داد. یافته‌ها نشانگر کارایی نشانگرهای ISSR در تشخیص ژنوتیپ‌های تمشک سیاه خاردار و بدون خار در دو گروه اصلی می‌باشد: اولی مرتبط با نسل‌های اولیه بدون خار *R. laciniatus*، و دومی شامل تلاقی‌های مربوط به رقم مرتون است. به طور کلی، تنوع ژنتیکی شناسایی شده در بین ارقام تمشک سیاه، کاربردهای ارزشمندی را در برنامه‌های اصلاحی و اصلاحی نشان می‌دهد.

کلمات کلیدی: *Rubus laciniatus*، مرتون بدون خار، تنوع ژنتیکی، نشانگر مولکولی.

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