Morphological and Molecular Characterization of Iranian Wild Blackberry Species Using Multivariate Statistical Analysis and Inter-Simple Sequence Repeats (ISSR) Markers

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Abstract

This study was carried out to estimate the genetic diversity and relationships of 74 Iranian blackberry genotypes assigned to 5 different species using inter-simple sequence repeats (ISSR) marker analysis and morphological trait characterization. Sixteen traits including phenological, vegetative and reproductive attributes were recorded, and 10 ISSR primers were screened. Results showed that yield and leaf width have the highest and lowest genetic diversity, (diversity index = 62.57 and 13.74), respectively. Flowering and ripening date recorded as traits having the strongest correlations (r = 0.98). The selected 10 ISSR primers produced a total of 161 amplified fragments (200 to 3500 bp) of which 113 were polymorphic. The highest, lowest and average PIC values were 0.53, 0.38 and 0.44, respectively. Principle component analysis (PCA) based on morphological traits showed that the first six components explained 84.9% of the variations of traits studied, whilst the principal coordinate analysis (PCoA) based on ISSR data implied the first eight principal coordinates explained 67.06% of the total variation. Cluster analysis based on morphological traits and ISSR data classified all genotypes into two and three major groups, respectively, and the distribution pattern of genotypes was mainly based on species and the geographic origins.

Introduction

Rubus is one of the most diverse genera in the plant kingdom with 12 subgenera and over 500 species[1]. These species are distributed widely in temperate regions of Europe, South and North America and Asia[2]. Red raspberries (*R. idaeus* L., Subgenus: *Idaeobatus*), black raspberries (*R. occidentalis* L., Subgenus *Idaeobatus*) and blackberries [*Rubus* spp., Subgenus *Rubus* (formerly *Eubatus*)], are the most widely grown commercial *Rubus* species[3].

Rubus species have been cultivated or harvested from wild stands, as a food source (fresh, pulp, jam, juice, etc.), and for medicinal and therapeutic properties[4]. In every region of the world that *Rubus* is native to, the local cultivation and industries were based on their local species, such as trailing raspberry (*R. parvifolius* L.) in Asia, and arctic raspberry (*R. strigosus* L.) in North America. Although, the species used in developing the cultivated types are largely American or European in their origin, Asia has a wealth of diversity that should be useful in breeding programs for creating new cultivars with superior traits[4]. The information about worldwide production of blackberries is limited but it has been reported that the production is rapidly increased in the past decades with an estimated 140,292 tons commercially harvested from 20,035 ha in 2005 year[1].

Like other crops, blackberry breeding programs have been limited by a lack of variation for important traits in germplasm[5]. Acquiring information about the genetic structure of wild populations as sources of genetic diversity is the best strategy for identification of superior and promising genotypes to create new cultivars and improve the breeding program efficiency[6]. Various techniques have been used to evaluate plant genetic variability including morphological trait characterization[7, 8, 9] (Ahmed et al., 2014; Maro et al., 2014; Dossett and Finn, 2016) and molecular marker analysis[6, 10, 11]. Dossett and Finn (2016) [9] evaluated the genetic diversity of wild populations of *R. occidentalis* L. and reported a wide range of variation in plant vigor, morphological traits, flowering and ripening date, and fruit weight beyond those of existing commercial cultivars for every trait, confirming great potential to be utilized in future breeding programs. High variation of berry weight and dimensions were reported in investigation of wild populations of *R. ellipticus* Smith.[12]. Innis et al. (2011)[13] assessed the genetic diversity of *R. argutus* and *R. phoenicolasius* using ISSR markers and reported higher variations between sites than within site in both species. Also Lee et al. (2016)[11] used SSR markers to determine the genetic relationships between 69 *Rubus* accessions of six *Rubus* species and showed that *Rubus* accessions were subdivided into six sub-populations.

Iran is one of the most important areas in terms of plant genetic resources in particular for wild edible fruits like blackberry. Based on the latest survey by Khatamsaz (1992)[14] *Rubus* germplasm in Iran comprise 8 species assigned to two subgenera. Seven out of these 8 species including *R. sanctus* Schreber (syn. *R. anatolicus* (Focke) Hausskn.), *R. caesius* L., *R. hirtus* Waldst. & Kit. (syn. *R. lanuginosus* Stev. ex Ser), *R. dolichocarpus* Juz. (syn. *R. ochtodes* Juz.), *R. discolor* Weihe and Nees (syn. *R. armeniacus* Focke), *R. persicus* Boiss (syn. *R. raddeanus* Focke), *R. hyrcanus* Juz., belong to the subgenus *Rubus*, whilst the only herbaceous type, *Rubus saxatilis* L., belongs to the Subgenus *Cylactis*). Five inter-specific natural hybrids also were reported[14].

While the genetic diversity of blackberry has been documented in many parts of the world, there are only a few reports with limited number of species (evaluating only one species) or limited geographic distribution of Iranian *Rubus* species[6, 15, 16]. Sedighi and Rahimmalek (2015)[6] used the ISSR marker and morphological trait characterization to assess genetic diversity in a few populations of *R. hyrcanus* in the Caspian area of Iran. They found a narrow genetic base and relatively high genetic differentiation for *R. hyrcanus* genotypes studied. They also concluded that the morphological analysis corresponded to those obtained through molecular analyses in most cases. Furthermore, Gharaghani et al. (2014) [15] evaluated the variation of fruit characteristics of two populations of *R. sanctus* Schreb., genotypes collected from two distinct regions in north and south of Iran. They observed high variation as well as significant differences in fruit attributes between the genotypes collected from two different regions of Iran. Recently, Yazdanpour et al. (2018) [16] investigate the phenotypic variations of one *Rubus* species in Babol county of Mazandaran province, in the Caspian region. They referred to this species as black raspberry which seems to be incorrect naming. Based on the photo presented in their paper this species seems to be *R. sanctus*, which is a blackberry rather than a black raspberry. They reported high levels of variation in almost all of measured phenotypic attributes including plant and fruit characteristics. Garazhian et al. (2020)[17] investigating the fruit bio-chemicals and antioxidant activity in same set of the genotype being studied herein, and reported that there is a vast majority of genetic diversity available for fruit bio-chemicals antioxidant activity in the Iranian wild *Rubus* species.

However, apart from the reports on taxonomic classification, mainly based on phenotypic characterization, there is not enough comprehensive information on the phenotypic and genotypic diversity as well as relationships of the available genetic resources of the wild *Rubus* species in Iran. Accordingly, the objectives of this study were to characterize phenotypic diversity along with ISSR molecular markers to evaluate genetic diversity and relationships of 72 accessions of the Iranian wild blackberries representing five species. Objectives also addressed conducting correlation analyses among the measured traits and determining the relationships between the genotypes and species by using cluster analysis based on morphological and molecular data. These were performed to provide necessary information for the domestication and further breeding of this valuable crop.

Materials And Methods

2.1. Plant Material

Seventy-two genotypes assigned to five Iranian wild *Rubus* species were selected from the first Iranian blackberry repository established in 2010 at the research field of the Department of Horticultural Science, School of Agriculture, Shiraz University, Shiraz, Iran. The geographic information of the experimental site is as follow; Longitude 52° 35' 0.73"; Latitude 29° 43' 43.56"; and Altitude 1791.4 (m). The average long-term annual rainfall is 336 mm, the soil type is silty-loam, and plants were irrigated using drip irrigation based on the common practices of the region. The accessions of this collection were collected from 10 provinces across Iran (Fig. 1) and include five different species, *R. sanctus, R. hirtus, R. caesius, R. discolor* and *R. persicus*. The geographic information of the origin of studied genotypes is presented in Table 1.

2.2. Morphological Characterization

Sixteen morphological traits of 57 genotypes (some of the accessions did not bear fruits so that, we merely recognized genotypes that their morphological traits were measurable) were studied during two growing seasons (2014 and 2015). Some of the characterizations were performed according to the guidelines provided by the International Union for the Protection of New Varieties of Plants[18]. Cane morphological traits included cane length (cm), internode length (mm), number of nodes, and spine density (No. of spine/cm of cane), were measured. Observations of flowering and fruit ripening date on floricanes were also recorded. Dates were recorded for the appearance of the full bloom and the fully colored (black color) fruits. Flowering and ripening dates were determined as date in which flowering or ripening took place. Number of fruit inflorescence/plant and number of fruits/ inflorescence, were counted as they emerged on the canes. Leaf dimensions included leaf length (mm), leaf width (mm), and petiole length (mm), of mature leaves (20 leaves/accession per replicate) representing the typical characteristics of each genotype were randomly collected from several parts of the shrubs in the summer. Fully mature fruits (black colored) were collected from different fruit clusters of the shrubs (20 fruits/accession per replicate), and immediately transferred to a refrigerator for future measurements of fruit weight (g), fruit length (mm), fruit width (mm), and number of drupelets/fruit. Characteristics such as leaf and fruit dimensions were measured by using a digital caliper. The fruit weight was measured with an electronic scale with 0.01 g precision. The total yield (g/plant) was calculated by summing the repeated harvest of ripened fruits as well as the weight of fruits sampled for other measurements.

2.3. DNA Extraction

DNA was extracted from fully expanded young leaves using the modified CTAB procedure as described by Khanuja et al. (1999)[19]. The quantity and quality of extracted DNA was estimated with electrophoretic and spectrophotometric methods. The DNA was diluted to a working concentration of 5 ng/µl with sterile, distilled water.

2.4. ISSR Primers and PCR Reaction

Fifteen ISSR primers screened and 11 primers that produced higher number of reproducible bands were selected (Table 4). PCR reactions were carried out in a volume of 20 µl that contained 2 µl of DNA (5 ng/µl), 3 µl of ISSR Primer (5 ng/µl), 8 µl of Master-Mix (Metabion-Germany) and 7 µl of sterile double distilled water. The optimum annealing temperature was determined for each primer (Table 4). The PCR cycling conditions for all studied primers were 3 min initial denaturation in 95 °C; followed by 35 cycles of 1 min at 95 °C, 1 min at the specific annealing temperature (52-57.5 °C), 1 min at 72 °C and the final extension step of 8 min at 72 °C. The amplified DNA fragments were separated in a 1% agarose gel at 100 W for 3 h in 1 × TBE buffer (100 mM Tris–Borate, 2 mM EDTA in pH 8.0) and stained with SimplySafe (EURX company).

2.5. Statistical Analysis

At first, morphological data pertaining to two years were compared to determine the significance of each year, since the effect of the year and year × genotype interaction was not significant at $p < 0.01$, for almost all of the measured traits, the average data of two years were used. The range and estimated diversity index (DI) were used as indicators of variability. The simple correlation coefficient was calculated to determine the relationships between the studied morphological variables using the Pearson correlation coefficient by Minitab, version 16[20]. Relationships among genotypes were investigated by principal component analysis (PCA) using Minitab statistics software. The morphological similarity coefficients according to Euclidian distance matrix using Ward method were calculated using Minitab, version 16 and the dendrogram was constructed.

A scatter plot was created according to the PC1 and PC2 using PAST statistics software[21]. The analysis of polymorphic ISSR bands in each gel were scored as absent (0) or present (1). The cluster analysis and principal coordinate analysis (PCoA) were conducted by the NTSYs software Version 2.01[22]. Polymorphic information content (PIC) was calculated using this formula; $PIC_i = 2f_i (1 - f_i)$, in which f_i is the percentage of the i^{th} present band (Anderson, 1993). Genetic similarity among all genotypes was calculated according to Simple Matching (SM) similarity index, using the similarity of qualitative data (Simqual) routine. The dendrogram was constructed using the unweighted pair group method average (UPGMA) clustering procedure. Also cophenetic correlation coefficients were calculated by using COPH and MXCOMP procedures for each combination.

Results

3.1. Morphological Diversity Analysis

3.1.1. Variations of Morphological Traits

Based on combined analysis of variance (ANOVA) there was no significant difference among studied traits in two years, but significant variations were observed in most of the measured traits among genotypes. Year × genotype interaction also was not significant (data not shown). Considering cane

characteristics, cane length varied from 73 cm (Jade Kandovan; *R. sanctus*) to 197 cm (Masule; *R. persicus*). The number of nodes were between 15.5 (Jade Kandovan; *R. sanctus*) to 48.5 (Kazerun-Eslamabad; *R. sanctus*) and internode length ranged from 30.0 mm (Chaloos; *R. sanctus*) to 85.6 mm (Masule; *R. persicus*). Variation of spine density was recorded between 0.4 (Gardane Heyran 5; *R. hirtus*) to 4.45 per/cm of cane (Aliabad Katul 1; *R. caesius*) (Table 2, A and B).

Regarding all the genotypes, the leaf length was from 30.2–94.2 mm, leaf width varied from 28.2 to 59.4 mm, and petiole length ranged from 14.5–52.6 mm (Table 2). The highest values of all leaf parameters were measured in Masule (*R. persicus*), while the lowest values of leaf length and width were measured in Kamfiruz (*R. sanctus*). Jadeh Kandovan (*R. sanctus*) had the lowest petiole length.

Phenological traits also showed high variation among genotypes. The flowering time varied from May 14th (Gerdbisheh, *R. hirtus*) to August 1st (Masule; *R. persicus*), while genotypes were harvested from May 30th (Gerdbisheh, *R. hirtus*) to August 24th (Anzali 2, *R. hirtus*) (Table 2).

Number of inflorescences/plant varied from 17.5–75 (Kamfiruz and Sanandaj; *R. sanctus*), while number of fruits/inflorescence ranged from 3–16 (in Babolsar 2 and Astara 4; *R. sanctus*) among all studied genotypes. The range of fruit length was 6.5–16.2 mm, while the fruit width was between 05.3–15.5 mm (Table 2). Collections from Ganjname and Abas Abad (both of which belong to the *R. hirtus*) were recorded as genotypes having the highest and lowest fruit dimensions, respectively. The highest value for fruit weight was 1.38 g (Sepidan-Roodbal; *R. sanctus*), while the lowest value for fruit weight was 0.14 g (Babolsar 2; *R. sanctus*). Number of drupelets/fruit showed a high level of variation ranging from 5.5–36.0 drupelets per fruit. Gardane Heyran 1 and Babolsar 2 (both of which belong to the *R. sanctus*) were the genotypes having the highest and lowest number of druplets/fruit, respectively (Table 2). The highest (944.7 g/plant) and lowest (16.8 g/plant) yield were harvested from Sanandaj 1 and Babolsar 2 genotypes, both of which are accessions of *R. sanctus*.

Considering the estimated diversity index (DI), the studied vegetative, phenological and pomological attributes could be categorized into three groups having high (DI ≥ 40), medium (DI from 20–40) and low (DI ≤ 20) diversity. Based on this grouping, yield (DI = 62.57) and spine density (DI = 53.54) recorded as characteristics having the highest diversity, while ripening date, leaf width, fruit width, flowering date, fruit length, leaf length, cane length and internode length were categorized as traits having low diversity. Other groups of evaluated characteristics including number of nodes, petiole length, fruit weight, number of fruits/inflorescence, number of drupelets/fruit and number of inflorescences/plant were found to have medium diversity (Table 2).

3.1.2. Correlation among Traits, Principle Component Analysis (PCA) and Cluster Analysis

The analyses of correlation between traits are presented in Table 5. As expected the cane lengths showed a significant positive correlation (r = 0.76) with number of nodes and interestingly a significant but negative correlation (r = − 0.32) with spine density. Leaf length, leaf width, and petiole length were also highly correlated to each other (ranging from r = 0.71 to r = 0.79). Leaf length and petiole length also had positive correlation with internode length (r = 0.32 and r = 0.37, respectively). Flowering and ripening dates were highly correlated to each other (r = 0.98).

Not surprisingly yield was positively correlated to its components including number of inflorescences (r = 0.60), number of fruits/inflorescence (r = 0.69), fruit weight (r = 0.54) and number of drupelets/fruit (r = 0.48). Interestingly, yield and many of its component were negatively correlated to spine density (ranging from r = − 0.25 in number of fruits/inflorescence to r = − 0.50 in fruit weight). Fruit weight had positive correlations with fruit length (r = 0.42), fruit width (r = 0.40), and number of drupelets/fruit (r = 0.42).

The results of principle component analysis (PCA) showed the first six components explained 84.9% of the variations of traits in the studied genotypes (Table 3). The first component, which accounted for 25.3% of total variations, featured mainly reproductive attributes including fruit weight, yield, and number of drupelets, and fruit length and leaf length. The second component, explained 15.8% of total variations (flowering date, ripening date and number of nodes) (Table 5).

The bi-plot of PCA grouped the samples into their phenotypic resemblance and morphological characteristics. Results showed that all genotypes were divided into two main categories based on the first two components according to loading plot (Fig. 2);

The cluster analysis of genotypes on the basis of morphological characteristics using Wards method is presented in Fig. 3. According to the dendrogram, the studied genotypes were divided into two main clusters. The first cluster which contains the majority of *R. sanctus* accessions, was divided into 2 sub-clusters. In the first sub-cluster despite the Abidar and Sanandaj1 (from Kurdistan Province in west of Iran) other genotypes were from Caspian Sea region in north of Iran. Most of the genotypes originated from west and south of the country and a few genotypes from northern Iran (including Talesh 2, Namak Abrud 3, Jadeh Haraz and Kelachay-Polrood) were placed in the second sub-cluster (Fig. 3). The second cluster was contained 20 genotypes including 6 out of 8 genotypes of *R. hirtus* (except Gerdbisheh and Ashkvarat 1), 2 genotype of *R. persicus*, 2 out of 4 genotypes of *R. caesius* (except Gorgan 5 and Aliabad Katul 2) and 10 genotypes of *R. sanctus* species. Except for Nourabad, which is from Fars province, other *R. sanctus* genotypes in this cluster were from Caspian Sea region in Golestan, Mazandaran and Guilan provinces.

3.2. ISSR Analysis

3.2.1. Primers Amplification and Diversity Indices

The selected primers produced a total of 161 amplified fragments in which 113 were polymorphic. The sizes of amplified products were ranged from 200 to 3500 bp. The number of amplified bands per primer varied from 11 (P9) to 25 (P7), while the number of polymorphic bands per primer ranged between 9 (in P9, P11 and P13) to 17 (in P7) with an average of 11.3 polymorphic fragments per primer considering all genotypes. The rate of polymorphism ranged from 69.23% (in P11) to 90.2% (P9) with an average of 78.72% (Table 4). The highest and lowest PIC values were observed in P5 (0.53) and P6 (0.38), respectively and the average PIC was 0.44 (Table 4).

3.2.2. Genetic Diversity, Cluster Analysis and PCo Analyses

A dendrogram generated by the Jaccard similarity matrix and UPGMA method revealed genetic relationships among genotypes of different species (Fig. 4). Jaccard similarity coefficients were ranged from 0.33 to 0.90. The highest genetic similarity (90%) was observed between Astara 1 and Astara 2 genotypes (both belong to *R. hirtus* species). This is while, genotypes of *R. persicus* had the highest genetic distance (67%) to the rest of the genotypes.

According to the generated cluster based on similarity matrix, genotypes were placed in three major groups based on the species. All genotypes of *R. sanctus* originating from different parts of the country were located in the first large cluster. The second cluster contains 20 genotypes including 10 genotypes of *R. hirtus*, 9 genotypes of *R. caesius* and one genotype of *R. discolor.* Cluster 3 consisted of four genotypes belonged to *R. persicus*.

The PCo analysis implied the first eight principal coordinates totally explained 67.06% of the total variation (54.20% in the first three principal coordinates). In most cases, PCo analysis showed that the result was corresponded to those obtained through cluster analysis and classified genotypes into four groups as follows: group1; all of *R. sanctus* genotypes, group 2; all of *R. hirtus* genotypes, group 3; all of *R. caesius* genotypes and the only genotype of *R. discolor* and group 4; all of *R. persicus* genotypes, this is while the majority of *R. hirtus*, *R. caesius* and *R. discolor* genotypes were classified in one group based on morphological cluster analysis (Fig. 3).

Discussion

4.1. Morphological Diversity Analysis

4.1.1. Variations of Morphological Traits

Like other fruit crops, blackberries commercial cultivars have narrow genetic back ground making them not only vulnerable against new biotic and abiotic stresses, but also limits breeding programs with lack of variation for important plant and fruit traits in domesticated germplasm[5]. Acquiring comprehensive information about the genetic diversity and population structure of wild species is a good strategy for identification of superior genotypes to be introduced as new cultivars or promising parents in future breeding programs[6]. Results of this study revealed that there is high level of genetic variations among and within Iranian wild blackberry species concerning vegetative, phenological and reproductive traits (Table 2). Plants vegetative and reproductive traits, in addition to genetic factor, greatly depended on environment. Usually, species or accessions from the regions that received greater annual precipitation always had greater leaf dimensions[23]. Ryabova (2007)[24] showed a high level of morphological, phenological and genetic variations of wild raspberry and concluded that these variations might be affected by prevailing soil and climatic conditions as well as topography of the location. Furthermore, vegetative traits such as the number of nodes, shoot length, and plant size are depending on light penetration to the plant[25]. Also, hybridization between close species and changes in ploidy level, could be one reason for the wide range of vegetative trait diversity[10].

Ahmed et al. (2014)[7] studied the genetic diversity of wild raspberry and reported leaf lengths and leaf widths between 91–124 mm and 94–122 mm, respectively, which were higher than those obtained in this study. Sedighi and Rahimmalek (2015)[6] studied the morphological traits of wild *R. hyrcanus* and reported the range of leaf length, leaf widths, and petiole lengths between 53 to 73 mm, 21.3 to 48.1 mm and 10.9 to 18.7 mm, respectively, which were in accordance but much lower than values recorded in current study. This differences could be mainly due to the higher number of species included and larger geographical area covered in this study compared to their study covering only one species in a limited habitat.

Graham et al. (2003)[26] showed that wild *R. ideaus* population had the range of 21 days for flowering and 65 days for fruit ripening which was much lower than those recorded in this study (79 and 86 days on average, respectively). Also Graham et al. (2009)[27] investigated fruit ripening time in different populations of raspberry during 4 years that grown at different locations. They reported that the ripening time was from 21 April to 24 July in 2003, 18 May to 7 July in 2004, 10 May to 23 July in 2005 and 19 May to 6 July in 2006. They expressed this variation were affected by longitude and altitude. In the higher altitude, flowering and ripening dates are later than other areas. Also the cultivated raspberries have completed flowering and fruit set before any of wild populations[26]. In addition, geographic condition and pollen grain also can effect on flowering and ripening time[27].

Some reproductive traits such as fruit size and the number of drupelets (as a yield component) has been a primary objective in all blackberry breeding programs[4]. Unlike vegetative traits, fruit traits appear to be less influenced by rainfall or elevation. Yilmaz et al. (2009)[28] reported the fruits' length and width in wild populations of *R. fruticosus* L. ranged from 7.8 to 11.4 mm and 9.4 to 13.0 mm, respectively. Furthermore, they showed fruit weight varied from 0.4 to 1.2 g in wild genotypes. These results are generally in agreement with finding of current research and minor difference may be due to different species and varied number of genotypes in two studies. Maro et al. (2014)[8] evaluated genetic diversity of Brazilian raspberry cultivars and showed that fruit length in mountains and plains area ranged from 13.5 to 23.8 mm and 10.5 to 23.5 mm, respectively. Moreover, they reported the fruit diameter ranged from 16.5 to 21.6 mm and 13.8 to 21.5 mm in mountains and plains area, respectively. These differences could be due to higher temperatures in the plains[8]. The number of drupelets has a direct effect on fruit size and yield and ranged from 50 to 80 drupelets/fruit in commercial cultivars[8], that was twice more than that of best genotype studied herein. Various factors can affect the number of druplets in wild genotypes, including pollen source (weakness of pollen grain in wild genotype), pollination, pollinator incompatibility and ploidy level[27].

Considering the estimated diversity index (DI), yield and spine density length, leaf length recorded as characteristics having high diversity ($DI \geq 40$), while ripening date, leaf width, fruit width, flowering date, fruit, cane length and internode length were categorized as traits having low diversity ($DI \leq 20$). Other groups of evaluated characteristics including number of nodes, petiole length, fruit weight, number of fruits/inflorescence, number of drupelets/fruit and number of inflorescences/plant were found to have medium (DI from 20–40) diversity (Table 2).

Maro et al. (2014) [8] studied genetic diversity in raspberry and reported the diversity index of fruit length, fruit width, fruit mass and number of drupelets were 9.63, 7.78, 20.29% and 19.37%, respectively. Singh et al. (2009) reviewed the genetic diversity of wild genotypes in the north-western Himalayan region and reported coefficients of variation for berry weight, berry length, and berry widths were 24.11 g, 13.3 mm, and 11.5 mm, respectively. They concluded that the climatic conditions like temperature, sunlight, rainfall and snowfall can affect these traits. Blackberry yield varied substantially among cultivars as well as from management practices and the locations of production. In floricane blackberry the yield depends on cane number, fruiting laterals per cane, and fruit weight, while in primocane types yield depends on cane number and amount of branching[4, 3]. Moreover, Weber et al. (2005)[29] reported that blackberry yield ranged from 6.8 to 2.6 t/ha which was much more than values recorded in wild genotypes evaluated in current study. They explained the differences between yields as a result of genetics and environmental conditions, which could be affected also by the sensitivity to diseases, pests and the production system[29].

4.1.2. Correlation among Traits, Principle Component Analysis (PCA) and Cluster Analysis

Association between traits indicate whether the selection of one trait has an effect on another. Strong correlations between traits will assist breeders select the important traits indirectly. This can accelerate and facilitate breeding programs. Established relationships between desirable traits can also help breeders with parental partner selection in breeding programs[17].

According to the results, a significant positive correlation was detected between cane lengths and number of nodes. Interestingly a significant but negative association was proved between cane length and spine density. Almost all of leaf attributes including Leaf length, leaf width, and petiole length were also highly correlated to each other as well as to internode length. The high positive correlations between the leaf characteristics and traits related to plant growth indicate that more leaf expansion can lead to stronger shrub vegetative growth[30].

Flowering and ripening dates were highly correlated to each other. Yazdanpour et al. (2018)[16] also reported a high and positive correlation between flowering and fruit ripening times (r = 0.62), which is in accordance but weaker than that reported in this study (r = 0.98). These differences can arise from the fact that current study evaluated all of the genotypes in a single site thus eliminating the environmental effects. Although later flowering accessions tended to have later fruit, the fruit growth and development was slightly accelerated, perhaps due to increased temperatures later in spring and summer[31].

Not surprisingly yield was positively correlated to its components including number of inflorescences, number of fruits/inflorescence, fruit weight and number of drupelets/fruit. Interestingly, yield and many of its component were negatively correlated to spine density. Sonsteby et al. (2009)[32] reported that yield was associated with cane height and the number of lateral buds in 'Glen Ample' raspberry. Perasovic (2013)[33] reported that yield was highly correlated with the number of lateral shoots per cane. However, in current study, no correlation was observed between cane length and yield. Also, they showed that raspberry yield was highly and positively correlated to fruit size (r = 0.497) which was consistent with our findings (r = 0.54). Negative correlation between spine density and some vegetative and reproductive traits could be explained in this way that production of spines consumes some of plants potential carbohydrate sources to produce protective organs against probable biotic and abiotic hazards.

Fruit weight had positive correlations with fruit dimentions and number of drupelets/fruit. Gharaghani et al. (2014)[15] and Yazdanpour et al. (2018)[16] reported a positive correlation between fruit weight and fruit dimensions in *Rubus* species from Iran. Detection of useful and highly significant correlations between traits can help breeders with indirect selection of traits (easily or even cheaper) in germplasm, parental materials and seedling population's characterization. It also can help to improve the selection efficiency by using the proper combination of the traits.

The PCA is a multivariate statistical analysis which can be used to determine the number of main factors with the purpose of reducing the number of effective parameters to discriminate genotypes. Previously, PCA had been used to establish genetic relationships among cultivars and genotypes as well as to study the correlations among plant characteristics of different *Rubus* species in Iran[16, 17] and some other countries[28, 34].

The results of principle component analysis (PCA) showed the first six components explained 84.9% of the variations of traits in the studied genotypes (Table 3). The first component (25.3% of total variations), featured mainly reproductive attributes and second component (15.8% of total variations) includes phonological traits (flowering and ripening date) and number of nodes) (Table 5). The bi-plot of PCA showed that all genotypes were divided into two main categories based on the first two components according to loading plot (Fig. 2); the cosine of the angles between vectors shows the extent of correlation between traits which is in accordance to the correlation analysis in almost all of the traits studied. The acute angles (< 90°) represent positive correlations, whereas wide obtuse angles (> 90°) show negative correlations. The length of the vectors connecting traits to the origin shows the extent of variability. The results of PCA and bi-plot are in line with those reported by Yazdanpour et al. (2018)[16].

According to the cluster analysis, the genotypes being studied were divided into two main clusters (Fig. 3). These results show relatively good differentiation of genotypes based on species and to some extent based on their origin. Because of possibility of plant exchange between the studied regions, genotypes of different geographic origin may group together whereas genotypes of same geographic origin may not necessarily have placed in the same cluster. However, it should be mentioned that this analysis is based on a limited number of morphological traits and their corresponding data. Therefore, it may not be as reliable as larger numbers of morpho-chemical traits or molecular data. In the other hand morphological markers are greatly affected by environmental conditions, thus using molecular marker (which has generated in current study and will be discussed later) can help to complete and refine the results[4]. Ryu et al. (2014)[35] evaluated the genetic diversity among fifty-six blackberries (*R. fructicosus*) using morphological characteristics. Based on their report the studied blackberry germplasm grouped into six clusters and two independent groups that indicated an unclear pattern of division among the groups. Amsellem et al. (2001)[10] studied genetic diversity of local and introduced genetic resources of *R. alceifolius* by cluster analysis and showed that studied germplasm clustered into two main groups of native and exotic genotypes. Also Gharaghani et al. (2014)[15] studied genetic diversity of *R. sanctus* genotypes grown in two distinct climatic conditions of Iran and found that 16 blackberry genotypes were grouped in to two main clusters regardless of their origins, their result is largely consistent with results of current study. Yazdanpour et al. (2018)[16] also used hierarchical clustering based on the dissimilarity of the genotypes to group *Rubus*

germplasm of Babol County in northern Iran and showed that the clustering pattern is mainly based on the location of genotypes sampled. Garazhian et al. (2020)[17] investigating the fruit bio-chemicals and antioxidant activity in same set of the genotype being studied herein, reported differentiation of genotypes based on species and to some extent based on their origin. Graham et al. (2003)[26] analyzed genetic similarity among wild accessions and cultivar of *Rubus ideaus* from different sites and showed that the clustering pattern of wild plant materials is largely according to their origin and the cultivars were genetically distinct from the wild populations.

4.2. ISSR Analysis

4.2.1. Primers Amplification and Diversity Indices

The ISSR markers analysis also provided a comprehensive insight of the genetic diversity among Iranian wild species. Considering all genotypes the average polymorphic fragments per primer was of 11.3 with an average polymorphism of 78.72% (Table 4). These results are concordant with some results in similar studies in Rosaceae using ISSR markers[36], especially in *Rubus*. In this regard, Sedighi and Rahimmalek (2015)[6] used ISSR primer to assess the genetic diversity in several populations of *R. hyrcanus* from various geographical regions of the Caspian Sea in Iran. They reported an average polymorphism of 77% that was similar to result being generated in this study (78.72%). They reported relatively low levels of genetic diversity within Iranian wild blackberry populations, with high variation obtained among populations collected from different geographical regions. This is while, the results of current research indicated a high genetic diversity among Iranian wild blackberry genotypes. These differences can be due to covering several species in current study compared to only one species (*R. hyrcanus*) in their research. Innis et al. (2011)[13] surveyed genetic diversity in *R. phoenicolasius* and *R. argutus* using ISSR markers and concluded that the lack of genetic diversity in these clonal invasive species was due to fewer introductions into their invaded habitat or frequent self-fertilization and clonal reproduction. Lee et al. (2016)[11] also estimated the genetic relationships among 69 *Rubus* accessions and reported that the average polymorphism was 0.76 which is largely in accordance with the findings of this research.

The highest and lowest PIC values were observed in P5 (0.53) and P6 (0.38), respectively and the average PIC was 0.44 (Table 4). The average PIC of 0.55 and 0.49 were reported respectively in red raspberry[37] and wild and cultivated black raspberry[5] which is largely in agreement with the results of current study.

4.2.2. Genetic Diversity, Cluster Analysis and Principal Coordinate Analyses (PCoA)

A dendrogram generated by the Jaccard similarity matrix and UPGMA method revealed genetic relationships among genotypes of different species (Fig. 4). The high cophenetic correlation coefficient (0.897) was observed, indicating a good fit between the dendrogram clusters and the similarity matrices, which is higher than that (0.80) reported by Sedighi and Rahimmalek (2015)[6] using the same molecular marker (ISSR). Jaccard similarity coefficients were ranged from 0.33 to 0.90. The highest genetic similarity (90%) was observed between Astara 1 and Astara 2 genotypes (both belong to *R. hirtus* species). This is while, genotypes of *R. persicus* had the highest genetic distance (67%) to the rest of the genotypes. High molecular variation among wild Iranian *Rubus* species could be explained in this way that, they have not undergone any domestication process (suffering less selection pressure). Also, plant propagation system and cross-pollination can effect on variation among species[6, 10]. It has been reported that wild red raspberries form discrete populations which are adapted to local conditions may lead to reproductive isolation without a geographic separation[6, 26].

According to the generated cluster based on similarity matrix, genotypes were placed in three major groups based on their species. Separation of the genotypes within this cluster is based on the species. With a few exceptions the pattern of grouping within the main clusters was also based on the species and origin of the accessions, showing that clustering based on the molecular data not only differentiated among species but also can assign genotypes with relatively similar origin in the identical groups within the main clusters. For instance, all genotypes of *R. sanctus* originating from different parts of the country were located in the first large cluster. Within this large clade, the majority of *R. sanctus* genotypes from southern (Fars and Kohgiluyeh-Boyrahmad), western (Hamadan, Kurdistan and West Azarbayejan) and northern (Guilan, Mazandaran and Golestan) Iran were located in separate sub-clusters. However, some genotypes clustered irrespective of their origin (for instance; grouping of Jahrom-Khafr and Seyedan (Bagh Bonyad) from Fars province in south among genotypes of northern Iran) which could be due to germplasm transmission and exchange between regions[15]. The second cluster contains 20 genotypes including 10 genotypes of *R. hirtus*, 9 genotypes of *R. caesius* and one genotype of *R. discolor*. Further our finding suggested that the only genotype belonged to *R. discolor* was placed in the sub-cluster of *R. caesius* genotypes, but quite distantly (Figs. 4 and 5).

The PCo analysis implied the first eight principal coordinates totally explained 67.06% of the total variation (54.20% in the first three principal coordinates). In most cases, PCo analysis showed that the result was corresponded to those obtained through cluster analysis and classified genotypes into four groups as follows: group1; all of *R. sanctus* genotypes, group 2; all of *R. hirtus* genotypes, group 3; all of *R. caesius* genotypes and the only genotype of *R. discolor* group 4; all of *R. persicus* genotypes, this is while the majority of *R. hirtus*, *R. caesius* and *R. discolor* genotypes were classified in one group based on morphological cluster analysis (Fig. 3). Result of PCoA confirmed the result of cluster analysis based on similarity matrix (Fig. 4). The comparison between the clusters obtained from morphological and molecular data showed the higher and stronger discrimination ability of molecular markers in separation of the genotypes. The results of morphological analysis were in general agreement but having lower resolution compared to those obtained through molecular analyses. There are several reports on application of molecular marker for evaluation of the genetic variations and relationships in *Rubus* species[6, 11, 13].

Lee et al. (2016) [11] estimated the genetic relationships among sixty-nine *Rubus* accessions and found all the 69 *Rubus* accessions belonged to six species, were classified into three groups and concluded that undertrained diversity of species and artificial groups of the *Rubus* genus have brought some kind of confusions to the mind with respect to the correct classification of the species at both commercial and scientific levels.

Sedighi and Rahimmalek (2015) [6] showed relatively a high agreement between the morphological and molecular data in *R. hyrcanus* and concluded that this may be due to low environmental variation in the sampling regions. Also Amsellem et al. (2001) [10] illustrated a strong congruence in taxonomy and genetic diversity based on morphological and molecular markers studying the wild populations of *R. alceifolius*. In contrary, Ryu et al. (2014) [35] reported a low

correlation between the cluster analysis of morphological and molecular marker. The low correlation between molecular and morphological markers might be arisen from using primers of the intron regions of the genome. Also, it is specified that plant's biological characteristics and habitat fragmentation could affect the performance of morphological traits and might therefore; play an important role in the weak associations between the results obtained by molecular and morphological marker[6].

Conclusion

The results of this study revealed that there is high level of genetic variations among and within Iranian wild blackberry species concerning vegetative, phenological and reproductive traits, which could be utilized through breeding programs. Also, the result implied some positive/negative correlation between evaluated traits which could be very useful in efficient screening of germplasm and breeding populations. Analysis of morphologic data with PCA showed that the genotypes were divided into two main categories which reflected known differentiation among species and their origin. The ISSR markers analysis also provided a comprehensive insight of the genetic diversity among Iranian wild species. The results of morphological cluster analysis were in general agreement but having lower resolution compared to those obtained through molecular analyses. The current study provided basic information for phylogeny, taxonomy and breeding programs in some Iranian *Rubus* species. However, it seems that more researches on genetic diversity including more genotypes as well as commercial cultivars are needed in order to categorize, clarify and classify more precisely, confirm and approve the current results.

Declarations

DATA AVAILABILITY STATEMENT

All necessary data are presented in the manuscript and no data are publicly available.

AUTHOR CONTRIBUTIONS

AG and SE design of the work. MG and AT carried out the analysis. AG and MG carried out the interpretation of data. MG, AG, and SE have drafted the work.

Compliance with Ethical Standards

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Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1.

Geographic information of wild blackberry genotypes being used in this study

Species	Number	Genotype	Province	E (Longitude)	N (Latitude)	Altitude
R. sanctus	1	Shirgah	Mazandaran	52°55'16.42"	36°20'55.67"	361.4 m
	2	Sisangan	Mazandaran	51°47'45.31"	36°35'58.86"	-13.0
	3	Sari1(Jade Khazar)	Mazandaran	53° 1'8.24"	36°40'8.83"	544.6 m
	4	Jade Haraz	Mazandaran	52°17'17.34"	35°56'42.56"	403.5 m
	5	Chaloos	Mazandaran	51°26'4.23"	36°38'0.01"	1547.7 m
	6	Tonekabon (Nematabad)	Mazandaran	50°55'11.70"	36°45'9.33"	1821.7 m
	7	Tonekabon	Mazandaran	50°55'14.02"	36°45'10.330"	1803.5 m
	8	Namak Abrud 1	Mazandaran	51°20'47.38"	36°38'24.21"	1625.1 m
	9	Namak Abrud 3	Mazandaran	51°15'43.71"	36°39'8.39"	1376.4 m
	10	Babolsar 1	Mazandaran	52°45'29.09"	36°38'26.80"	-22.2 m
	11	Babolsar 2	Mazandaran	52°34'12.71"	36°37'42.13"	-24.6 m
	12	Sari 3	Mazandaran	53° 3'21.17"	36°34'48.79"	96.1 m
	13	Sefid Tameshk	Mazandaran	50° 38'14.81"	36° 54'57.65"	56.8 m
	14	Behshahr 2	Mazandaran	53°34'22.62"	36°40'41.79"	359.0 m
	15	Bandar Gaz	Golestan	53°56'13.67"	36°46'28.74"	409.0 m
	16	Gorgan 2	Golestan	54°25'58.79"	36°50'35.75"	501.3 m
	17	Aliabad Katul 3	Golestan	54°55'15.10"	36°52'50.12"	147.5 m
	18	Naharkhoran	Golestan	54°27'45.60"	36°47'2.595"	413.4 m
	19	Roodbar	Guilan	49°27'2.04"	36°48'10.00"	1639.5 m
	20	Kelachay	Guilan	50°22'53.58"	37° 0'22.53"	1804.1 m
	21	Roodsar	Guilan	50°16'33.97"	37° 8'24.95"	1630.3 m
	22	Kelachay (Polrood)	Guilan	50°22'29.17"	37° 4'40.88"	1680.0 m
	23	Talesh 2	Guilan	48°52'6.85"	37°48'40.60"	766.8 m
	24	Talesh 1	Guilan	48°52'6.08"	37°48'41.160	754.5 m
	25	Astara 4	Guilan	48°46'24.82"	38°25'21.13"	531.2 m
	26	Gardane Heyran 1	Guilan	46°57'4.84"	38°49'20.26"	645.8 m
	27	Gardane Heyran 4	Guilan	46°47'37.52"	38°47'13.90"	776.5m
	28	Lahijan	Guilan	49°59'5.50"	37°11'34.92"	4.0 m
	29	Anzali 3	Guilan	49°27'58.03"	37°27'32.69"	-24.2 m
	30	Jade Kandovan	Alborz	46°37'36.78"	37°59'25.16"	801.0 m
	31	Urmia 1	West Azarbayejan	44°54'20.88"	37°25'56.32"	1794.6 m
	32	Urmia 2	West Azarbayejan	44°55'30.15"	37°39'34.54"	2032.1 m
	33	Sanandaj 1	Kurdistan	47°03'40.14"	35°13'1.75"	1588.6 m
	34	Abidar	Kurdistan	50°32'40.61"	36° 2'25.56"	1890.6 m
	35	Malayer	Hamedan	48°50'41.89"	34°14'3.40"	1862.6 m
	36	Seyedan (Bag Bonyad)	Fars	52°35'3.47"	30°57'3.11"	1689.4 m
	37	Shiraz (Chamran)	Fars	52°29'41.80"	29°39'12.50"	1674.5 m
	38	Kazerun (Fathabad)	Fars	51°31'21.72"	29°43'42.59"	755.2 m
	39	Kazerun (Eslamabad)	Fars	51°34'26.50"	29°46'58.43"	797.3 m
	40	Nourabad (Bavan)	Fars	51°32'22.98"	30° 7'36.14"	1079.9 m
	41	Dasht Arzhan	Fars	51°58'46.66"	29°39'10.06"	2142.1 m

	42	Sepidan (Roodbal)	Fars	52° 2'20.75"	30° 6'0.10"	1920.2 m
	43	Beyza (Hoseinabad)	Fars	52°23'24.53"	29°58'14.28"	1637.0 m
	44	Kamfiruz	Fars	52°11'32.76"	30°19'35.13"	1783.0 m
	45	Firouzabad	Fars	52°32'21.76"	28°52'37.76"	1347.2 m
	46	Jahrom (Khafr)	Fars	53°31'31.93"	28°29'15.77"	1049.4 m
	47	Sivand	Fars	52°55'1.58"	30° 5'12.83"	1802.8 m
	48	Dena (Karyak)	Kohgiluyeh-Boyrahmad	51°25'5.97"	30°49'1.80"	2018.9 m
	49	Kakan	Kohgiluyeh-Boyrahmad	51°48'3.78"	30°37'31.62"	2013.2 m
	50	Yasuj (Naregah)	Kohgiluyeh-Boyrahmad	51°34'8.08"	30°36'55.97"	2105.2 m
R. hirtus	51	Abas Abad	Mazandaran	51° 7'24.50"	36°42'34.78"	1722.7 m
	52	Gardane Heyran 5	Guilan	46°53'1.14"	38°48'17. 6"	660.5 m
	53	Rezvanshahr	Guilan	49° 8'32.03"	37°33'33.37"	950.3 m
	54	Ashkvarat 1	Guilan	50°16'7.76"	36°48'5.24"	1835.2 m
	55	Ashkvarat 2	Guilan	50°14'7.40"	36°46'26.55"	1839.9 m
	56	Anzali 2	Guilan	49°28'44.39"	37°27'12.35"	1173.7 m
	57	Astara 1	Guilan	48°46'45.47"	38°26'56.21"	490.4 m
	58	Astara 2	Guilan	48°47'43.30"	38°22'49.72"	558.6 m
	59	Ganjname	Hamedan	48°26'6.01"	34°45'34.13"	1795.2 m
	60	Gerdbisheh	Char Mahal-Bakhtiari	50°39'37.12"	32° 8'12.91"	2062.8 m
R. caesius	61	Aliabad Katul 1	Golestan	54°54'45.18"	36°54'54.92"	150.4 m
	62	Aliabad Katul 2	Golestan	54°51'2.03"	36°53'32.20"	159.7m
	63	Gorgan 5	Golestan	54°26'21.73"	36°50'44.32"	499.8 m
	64	Gorgan 4	Golestan	54°25'11.23"	36°50'49.48"	484.2 m
	65	Naharkhoran 1	Golestan	54°28'20.19"	36°48'28.69"	360.2 m
	66	Gonbad-e Kavus	Golestan	55°10'31.47"	37°12'37.55"	43.0 m
	67	Anzali 1	Guilan	49°27'7.99"	37°27'16.12"	-29.0 m
	68	Fuman 1	Guilan	49°18'41.58"	37°12'45.17"	1325.5 m
	69	Gerdbisheh 1	Char Mahal-Bakhtiari	50°37'14.22"	32° 8'88.03"	2051.5 m
R. discolor	70	Kelachay 1	Guilan	50°22'25.3"	37° 4'41.24"	1676.0 m
R. persicus	71	Masuleh	Guilan	49° 00'2.22"	37° 9'40.34"	1231.3m
	72	Fuman	Guilan	49°17'51.93"	37° 12'32.07"	39.6 m
	73	Rasht Abad	Guilan	49° 50'25.21"	37° 15'32.05"	-4.4 m
	74	Ramsar 1	Mazandaran	50° 38'13.89"	36° 54'53.75"	95.5 m

Table 2(A).

Descriptive Statistics of morphological traits of studied blackberry genotypes from Iran

Code	Traits	Mean	Std. error	Range	Max	Min	Std. dev.	Diversity Index*
1	Cane length (cm)	123.71	1.35	124.00	197.00	73.00	20.78	16.79
2	Leaf length (mm)	50.00	0.50	64.00	94.20	30.20	8.10	16.11
3	Leaf width (mm)	44.50	0.40	31.20	59.40	28.20	6.10	13.74
4	Petiole length (mm)	23.10	0.40	38.10	52.60	14.50	5.70	28.84
5	Internodes length (mm)	42.70	0.50	55.60	85.60	30.00	7.90	18.49
6	Number of nodes	29.88	0.47	33.00	48.50	15.50	7.23	24.19
7	Spine density (No. in 1 cm of cane)	1.21	0.04	4.05	4.45	0.40	6.49	53.54
8	Flowering date	107.96	1.22	79.00	August 1th	May14th	15.91	14.74
9	Number of inflorescences	36.50	1.01	57.50	75.00	17.50	13.21	36.18
10	Fruits/ inflorescences	7.49	0.19	13.00	16.00	3.00	2.44	32.51
11	Ripening date	127.75	1.32	86.00	August 24th	May 30th	17.20	13.47
12	Number of drupelets/Fruit	20.05	0.51	30.50	36.00	5.50	6.68	33.32
13	Fruit width (mm)	11.70	0.10	10.20	15.50	5.30	1.60	14.06
14	Fruit length (mm)	11.90	0.10	9.70	16.20	6.50	1.80	14.80
15	Fruit weight (g)	0.82	0.02	1.19	1.33	0.14	0.25	30.25
16	Yield (g)	233.90	11.20	927.90	944.70	16.80	146.30	62.57

*Diversity Index = (Std. dev./Mean)×100, Std. dev. = Std. error×√n, n = (79 for vegetative traits and 57 for reproductive traits).

Table 2(B).

Leven's statistic

Variable	Leven's statistic	P-value
Number of nodes	3.02	0.074
Internodes length	0.25	0.614
Cane length	3.08	0.080
Spine density	0.11	0.739
Leaf length	0.00	0.951
Leaf width	0.01	0.924
Petiole length	0.64	0.424
Flowering date	0.03	0.862
Ripening date	0.04	0.847
Fruit length	0.04	0.844
Fruit width	0.07	0.786
Number of drupelets/Fruit	0.14	0.710
Number of inflorescences	2.37	0.124
Fruits/inflorescences	0.02	0.901
Fruit weight	0.46	0.496
Yield	3.31	0.095

Table 3.

Eigenvectors of the eight principal component axes from PCA analysis of morphological variables in studied Iranian blackberry accessions

Traits	Component					
	1	2	3	4	5	6
Cane length	0.056	0.384	0.067	0.369	-0.129	-0.228
Leaf length	-0.284	0.279	0.406	0.064	-0.046	-0.07
Leaf width	-0.265	0.218	0.355	0.147	-0.035	0.075
Petiole length	-0.266	0.217	0.407	-0.059	0.155	0.067
Internodes length	-0.127	-0.077	0.349	-0.380	-0.161	-0.348
Number of nodes	0.123	0.353	-0.161	0.509	0.003	0.051
Spine density	-0.256	-0.280	0.139	0.055	0.127	0.381
Flowering date	0.046	0.429	-0.122	-0.388	-0.079	0.356
Number of inflorescences	0.080	0.015	0.115	0.115	0.705	0.206
Fruits/inflorescences	0.284	0.018	0.154	-0.300	0.225	-0.289
Ripening date	0.065	0.425	-0.136	-0.384	-0.045	0.361
Number of drupelets	0.398	0.069	0.131	-0.048	-0.130	-0.026
Fruit width	0.276	-0.203	0.316	0.103	-0.298	0.314
Fruit length	0.290	-0.189	0.333	0.096	-0.165	0.391
Fruit Weight	0.362	0.135	0.197	0.055	-0.160	-0.133
Yield	0.367	0.079	0.185	-0.039	0.446	-0.105
Proportion of total variance	0.253	0.157	0.132	0.128	0.097	0.082
Cumulative % of total variance	0.253	0.410	0.542	0.670	0.767	0.849

Table 4.

Characteristics of ISSR primer being used in this study

Primer name	Motif	Annealing temperature	Number of band	Number of polymorphic band	Polymorphisms%	Pic/Primer
P1	5′-(CA)8 G-3′	52.5	21	14	77.57	0.46
P2	5′-(AC)8 G-3′	52.0	18	13	81.25	0.44
P3	5′-(CA)7 CTA-3′	54.0	12	10	84.61	0.42
P5	5′-(AG)8 C-3′	53.5	15	11	71.66	0.53
P6	5′-(GA)8 C-3′	52.5	17	11	73.63	0.38
P7	5′-(AC)8 C-3′	52.5	25	17	74.41	0.48
P9	5′-(CA)8 CCCT-3′	53.5	11	9	90.20	0.44
P10	5′-(CA)8 GGGT-3′	53.5	13	10	82.90	0.45
P11	5′-T(AG)7-3′	55.5	14	9	69.23	0.46
P13	5′-G(TC)7-3′	57.5	15	9	81.81	0.39
Total	-	-	161	113	-	-
Average	-	-	16.1	11.3	78.72	0.44

Table 5.

Correlation between studied morphological traits.

	1	2	3	4	5	6	7	8	9	10	11	12	13	1
1-Cane length	1.00	0.257 ns	0.142 ns	0.144 ns	-0.035 ns	0.766**	-0.32*	0.054 ns	-0.031 ns	-0.055 ns	0.043 ns	0.138 ns	0.011 ns	-0 ns
2-Leaf length		1.00	0.792**	0.714**	0.326*	0.009 ns	0.178 ns	0.059 ns	-0.043 ns	-0.22 ns	0.026 ns	-0.290*	-0.212 ns	-0 ns
3-Leaf width			1.00	0.561**	0.096 ns	0.088 ns	0.277*	0.003 ns	-0.035 ns	-0.225 ns	-0.026 ns	-0.247 ns	-0.145 ns	-0 ns
4-Petiole length				1.00	0.373**	-0.131 ns	0.221 ns	0.131 ns	0.200 ns	-0.130 ns	0.094 ns	-0.300*	-0.159 ns	-0 ns
5-Internodes length					1.00	-0.658**	0.058 ns	-0.014 ns	-0.241 ns	0.211 ns	-0.046 ns	-0.071 ns	0.023 ns	-0 ns
6-Number of nodes						1.00	-0.282*	0.073 ns	0.121 ns	-0.151 ns	0.084 ns	0.150 ns	-0.004 ns	-0 ns
7-Spine density							1.00	-0.257 ns	0.120 ns	-0.270*	-0.254 ns	-0.380**	0.016 ns	0 ns
8-Flowering date								1.00	-0.074 ns	0.119 ns	0.980**	0.139 ns	-0.130 ns	-0 ns
9-Number of inflorescences									1.00	0/103 ns	-0.030 ns	0.016 ns	-0/028 ns	0
10-Fruits/ inflorescences										1.00	0.13 ns	0.427**	0.125*	0
11-Ripening date											1.00	0.150 ns	-0.140 ns	-0 ns
12-Number of drupelets												1.00	0.439**	0
13-Fruit width													1.00	0
14-Fruit length														1
15-Fruit weight														
16-Yield														
*and **: significant different at 5% and 1% probability levels respectively; ns: non-significant.														

Figures

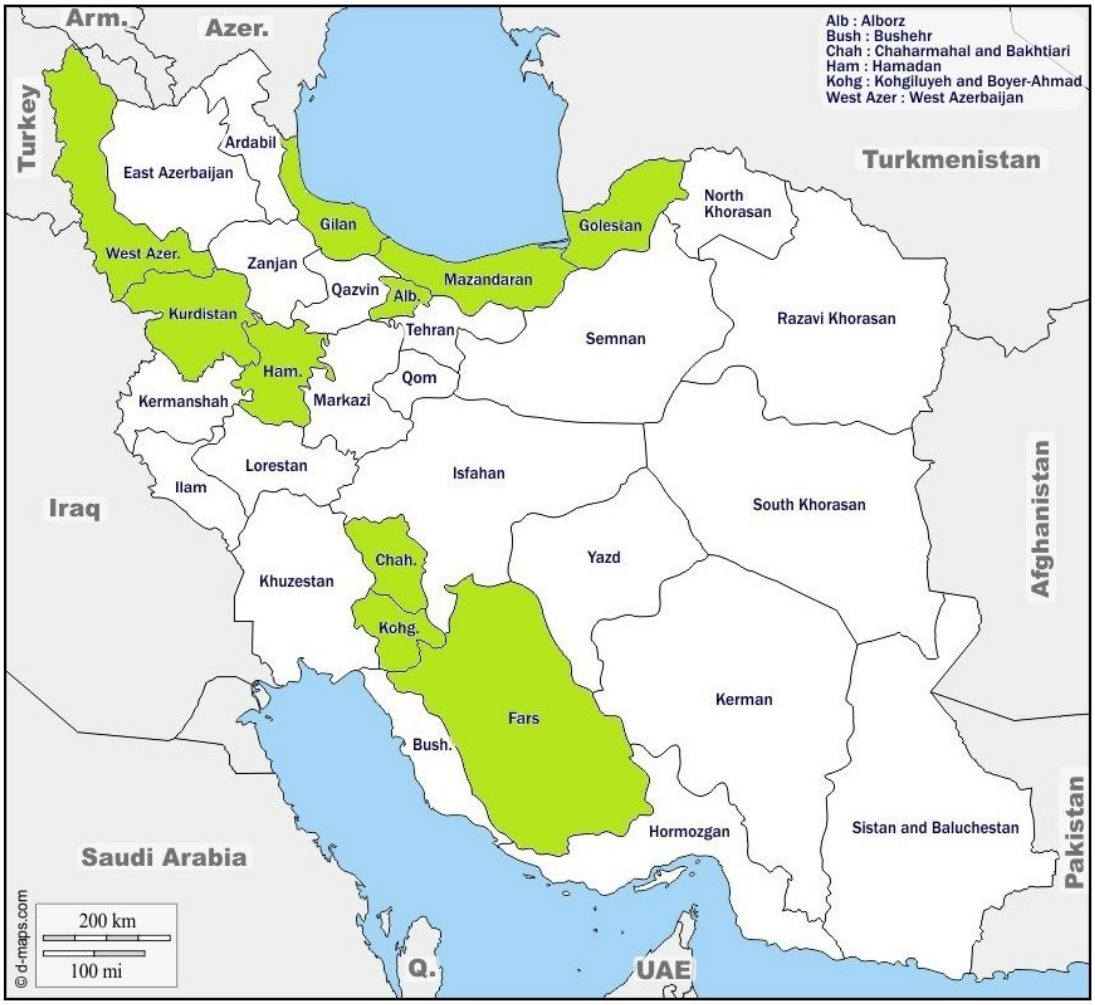

Figure 1

Approximate distribution of blackberry genotypes being used in this study within Iran (the green colored provinces). The original map is obtained from d-map (https://d-maps.com/carte.php?num_car=5496&lang=en) and modified (colored) using paint software of Microsoft Windows 10. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

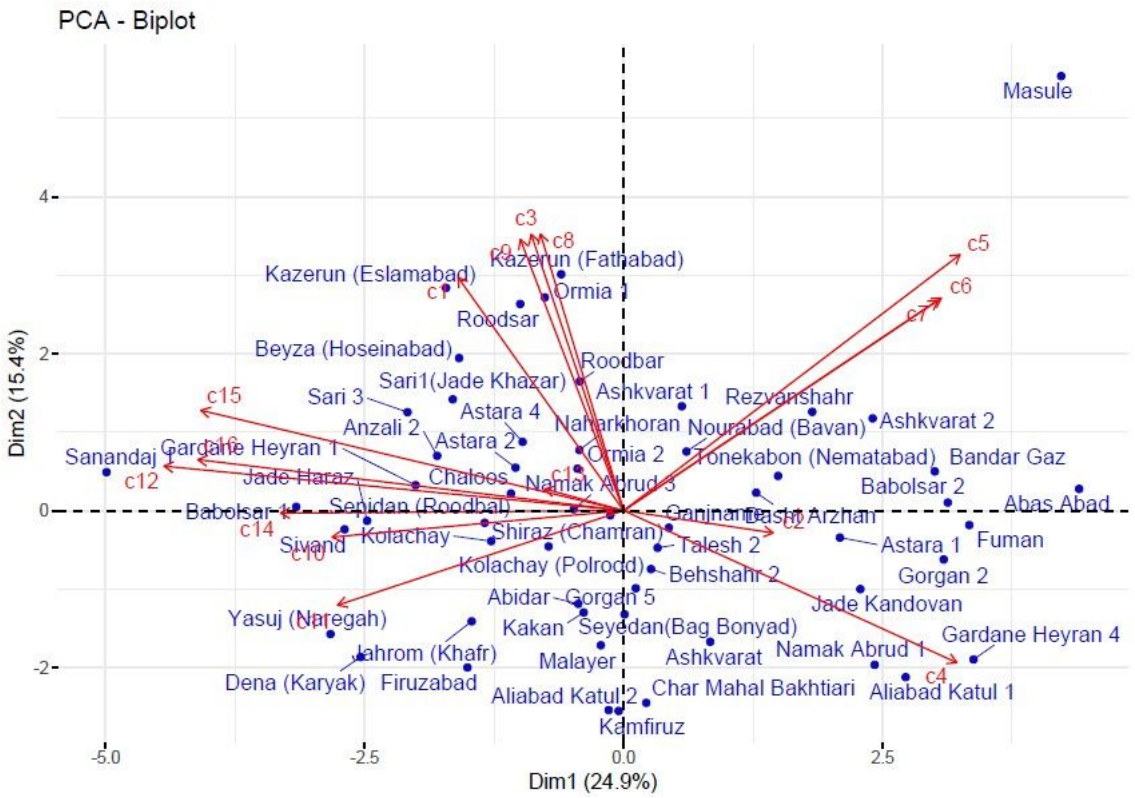

Figure 2

Projection of genotypes on the first (PC1) and second (PC2) principal components based on bi-plot. Discrimination vector of different morphological traits based on loading plot.

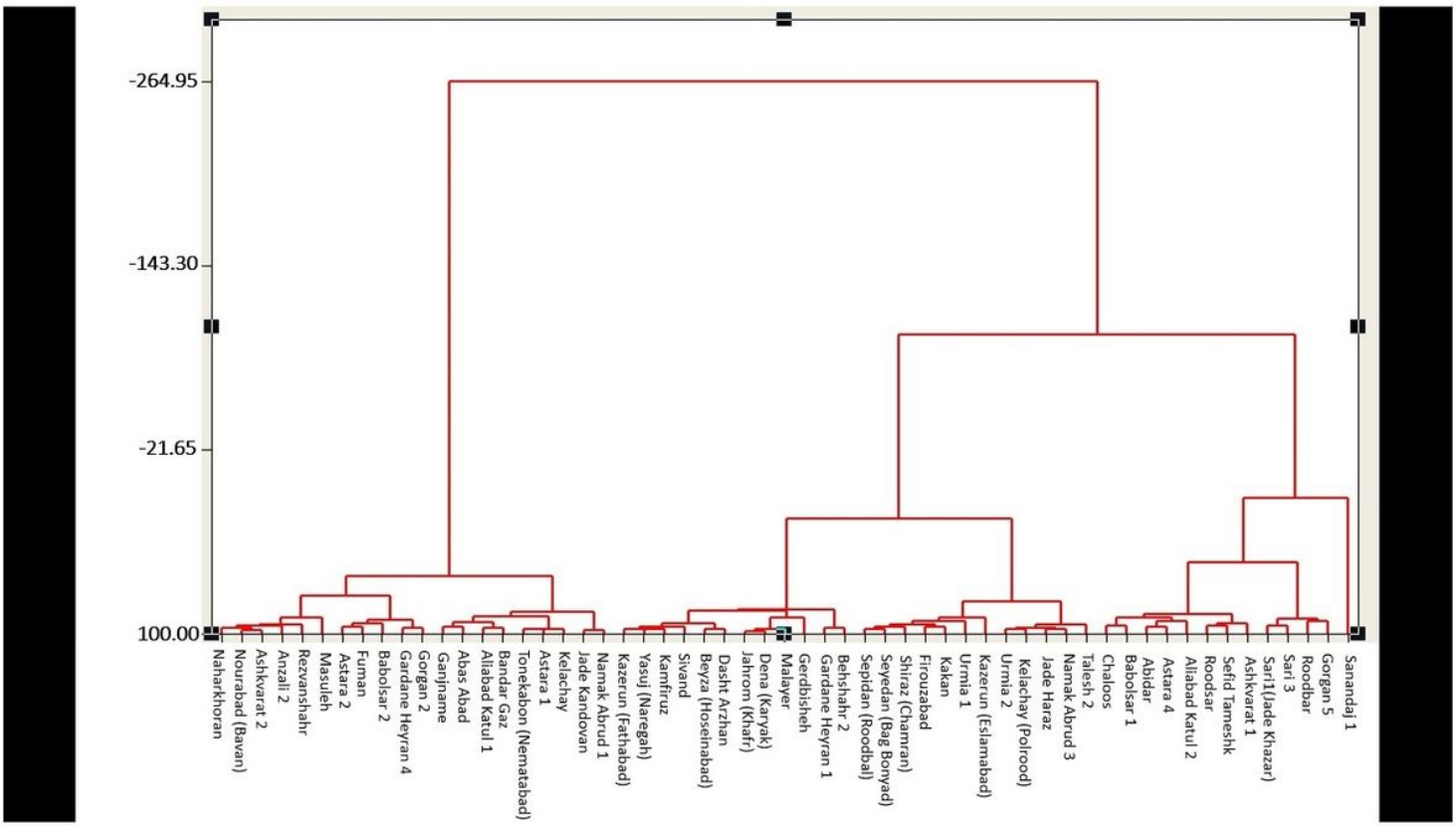

Figure 3

Dendrogram of blackberry genotypes based on cluster analysis of morphological traits.

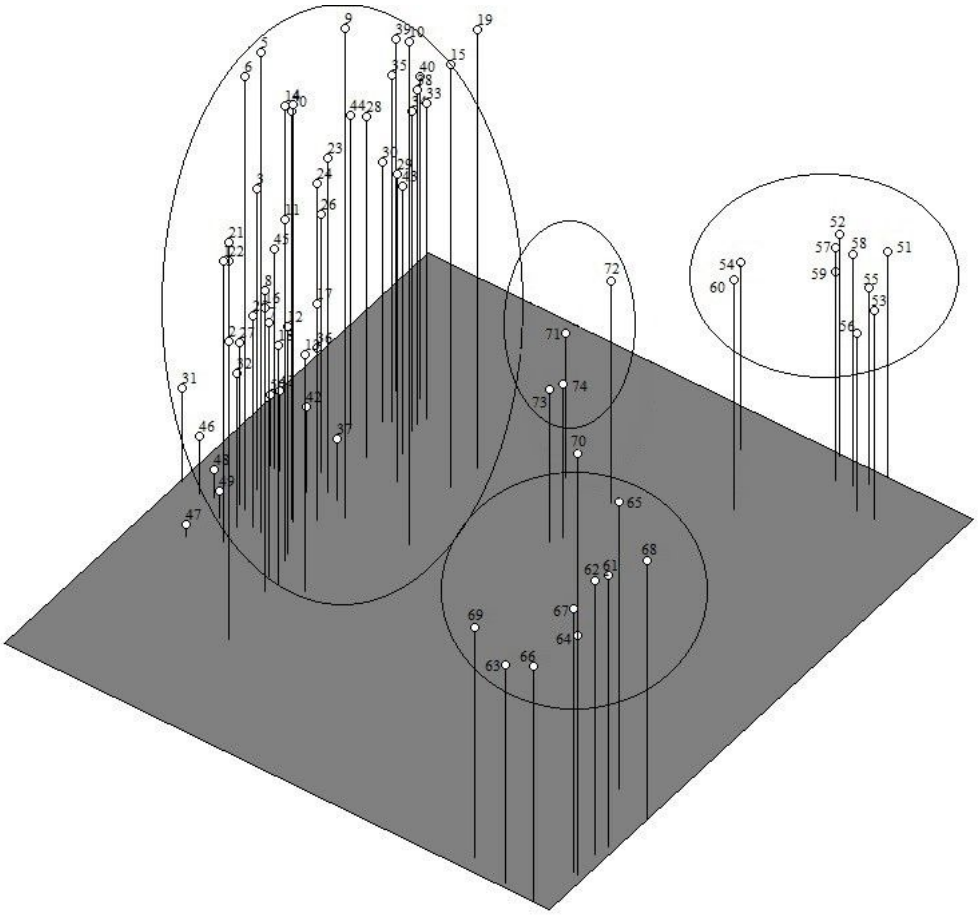

Figure 4

Patterns of relationships among blackberry genotypes being used in this study based on ISSR data that revealed by PCoA.

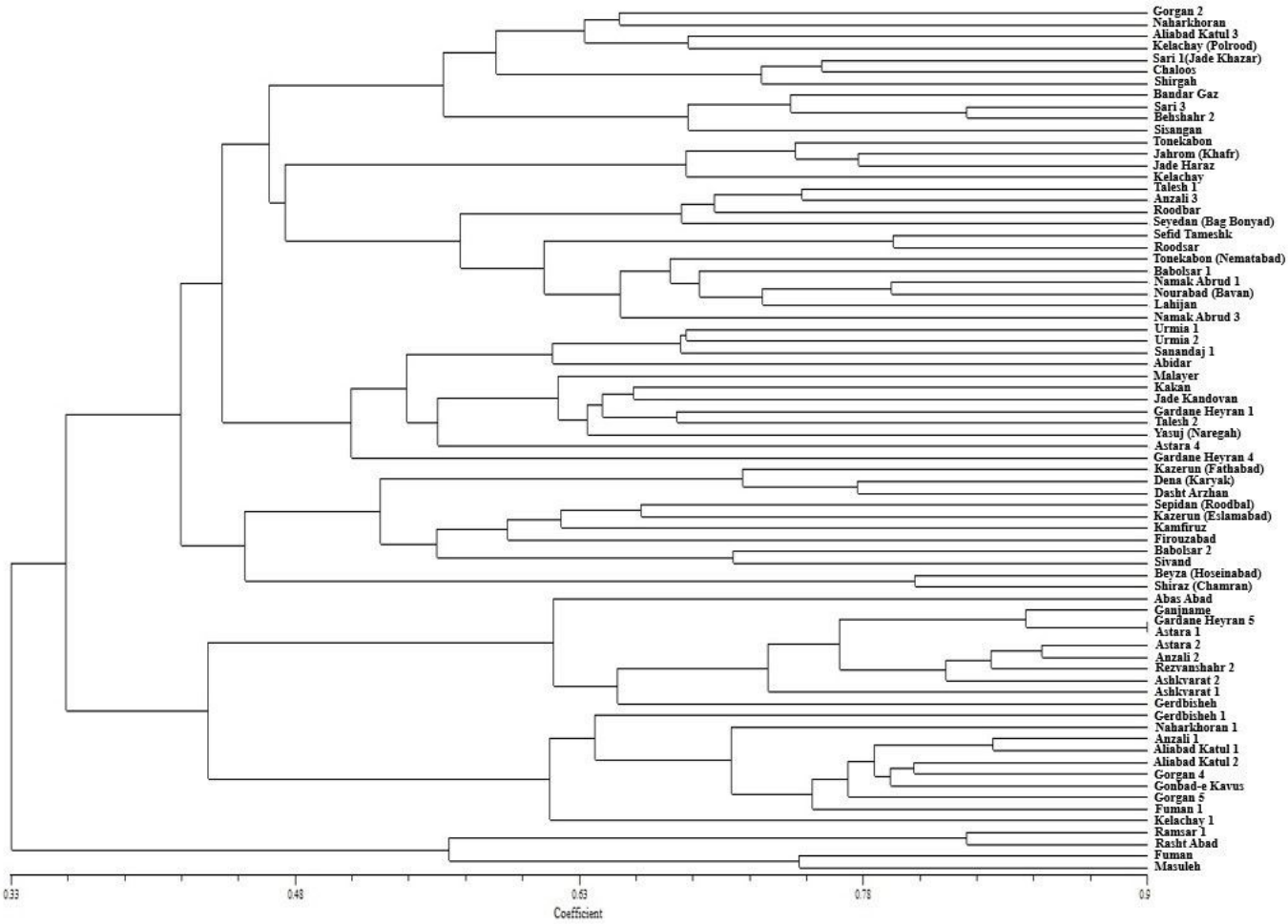

Figure 5

UPGMA dendrogram generated based on ISSR data using Jaccard similarity coefficient for blackberry genotypes being used in this study.