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# Genetic structure and diversity of *Adonis* L. (Ranunculaceae) populations collected from Turkey by inter-primer binding site (iPBS) retrotransposon markers

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**Abstract:** The genus *Adonis* L. is a member of Ranunculaceae and consists of perennial and annual herbaceous plants included in the tribe Adonideae under the subfamily Ranunculoideae. Botanically, Ranunculaceae comprises vital medicinal plants. Molecular markers are one of the most effective tools for exploring genetic variation that can enhance breeding efficiency. To identify the genetic diversity of 62 *Adonis* ecotypes collected from different regions in Turkey, the interprimer binding site (iPBS) retrotransposon system was used. Of the 83 iPBS primers used, 10 provided sufficient polymorphic data, generating a total of 204 alleles. The number of iPBS bands per individual was 3.29, and the number of alleles per polymorphic locus ranged from 8 to 35, with an average of 20.30. The average polymorphism percentage was 99.50%, and polymorphic information content ranged from 0.16 to 0.39. The highest average number of alleles, Nei's genetic diversity (h), and Shannon's information index (I) were obtained from *A. volgensis* species (1.64, 0.39, and 0.58, respectively), whereas the lowest values (1.41, 0.29, and 0.46, respectively) were found in *A. flammea* species. The analysis of molecular variance revealed significant variance within the population (71%), whereas no significant genetic variation was observed among the different species (29%). Cluster analysis according to unweighted pair-group mean average (UPGMA) divided 62 *Adonis* ecotypes into four major clusters. According to the principal coordinate analysis, the first three principal coordinates accounted for 81.51% of total variation. Genetic structure analysis of the studied germplasm using the Bayesian method revealed four subpopulations with an average of 0.2634 for expected heterozygosity and 0.2154 for population differentiation measurements. The results of this study suggested that iPBS markers could be used in the identification of genetic diversity among the *Adonis* species.

**Key words:** *Adonis* L., iPBS markers, genetic diversity, population structure, AMOVA

## 1. Introduction

Ranunculaceae is a family of flowering plants known as the buttercup family and consists of 59 genera and about 2500 species (Tamura, 1993). The ornamentally important genus *Adonis* L. comprises approximately 40 species of annual and perennial plants, which are widely distributed in southwestern Asia and Europe, northern Africa, and the Mediterranean region (Ghorbani Nahoojei et al., 2008). The genus is represented by 11 taxa in Russia (Komarov and Schischkin, 1937), 13 taxa in Iran (Rechinger, 1992), 11 taxa in China (Fu and Robinson, 2001), 8 taxa in Syria and Palestine (Post, 1932), 5 taxa Israel (Heyn and Pazy, 1989), and 10 taxa in Europe (Tutin et al., 1993). The genus has been divided into two distinct sections by different authors: sect. *Adonis* (annual herbs) and sect. *Consiligo* DC. (perennial plants) (Wang, 1980). In Turkey, it is represented by 10 taxa (nine species and one subspecies) and two natural hybrids (Davis, 1965).

A wide degree of genetic diversity is a first step for the effective selection of superior genotypes and development of novel varieties. Molecular markers are one of the most effective tools for exploring genetic variation to enhance breeding efficiency (Erayman et al., 2014). Morphological (Son and Ko, 2013), ecological (Erfanzadeh et al., 2013), palynological (Fernández and Sánchez, 1988), and genetic diversity (Ro et al., 1997; Suh et al., 2002; Boronnikova and Kalendar, 2010; Kalendar et al., 2010) studies on the genus *Adonis* have been undertaken in various regions of the world. However, to date, in *Adonis* there has been limited research regarding the use and development of molecular markers such as random amplified polymorphic DNA (Suh et al., 2002)

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and amplified fragment length polymorphism (Hirsch et al., 2015; Meyer et al., 2015).

The inter-primer binding site (iPBS) retrotransposon technique has been employed successfully in flax (Smykal et al., 2011), apricot (Baránek et al., 2012), *Saussurea esthonica* (Gailite and Rungis, 2012), chickpea (Andeden et al., 2013), guava (Mehmood et al., 2016), grape (Guo et al., 2014), okra (Yıldız et al., 2015), rice (Comertpay et al., 2016), lentil and pea (Baloch et al., 2015), tea (Phong et al., 2016), saffron (Gedik et al., 2017), and *Leonurus cardiaca* L. (Borna et al., 2017). It is an easy-to-use technique that requires no sequence data (Nemli et al., 2015). Therefore, to simplify the application of molecular tools and offer a better understanding of the genetic diversity of *Adonis* species, we utilized iPBS molecular markers in these species for the first time in the literature. In addition, to the best of our knowledge, there is no available data in the literature on the analysis of genetic relationships among and within *Adonis* ecotypes distributed in Turkey. Thus, the purposes of this study were to investigate the genetic diversity of 62 *Adonis* species using iPBS molecular markers, evaluate the structure of diversity in the germplasm, and generate useful data for future breeding programs.

## 2. Materials and methods

### 2.1. Collection of plant material

The materials used in this study consisted of the following nine *Adonis* L. taxa: *A. volgensis* Stev. ex DC., *A. aleppica* Boiss., *A. annua* L., *A. microcarpa* DC., *A. dentata* Del., *A. aestivalis* L. subsp. *aestivalis* L., *A. aestivalis* L. subsp. *parviflora* (Fisch. ex DC.) Busch., *A. eriocalycina* Boiss., and *A. flammea* Jacq. The samples were collected from 15 different locations in Turkey between 2014 and 2016 (Table 1; Figure 1). Botanical identification was carried out by Dr. A. İlçim from the Department of Biology, Faculty of Sciences and Arts, Mustafa Kemal University, based on the classification by Davis (1965).

### 2.2. Genomic DNA isolation

Genomic DNA from each accession was extracted from young leaf tissues using the method described by Zeinalzadehtabrizi et al. (2015). The quality of the DNA was confirmed by electrophoresis in 0.8% agarose gel, and DNA concentration was measured using the NanoDrop® ND-1000 UV/Vis spectrophotometer. The final DNA concentration was adjusted to 50 ng/µL for iPBS analysis, and the diluted DNA was stored at –20 °C for PCR reactions.

### 2.3. iPBS marker analysis

Initially, five randomly chosen ecotypes from 62 *Adonis* L. ecotypes were used to select polymorphic primers from 83 iPBS primers developed by Kalendar et al. (2010). Ten primers with good-to-excellent PCR products were selected for genotyping of the entire set of *Adonis* L. ecotypes. Detailed information about the primers used in this study is given in Table 2. PCR amplifications were performed in a thermal cycler (Labcycler). The PCR mixture consisted of 1X buffer; 2 mM $MgCl_2$; 0.25 mM of each: dNTP, 1 µM (20 pmol) primer, 0.5 U Taq polymerase, and 50 ng/µL DNA template in a 20 µL reaction mixture. The amplification conditions were as follows: an initial denaturation step of 3 min at 95 °C, 38 cycles of 60 s at 95 °C, 60 s at 50–56 °C, 120 s at 72 °C, and a final extension step of 10 min at 72 °C. The amplification products were resolved in 1.5% agarose gel in 1X SB buffer at 6 V/cm for 120 min, stained with ethidium bromide (0.5 µg/mL), and visualized under a UV-trans illuminator. The sizes of the base pairs were determined based on a DNA ladder between 50 and 1000 bp (Vivantis, product no.: NM2421).

### 2.4. Data analysis

The iPBS band patterns were evaluated using TotalLab TL120 software. The products of iPBS amplification were recorded as present (1) or absent (0). Only clear and strong bands were noted and used for further analysis.

The association between genetic dissimilarity was determined using the numerical taxonomy and multiware analysis system (NTSYS-pc, version 2.0), according to Dice similarity matrix (Dice, 1945). A UPGMA tree was constructed using the same software (Rohlf, 1998). Diversity of each iPBS marker was calculated using polymorphism information content (PIC) according to the following equation: $PIC = 1 - \sum_{f=1}^{n} (Pij)^2$, where $P_{ij}$ is the frequency of the patterns (j) for each marker (i) (Anderson et al., 1993).

To determine genetic parameters, the number of alleles (ne), Nei's genetic diversity (h), and Shannon's information index (I) were calculated by POPGEN1.32 (Yeh et al., 1997). Molecular variances within and between the 15 locations were estimated by the analysis of molecular variance (AMOVA) and the principal coordinate analysis (PCoA) using GenAlEx 6.5 software (Peakall and Smouse, 2012).

The genetic structure of the ecotypes was determined using model-based cluster analysis (STRUCTURE v. 2.2) (Pritchard et al., 2000a, 2000b). The number of populations (K) was expected every ten runs for every population, which varied from 2 to 10, characterized by a set of distinctive allele frequencies at each locus, and the individuals were sited in K clusters. Using this method, Markov chain Monte Carlo (MCMC) posterior probabilities were estimated. The MCMC chains were run with a 10,000-iteration burn-in period, followed by 100,000 iterations using a model allowing for admixture and correlated allele frequencies. The most expected value

**Table 1.** List of *Adonis* ecotypes collected from Turkey and their coordinates.

| NO. | Name of taxa | Latitude | Longitude | Altitude (m) | Location |
|---|---|---|---|---|---|
| 1 | *A. volgensis* | 39°76′870″ | 44°14′774″ | 1580 | Iğdır-Elmagöl village |
| 2 | *A. volgensis* | 39°76′870″ | 44°14′774″ | 1580 | Iğdır-Elmagöl village |
| 3 | *A. volgensis* | 39°76′870″ | 44°14′774″ | 1580 | Iğdır-Elmagöl village |
| 4 | *A. volgensis* | 40°46′264″ | 42°95′340″ | 1827 | Iğdır-Elmagöl village |
| 5 | *A. aleppica* | 37°39′885″ | 38°44′698″ | 576 | Urfa to Bozova road |
| 6 | *A. aleppica* | 37°39′885″ | 38°44′698″ | 576 | Urfa to Bozova road |
| 7 | *A. aleppica* | 37°39′885″ | 38°44′698″ | 576 | Urfa to Bozova road |
| 8 | *A. aleppica* | 36°93′452″ | 37°38′829″ | 803 | Antep to Kilis road |
| 9 | *A. aleppica* | 36°93′452″ | 37°38′829″ | 803 | Antep to Kilis road |
| 10 | *A. aleppica* | 36°93′452″ | 37°38′829″ | 803 | Antep to Kilis road |
| 11 | *A. aleppica* | 36°93′452″ | 37°38′829″ | 803 | Antep to Kilis road |
| 12 | *A. annua* | 36°93′452″ | 37°38′829″ | 803 | Antep to Kilis road |
| 13 | *A. annua* | 36°93′452″ | 37°38′829″ | 803 | Antep to Kilis road |
| 14 | *A. annua* | 36°93′452″ | 37°38′829″ | 803 | Antep to Kilis road |
| 15 | *A. annua* | 36°93′452″ | 37°38′829″ | 803 | Antep to Kilis road |
| 16 | *A. annua* | 37°53′903″ | 36°82′373″ | 489 | K. Maraş-Süleymanlı village |
| 17 | *A. annua* | 37°53′903″ | 36°82′373″ | 489 | K. Maraş-Süleymanlı village |
| 18 | *A. annua* | 37°53′903″ | 36°82′373″ | 489 | K. Maraş-Süleymanlı village |
| 19 | *A. annua* | 37°53′903″ | 36°82′373″ | 489 | K. Maraş-Süleymanlı village |
| 20 | *A. dentata* | 36°80′804″ | 36°93′789″ | 476 | Kilis to Hassa road |
| 21 | *A. dentata* | 36°80′804″ | 36°93′789″ | 476 | Kilis to Hassa road |
| 22 | *A. dentata* | 36°80′804″ | 36°93′789″ | 476 | Kilis to Hassa road |
| 23 | *A. dentata* | 36°80′804″ | 36°93′789″ | 476 | Kilis to Hassa road |
| 24 | *A. dentata* | 37°01′059″ | 38°03′994″ | 494 | Urfa-Bentbahçesi-Bozdere |
| 25 | *A. dentata* | 37°01′059″ | 38°03′994″ | 494 | Urfa-Bentbahçesi-Bozdere |
| 26 | *A. dentata* | 37°01′059″ | 38°03′994″ | 494 | Urfa-Bentbahçesi-Bozdere |
| 27 | *A. microcarpa* | 36°61′688″ | 36°57′067″ | 224 | Hatay-Köseler village |
| 28 | *A. microcarpa* | 36°61′688″ | 36°57′067″ | 224 | Hatay-Köseler village |
| 29 | *A. microcarpa* | 36°61′688″ | 36°57′067″ | 224 | Hatay-Köseler village |
| 30 | *A. microcarpa* | 36°61′688″ | 36°57′067″ | 224 | Hatay-Köseler village |
| 31 | *A. microcarpa* | 38°27′653″ | 30°16′245″ | 1120 | Afyon to Denizli road |
| 32 | *A. microcarpa* | 38°27′653″ | 30°16′245″ | 1120 | Afyon to Denizli road |
| 33 | *A. microcarpa* | 38°27′653″ | 30°16′245″ | 1120 | Afyon to Denizli road |
| 34 | *A. aestivalis* subsp. *aestivalis* | 38°01′864″ | 34°05′018″ | 1174 | Adana to Aksaray road |
| 35 | *A. aestivalis* subsp. *aestivalis* | 38°01′864″ | 34°05′018″ | 1174 | Adana to Aksaray road |
| 36 | *A. aestivalis* subsp. *aestivalis* | 38°01′864″ | 34°05′018″ | 1174 | Adana to Aksaray road |
| 37 | *A. aestivalis* subsp. *aestivalis* | 38°01′864″ | 34°05′018″ | 1174 | Adana to Aksaray road |
| 38 | *A. aestivalis* subsp. *aestivalis* | 39°97′982″ | 41°47′076″ | 1830 | Erzurum to Pasinler road |
| 39 | *A. aestivalis* subsp. *aestivalis* | 39°97′982″ | 41°47′076″ | 1830 | Erzurum to Pasinler road |
| 40 | *A. aestivalis* subsp. *aestivalis* | 39°97′982″ | 41°47′076″ | 1830 | Erzurum to Pasinler road |
| 41 | *A. aestivalis* subsp. *aestivalis* | 39°97′982″ | 41°47′076″ | 1830 | Erzurum to Pasinler road |
| 42 | *A. aestivalis* subsp. *parviflora* | 37°01′059″ | 38°03′994″ | 494 | Urfa to Bentbahçesi-Bozdere |

**Table 1.** (Continued).

| | | | | | |
|---|---|---|---|---|---|
| 43 | *A. aestivalis* subsp. *parviflora* | 37°01′059″ | 38°03′994″ | 494 | Urfa to Bentbahçesi-Bozdere |
| 44 | *A. aestivalis* subsp. *parviflora* | 37°01′059″ | 38°03′994″ | 494 | Urfa to Bentbahçesi-Bozdere |
| 45 | *A. aestivalis* subsp. *parviflora* | 37°05′282″ | 38°08′719″ | 554 | Bilecik to Suruç road |
| 46 | *A. aestivalis* subsp. *parviflora* | 37°05′282″ | 38°08′719″ | 554 | Bilecik to Suruç road |
| 47 | *A. aestivalis* subsp. *parviflora* | 37°05′282″ | 38°08′719″ | 554 | Bilecik to Suruç road |
| 48 | *A. eriocalycina* | 37°37′213″ | 40°70′214″ | 980 | Mardin to Zinnar gardens |
| 49 | *A. eriocalycina* | 37°37′213″ | 40°70′214″ | 980 | Mardin to Zinnar gardens |
| 50 | *A. eriocalycina* | 37°37′213″ | 40°70′214″ | 980 | Mardin to Zinnar gardens |
| 51 | *A. eriocalycina* | 37°37′213″ | 40°70′214″ | 980 | Mardin to Zinnar gardens |
| 52 | *A. eriocalycina* | 40°28′104″ | 42°95′687″ | 1820 | Kars to Kağızman road |
| 53 | *A. eriocalycina* | 40°28′104″ | 42°95′687″ | 1820 | Kars to Kağızman road |
| 54 | *A. eriocalycina* | 40°28′104″ | 42°95′687″ | 1820 | Kars to Kağızman road |
| 55 | *A. eriocalycina* | 40°28′104″ | 42°95′687″ | 1820 | Kars to Kağızman road |
| 56 | *A. flammea* | 37°37′213″ | 40°70′214″ | 980 | Mardin to Zinnar gardens |
| 57 | *A. flammea* | 37°37′213″ | 40°70′214″ | 980 | Mardin to Zinnar gardens |
| 58 | *A. flammea* | 37°37′213″ | 40°70′214″ | 980 | Mardin to Zinnar gardens |
| 59 | *A. flammea* | 37°37′213″ | 40°70′214″ | 980 | Mardin to Zinnar gardens |
| 60 | *A. flammea* | 39°61′516″ | 32°65′337″ | 1063 | Ankara Agricultural Application Station |
| 61 | *A. flammea* | 39°61′516″ | 32°65′337″ | 1063 | Ankara Agricultural Application Station |
| 62 | *A. flammea* | 39°61′516″ | 32°65′337″ | 1063 | Ankara Agricultural Application Station |

for K was predicted with Evanno's ΔK method (Evanno et al., 2005) using STRUCTURE HARVESTER (Earl and vanHoldt, 2012).

## 3. Results and discussion

### 3.1. Polymorphism revealed by iPBS primers

In this experiment, 83 pairs of iPBS primers were used, and only 10 primers (12%) generated sufficiently clear polymorphic band profiles. Similarly, Mehmood et al. (2013), Guo et al. (2014), and Nemli et al. (2015) selected 6, 15, and 47 primers out of 83, 41, and 83 iPBS primers, respectively, for further analysis. According to our results, the iPBS-retrotransposon primers among the *Adonis* sp. used in this study were not likely to be conserved. This finding supported previous reports in other species, such as saffron (Gedik et al., 2017), common bean (Nemli et al., 2015), grape (Guo et al., 2014), guava (Mehmood et al., 2016), and those investigated by Kalendar et al. (2010).

There were a total of 204 alleles with an average of 20.4 per primer. The number of iPBS bands per species was 3.29. The number of alleles per polymorphic locus ranged from 8 (iPBS 2401) to 35 (iPBS 2381), with an average of 20.3. In a similar study, the average number of polymorphic iPBS-retrotransposon bands was reported to vary between 15 and 35 bands per primer (Gedik et al., 2017). These results may also indicate that iPBS-retrotransposon primers for *Adonis* L. are more conserved compared to apricot (Baránek et al., 2012), *Cicer* species (Andeden et al., 2013), guava (Mehmood et al., 2013), *Myrica rubra* (Fang-Yong and Ji-Hong, 2014), grape (Guo et al., 2014), common bean (Nemli et al., 2015), tea (Phong et al., 2016), and saffron (Gedik et al., 2017). In addition, iPBS-retrotransposon primers seemed to provide more data than other methods, such as inter-retrotransposon–amplified polymorphism (IRAP) (Boronnikova and Kalendar, 2010), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP). For example, Suh et al. (2002) reported a total of 91 polymorphic bands for 60 individuals from 12 Korean *Adonis* populations using 12 arbitrary RAPD primers. In another study that utilized AFLP loci, the number of polymorphic bands was 393 among 163 *A. aestivalis* individuals, with an average of 2.41 polymorphic bands per species (Hirsch et al., 2015).

In the current study, the polymorphism percentage varied between 95% (iPBS 2079) and 100% (the remaining iPBS primers) with an average of 99.5% (Table 3). In the genetic diversity study of *Adonis* conducted by Boronnikova and Kalendar (2010) using IRAP markers, the polymorphism percentage ranged 86%–96% with an average of 93%. Similarly, the average percentage of

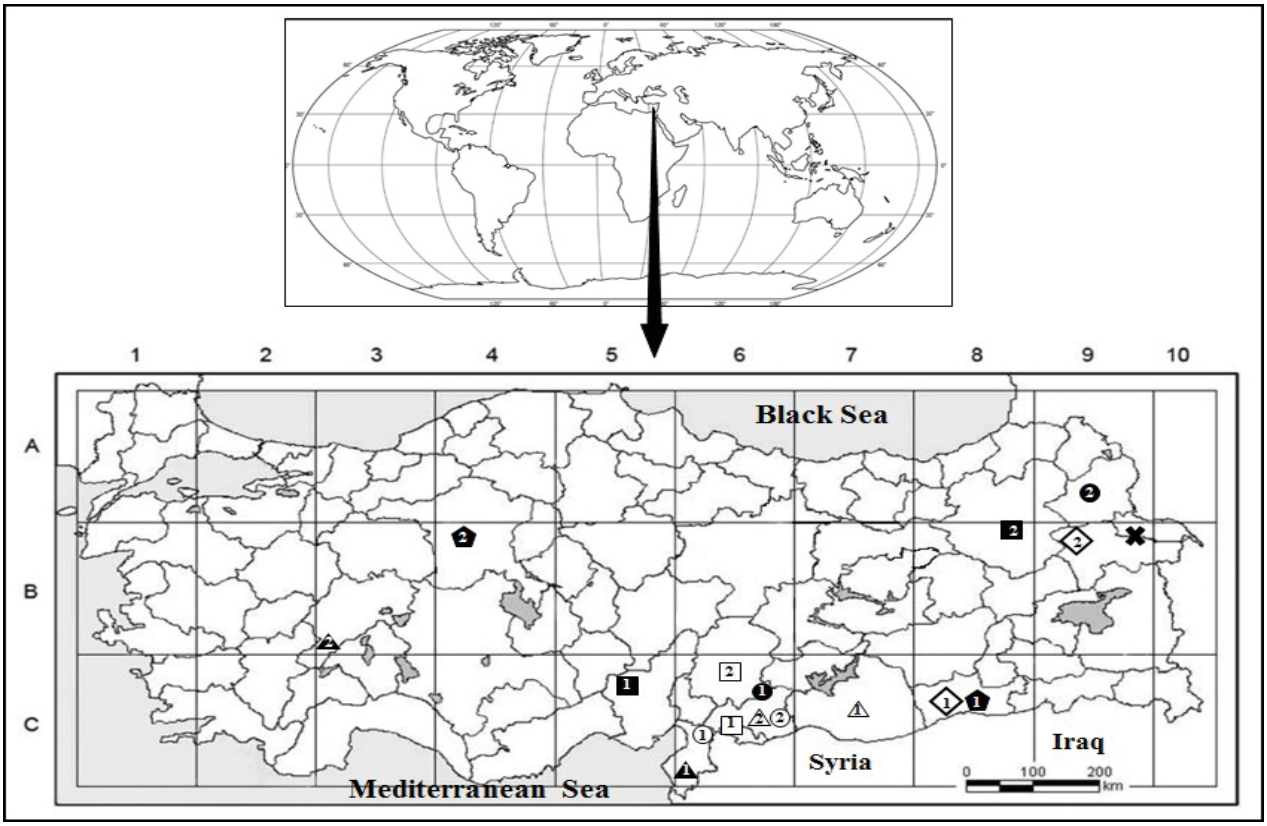


**Figure 1**. Map showing the location of study sites (Ó). *A. volgensis* (Iğdır-Elmagöl village, rocky slopes, 1580 m, 39°76′87″N, 44°14′77″E); r *A. aleppica* (1: Şanlıurfa-Bozova, field, 576 m, 37°39′88″N, 38°44′69″E; 2: Gaziantep-Kilis, field, 803 m, 36°93′45″N, 37°38′82″E; 3: Kilis-Hassa, roadside, 476 m, 36°80′80″N, 36°93′79″E); £ *A. annua* (1: Gaziantep-Kilis, field, 803 m, 36°93′45″N 37°38′82″E; 2: Kahramanmaraş-Süleymanlı village, slopes, 489 m, 37°53′90″N, 36°82′37″E); ™ *A. dentata* (1: Kilis-Hassa, roadside, 476 m, 36°80′80″N, 36°93′79″E; 2: Şanlıurfa-Bozdere village, slopes, 494 m, 37°01′05″N, 38°03′99″E); p *A. microcarpa* (1: Hatay-Köseler village, field, 224 m, 36°61′68″N, 36°57′06″E; 2: Afyon-Denizli highway, roadside, 1120 m, 38°27′65″N, 30°16′24″E); ¢ *A. aestivalis* ssp. *aestivalis* (1: Adana-Aksaray highway, field, 1174 m, 38°01′86″N, 34°05′01″E; 2: Erzurum-Pasinler highway, field, 1830 m, 39°97′98″N, 41°47′07″E); ˜ *A. aestivalis* ssp. *parviflora* (1: Şanlıurfa-Bozdere village, slopes, 494 m, 37°01′05″N, 38°03′99″E; 2: Kafkas University Campus, field, 1775 m, 40°35′03″N, 43°04′15″E); ¯ *A. eriocalycina* (1: Mardin-Zinnar gardens, slopes, 980 m, 37°37′21″N, 40°70′21″E; 2: Kars-Kağızman, roadside, 1820 m, 40°28′10″N, 42°95′68″E); À *A. flammea* (1: Mardin-Zinnar gardens, slopes, 980 m, 37°37′21″N, 40°70′21″E; 2: Ankara-İkizce Agricultural Application Station, field, 1063 m, 39°61′51″N, 32°65′33″E).

**Table 2.** Sequence, GC (%), and annealing temperature (°C) of iPBS markers used for genetic characterization of *Adonis* ecotypes.

| Number | iPBS primers | Sequence (5’ to 3’) | GC (%) | Tm (°C) | Optimal annealing, Ta (°C) |
|---|---|---|---|---|---|
| 1 | 2079 | AGGTGGGCGCCA | 75.0 | 56.0 | 55.0 |
| 2 | 2095 | GCTCGGATACCA | 58.0 | 49 | 53.0 |
| 3 | 2276 | ACCTCTGATACCA | 46.0 | 49.0 | 52.0 |
| 4 | 2377 | ACGAAGGGACCA | 58.0 | 49.0 | 53.0 |
| 5 | 2378 | GGTCCTCATCCA | 58.0 | 49.0 | 50.0 |
| 6 | 2381 | GTCCATCTTCCA | 50.0 | 46.0 | 50.0 |
| 7 | 2385 | CCATTGGGTCCA | 58.0 | 49.0 | 50.0 |
| 8 | 2390 | GCAACAACCCCA | 58.0 | 49.0 | 56.0 |
| 9 | 2392 | TAGATGGTGCCA | 50.0 | 46.0 | 52.0 |
| 10 | 2401 | AGTTAAGCTTTGATACCA | 33.0 | 58.0 | 50.0 |

**Table 3.** The number of alleles (ne), number of polymorphic alleles, percentage of polymorphism, and polymorphism information content (PIC) values of iPBS markers.

| Number | iPBS primers | Ne | Number of polymorphic alleles | Percentage of polymorphism (%) | PIC |
|---|---|---|---|---|---|
| 1 | 2079 | 20 | 19 | 95 | 0.31 |
| 2 | 2095 | 23 | 23 | 100 | 0.26 |
| 3 | 2276 | 18 | 18 | 100 | 0.16 |
| 4 | 2377 | 24 | 24 | 100 | 0.33 |
| 5 | 2378 | 14 | 14 | 100 | 0.36 |
| 6 | 2381 | 35 | 35 | 100 | 0.25 |
| 7 | 2385 | 15 | 15 | 100 | 0.39 |
| 8 | 2390 | 23 | 23 | 100 | 0.37 |
| 9 | 2392 | 24 | 24 | 100 | 0.19 |
| 10 | 2401 | 8 | 8 | 100 | 0.27 |
| Mean | | 20.40 | 20.30 | 99.5 | 0.30 |

polymorphism was 95.05% in tea (Phong et al., 2016). In a more recent study of *Adonis* sp., the percentage of polymorphism was 56%–81% for *A. vernalis* based on the AFLP marker (Hirsch et al., 2015).

The PIC value ranged from 0.16 (iPBS 2276) to 0.39 (iPBS 2385), with an average of 0.30 (Table 3). Due to its higher PIC values, iPBS 2385 was found to be the best marker for differentiating between the genotypes. The iPBS marker system used in this study revealed a wide range of genetic diversity in *Adonis* L. and its related species. These results are in agreement with earlier investigations in different plants, such as guava cultivars (*Psidium guajava* L.), with an average PIC of 0.28 (Mehmood et al., 2016), and tea cultivars (*Camellia sinensis*), with an average PIC of 0.30 (Phong et al., 2016).

### 3.2. Genetic diversity in *Adonis* L. (Ranunculaceae) and its close relative species

Table 4 presents a summary of the statistical results for each of the nine *Adonis* taxa. The highest average number of alleles (ne), Nei's genetic diversity, (h) and Shannon's information index (I) were obtained from *A. volgensis* species (1.64, 0.39, and 0.58, respectively), whereas the lowest values were observed in *A. flammea* species (1.41, 0.29, and 0.46, respectively). In addition, the total average ne, h, and I values were 1.55, 0.35, and 0.53, respectively. Gedik et al. (2017) reported that the average Nei's genetic diversity (h) and Shannon's information index (I) among saffron genotypes and its close relative species were 0.16 and 0.29, respectively, indicating a low level of differentiation. In other studies, the average Shannon's information index for iPBS-retrotransposon markers was 0.12 for okra species (Yıldız et al., 2015) and 0.27 for guava species (Mehmood et al., 2013).

### 3.3. Results of AMOVA

AMOVA revealed high genetic variation within the *Adonis* populations, and the percentage of total variance was 71% (Table 5). This may be due to variations in ecotypes, selection, adaptation, migration, genetic drift, gene flow, and pollination method. Other important factors could be the environment and human activities over time (Solouki et al., 2008). There was no significant genetic variation among populations (only 29%). Thus, the majority of genetic variation was attributed to differences within the population. The main reason could be the fertilization nature of *Adonis*. Adonis species usually have protandry or rarely protogyny. In their flowers, anthers ripen before stigma. Dichogamy function is supported by different (biological and morphological) mechanisms, and in this way, cross-fertilization is provided in this family, which increases genetic diversity (Denisow et al., 2014) within the population. Similar findings were reported for other plants, such as *A. vernalis* (Hirsch et al., 2015), *A. aestivalis* (Meyer et al., 2015), *Ajowan* (Heidari et al., 2016), *Nigella sativa* (Birhanu et al., 2015), and *Falcaria vulgaris* (Piya et al., 2014).

### 3.4. Cluster analysis and principal coordinate analysis

The Dice genetic similarity coefficient was used for the diversity estimation of the genotypes. This coefficient is commonly used to estimate genetic distances. The highest genetic dissimilarity (0.49) was found between the ecotypes *A. volgensis* and *A. aestivalis* subsp. *aestivalis*. The UPGMA tree constructed using the Dice genetic distance coefficient is shown in Figure 2. A relatively good separation was obtained between the species. The analysis divided the *Adonis* ecotypes into four main groups: group A consisting of two *A. flammea* ecotypes,

**Table 4.** Summary statistics for 62 *Adonis* ecotypes assessed with 10 iPBS primers.

| No. | Species | ne* | h* | I* |
|---|---|---|---|---|
| 1 | *A. volgensis* | 1.54 | 0.35 | 0.53 |
| 2 | *A. volgensis* | 1.78 | 0.44 | 0.63 |
| 3 | *A. volgensis* | 1.69 | 0.41 | 0.60 |
| 4 | *A. volgensis* | 1.53 | 0.34 | 0.53 |
| | Mean *A. volgensis* | 1.64 | 0.39 | 0.58 |
| 5 | *A. aleppica* | 1.49 | 0.33 | 0.51 |
| 6 | *A. aleppica* | 1.61 | 0.37 | 0.56 |
| 7 | *A. aleppica* | 1.53 | 0.34 | 0.53 |
| 8 | *A. aleppica* | 1.44 | 0.30 | 0.48 |
| 9 | *A. aleppica* | 1.31 | 0.23 | 0.40 |
| 10 | *A. aleppica* | 1.52 | 0.34 | 0.52 |
| 11 | *A. aleppica* | 1.53 | 0.34 | 0.53 |
| | Mean *A. aleppica* | 1.49 | 0.32 | 0.50 |
| 12 | *A. annua* | 1.71 | 0.41 | 0.60 |
| 13 | *A. annua* | 1.82 | 0.45 | 0.64 |
| 14 | *A. annua* | 1.68 | 0.40 | 0.59 |
| 15 | *A. annua* | 1.72 | 0.41 | 0.61 |
| 16 | *A. annua* | 1.61 | 0.37 | 0.56 |
| 17 | *A. annua* | 1.37 | 0.27 | 0.44 |
| 18 | *A. annua* | 1.38 | 0.27 | 0.45 |
| 19 | *A. annua* | 1.37 | 0.27 | 0.44 |
| | Mean *A. annua* | 1.58 | 0.36 | 0.54 |
| 20 | *A. dentata* | 1.39 | 0.28 | 0.45 |
| 21 | *A. dentata* | 1.52 | 0.34 | 0.52 |
| 22 | *A. dentata* | 1.48 | 0.32 | 0.50 |
| 23 | *A. dentata* | 1.63 | 0.38 | 0.57 |
| 24 | *A. dentata* | 1.61 | 0.37 | 0.56 |
| 25 | *A. dentata* | 1.57 | 0.36 | 0.55 |
| 26 | *A. dentata* | 1.61 | 0.37 | 0.56 |
| | Mean *A. dentata* | 1.54 | 0.35 | 0.53 |
| 27 | *A. microcarpa* | 1.63 | 0.38 | 0.57 |
| 28 | *A. microcarpa* | 1.58 | 0.37 | 0.55 |
| 29 | *A. microcarpa* | 1.64 | 0.39 | 0.58 |
| 30 | *A. microcarpa* | 1.46 | 0.31 | 0.49 |
| 31 | *A. microcarpa* | 1.49 | 0.33 | 0.51 |
| 32 | *A. microcarpa* | 1.63 | 0.38 | 0.57 |
| 33 | *A. microcarpa* | 1.62 | 0.38 | 0.57 |
| | Mean *A. microcarpa* | 1.58 | 0.36 | 0.55 |
| 34 | *A. aestivalis* subsp. *aestivalis* | 1.64 | 0.39 | 0.58 |
| 35 | *A. aestivalis* subsp. *aestivalis* | 1.58 | 0.36 | 0.55 |
| 36 | *A. aestivalis* subsp. *aestivalis* | 1.71 | 0.41 | 0.60 |
| 37 | *A. aestivalis* subsp. *aestivalis* | 1.81 | 0.44 | 0.64 |
| 38 | *A. aestivalis* subsp. *aestivalis* | 1.73 | 0.42 | 0.61 |
| 39 | *A. aestivalis* subsp. *aestivalis* | 1.66 | 0.39 | 0.58 |
| 40 | *A. aestivalis* subsp. *aestivalis* | 1.67 | 0.40 | 0.59 |
| 41 | *A. aestivalis* subsp. *aestivalis* | 1.67 | 0.40 | 0.59 |
| | Mean *A. aestivalis* subsp. *aestivalis* | 1.62 | 0.38 | 0.56 |
| 42 | *A. aestivalis* subsp. *parviflora* | 1.62 | 0.38 | 0.57 |
| 43 | *A. aestivalis* subsp. *parviflora* | 1.64 | 0.39 | 0.58 |
| 44 | *A. aestivalis* subsp. *parviflora* | 1.57 | 0.36 | 0.55 |
| 45 | *A. aestivalis* subsp. *parviflora* | 1.40 | 0.29 | 0.46 |
| 46 | *A. aestivalis* subsp. *parviflora* | 1.40 | 0.29 | 0.46 |
| 47 | *A. aestivalis* subsp. *parviflora* | 1.56 | 0.35 | 0.54 |
| | Mean *A. aestivalis* subsp. *parviflora* | 1.62 | 0.38 | 0.56 |
| 48 | *A. eriocalycina* | 1.58 | 0.37 | 0.55 |
| 49 | *A. eriocalycina* | 1.46 | 0.31 | 0.49 |
| 50 | *A. eriocalycina* | 1.48 | 0.32 | 0.50 |
| 51 | *A. eriocalycina* | 1.50 | 0.33 | 0.52 |
| 52 | *A. eriocalycina* | 1.38 | 0.27 | 0.45 |
| 53 | *A. eriocalycina* | 1.46 | 0.31 | 0.49 |
| 54 | *A. eriocalycina* | 1.42 | 0.29 | 0.47 |
| 55 | *A. eriocalycina* | 1.34 | 0.25 | 0.42 |
| | Mean *A. eriocalycina* | 1.45 | 0.31 | 0.49 |
| 56 | *A. flammea* | 1.32 | 0.24 | 0.40 |
| 57 | *A. flammea* | 1.14 | 0.12 | 0.25 |
| 58 | *A. flammea* | 1.47 | 0.32 | 0.50 |
| 59 | *A. flammea* | 1.60 | 0.37 | 0.56 |
| 60 | *A. flammea* | 1.52 | 0.34 | 0.52 |
| 61 | *A. flammea* | 1.46 | 0.31 | 0.49 |
| 62 | *A. flammea* | 1.37 | 0.27 | 0.44 |
| | Mean *A. flammea* | 1.41 | 0.29 | 0.46 |
| | Total mean | 1.55 | 0.35 | 0.53 |

**Table 5.** AMOVA analysis of nine *Adonis* taxa using iPBS markers.

| Source | Degrees of freedom (df) | Sum of squares (SS) | Variance component | % of total variance | P-value |
|---|---|---|---|---|---|
| Among pops. | 14 | 36.059 | 0.395 | 29% | 0.29 |
| Within pops. | 47 | 45.167 | 0.961 | 71% | 0.001 |
| Total | 61 | 81.226 | 1.356 | 100% | |

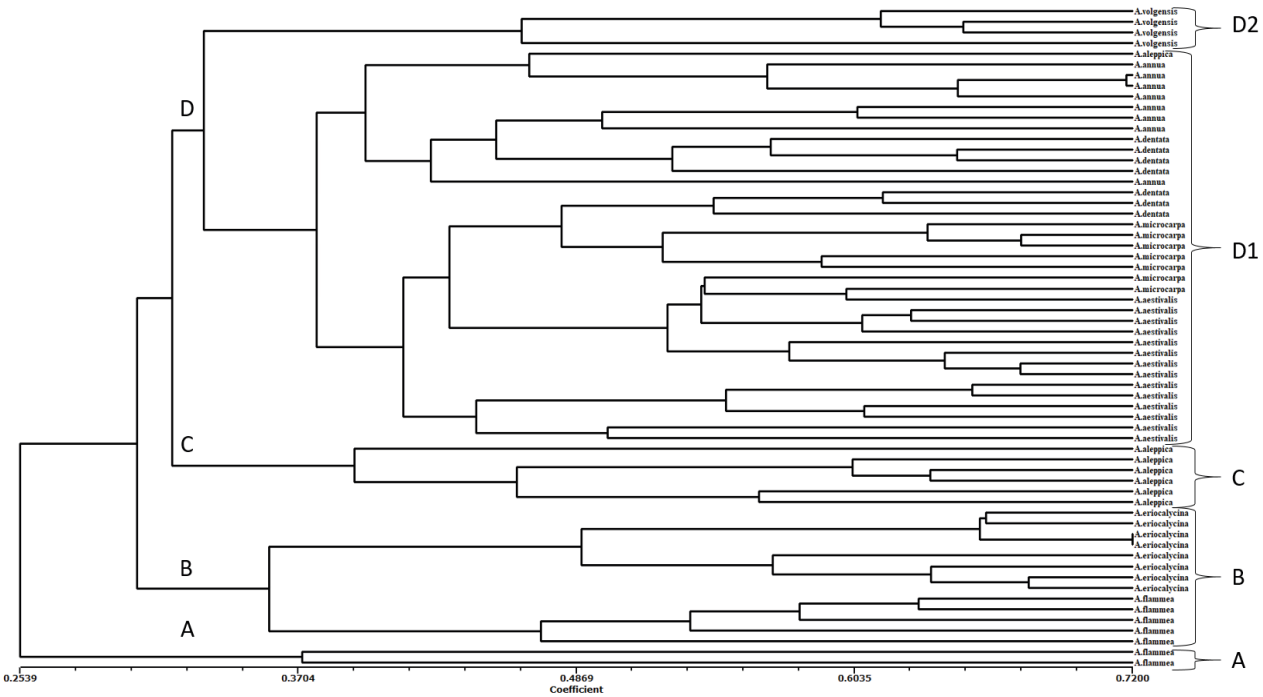


**Figure 2.** Dendrogram of 62 *Adonis* ecotypes based on iPBS markers according to UPGMA with the Dice similarity index.

group B comprising 13 *Adonis* ecotypes of five *A. flammea* ecotypes and eight *A. eriocalycina* ecotypes, group C containing six *Adonis* ecotypes of *A. aleppica* ecotypes, and group D containing 41 ecotypes. Group D had two subgroups: the first containing fourteen *A. aestivalis* subsp. *aestivalis* ecotypes, seven *A. microcarpa* ecotypes, seven *A. dentata* ecotypes, eight *A. annua* ecotypes, and one *A. aleppica*; and the second consisting of four *A. volgensis* ecotypes. Similarly, Gedik (2017) studied 28 *C. sativus* genotypes and 17 close relative species of saffron to identify their genetic diversity using 16 polymorphic iPBS-retrotransposon primers and stated that genotypes belonging to the same species were placed in different clusters of the dendrogram. Geographical distribution range is a major factor in determining the genetic diversity of varieties (Zecca et al., 2012). PCoA was performed to visualize variation of ecotypes within and among the populations in more detail. The results showed that the first three principal coordinates accounted for 81.51% of the total variation (first axis = 47.55%, second = 26.28%, and third = 7.38%) (Table 6; Figure 3). In a recent study, PCoA was used to determine the taxonomic identities of Korean *Adonis* species at specific and infraspecific levels (Lee et al., 2000). In another study, results for the reproductive character of Korean *Adonis* revealed three species: *A. amurensis*, *A. multiflora*, and *A. pseudoamurensis* (Suh et al., 2002). In our study, no relationship was found between

**Table 6.** PCoA analysis of *Adonis* ecotypes using the Dice similarity coefficients.

| Axis | 1 | 2 | 3 |
|---|---|---|---|
| % | 47.55 | 26.58 | 7.38 |
| Cum. % | 47.55 | 74.13 | 81.51 |

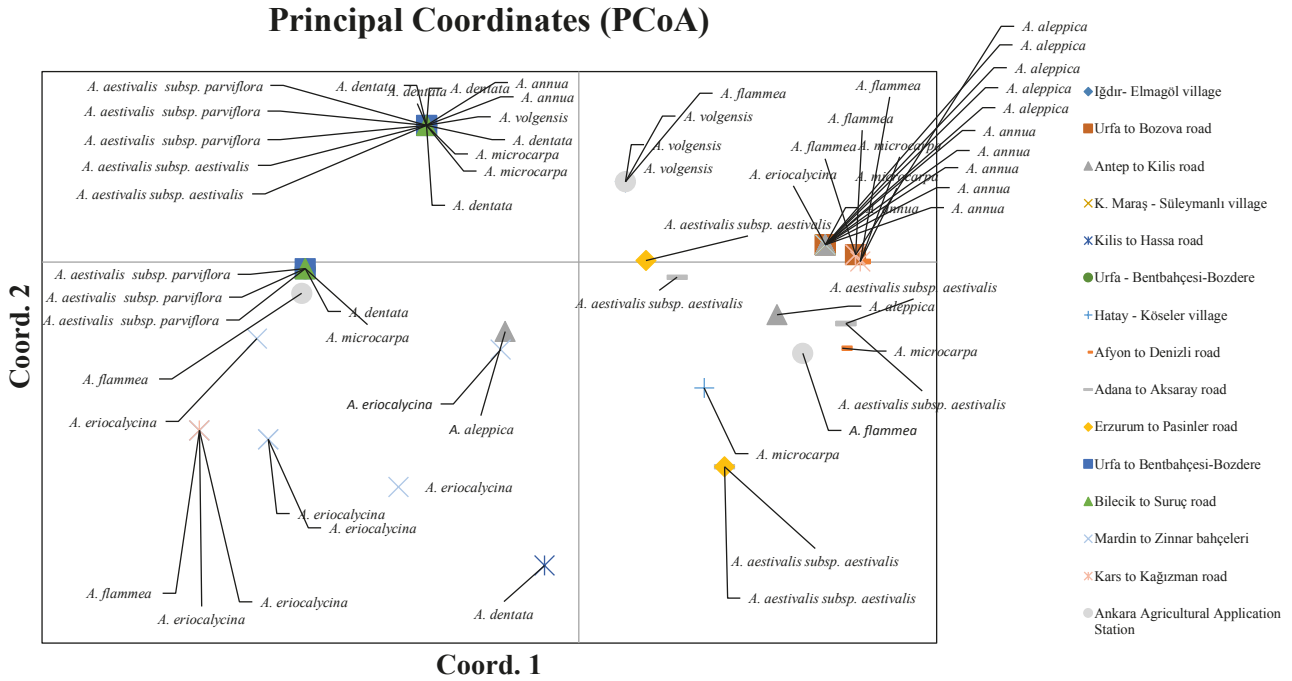


**Figure 3.** PCoA of nine species of *Adonis* based on 10 iPBS markers.

the PCoA grouping of genotypes and their geographical origin by cluster analysis. *Adonis* species had high genetic diversity based on the complex cluster results (Figure 2), and a wide scatter range was observed in the PCoA plot (Figure 3). This result was supported by earlier studies using molecular markers such as iPBS (Baloch et al., 2015), simple sequence repeat (Radinovic et al., 2017), and inter simple sequence repeat (Ballesta et al., 2015). This can be attributed to the highly cross-pollinated nature of *Adonis* species undergoing sexual reproduction.

### 3.5. Population genetic structure analysis

In this research, little congruence was found between UPGMA and geographical distances. The population structure of 62 *Adonis* ecotypes was categorized according to data produced from 10 iPBS markers. The Bayesian clustering method is considered a powerful computational tool for the estimation of various features of populations. STRUCTURE assigns individuals to different populations and hybrid zones on the basis of allele frequencies of genotypes. In this study, an admixture model with a correlated allele frequency was used to infer the genetic structure (testing from K = 2 to K = 10). The value of K was estimated by posterior probability of the data for a given K, Pr (X|K) (Pritchard et al., 2000b). ΔK is used to determine the best fit value of K. The highest value was obtained at K = 4 (Figure 4). The STRUCTURE analysis was conducted for K = 4, suggesting four clusters for 62 *Adonis* ecotypes as shown in Figure 5 (color scale: red [A], green [B], blue [C], and yellow [D]). According to Figure 5, cluster A (20.96% probability) contained 13 ecotypes (≠ 34, ≠ 38, ≠ 40, ≠ 39, ≠ 41, ≠ 37, ≠ 35, ≠ 33, ≠ 36, ≠ 32, ≠ 44, ≠ 43, and ≠ 42), cluster B (20.96% probability) included 13 ecotypes (≠ 23, ≠ 28, ≠ 20, ≠ 26, ≠ 22, ≠ 21, ≠ 24, ≠ 25, ≠ 29, ≠ 30, ≠ 19, ≠ 27, and ≠ 18), cluster C (27.41% probability) consisted of 17 ecotypes (≠ 55, ≠ 53, ≠ 54, ≠ 52, ≠ 50, ≠ 48, ≠ 58, ≠ 61, ≠ 49, ≠ 62, ≠ 51, ≠ 60, ≠ 59, ≠ 57, ≠ 47, ≠ 56, and ≠ 46), and cluster D (27.41% probability) comprised 17 ecotypes (≠ 3, ≠ 13, ≠ 7, ≠ 8, ≠ 10, ≠ 2, ≠ 14, ≠ 6, ≠ 1, ≠ 9, ≠ 11, ≠ 12, ≠ 15, ≠ 17, ≠ 4, ≠ 5, and ≠ 16). Furthermore, ≠45 was placed in all clusters, and ≠31 was observed in both cluster C and cluster D (3.2%; membership probability <0.8). Similar findings were reported for the population structure of common bean ecotypes (Nemli et al., 2014) and other edible seed plants, such as ajowan (Heidari et al., 2016) and fennel (Maghsoudi-kelardashti et al., 2015).

The expected heterozygosity, which measures the probability of two randomly chosen individuals being different (heterozygous) in a given locus, ranged from 0.2266 (subpopulation A) to 0.3034 (subpopulation B), with an average of 0.2634 (Table 7). Similarly, population differentiation measurements ($F_{st}$) which provide the summary of genetic differentiation between the groups ranged from 0.1170 (subpopulation B) to 0.3010 (subpopulation A) with a relatively high average 0.2154 (Table 8), which confirms the separation of all subpopulations and their diversity in iPBS alleles.

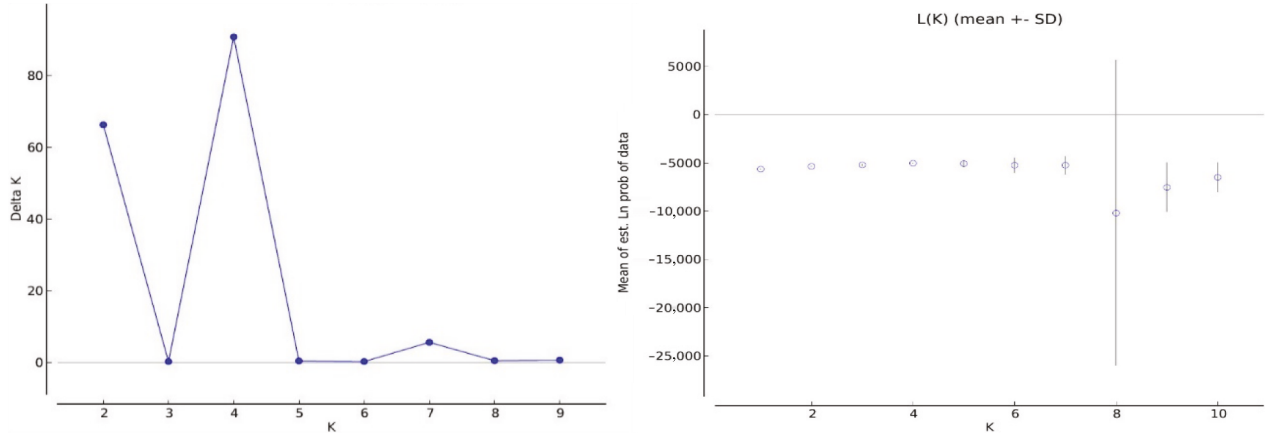


**Figure 4.** Line graphs from the admixture model of structure of Ln P(D) [a measure of the natural logarithm of the posterior probability (P) of the data (D)] and ΔK for *Adonis* populations. **a:** Mean value of the statistic Ln P(D) produced by STRUCTURE at each value of K; **b:** DK.

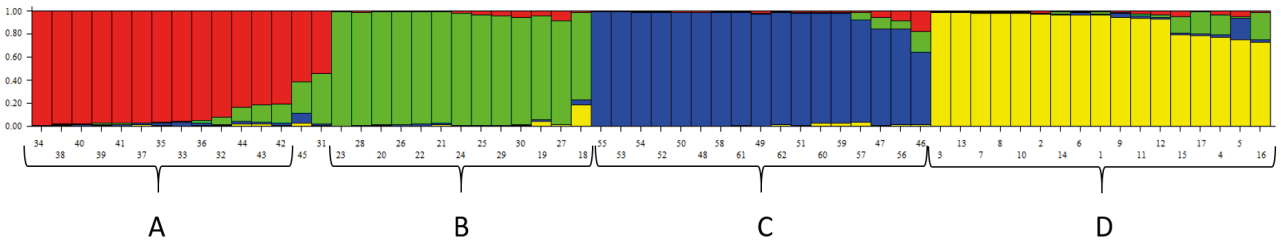


**Figure 5.** Genetic structure of 62 *Adonis* L. ecotypes as inferred by STRUCTURE software with 10 iPBS marker data sets. Single vertical line represents an individual accession, and different colors represent genetic stocks/gene pools. Segments of each vertical line show extent of admixture in an individual.

**Table 7.** Heterozygosity and $F_{st}$ values of 4 *Adonis* subpopulations.

| Subpopulation (K) | Expected heterozygosity | Fst value |
|---|---|---|
| A | 0.2266 | 0.3010 |
| B | 0.3034 | 0.1170 |
| C | 0.2451 | 0.2509 |
| D | 0.2787 | 0.1929 |
| Average | 0.2634 | 0.2154 |

**Table 8.** Genetic differentiation based on $F_{st}$ values among four *Adonis* subpopulations identified by population structure analysis.

| | Subpop. A | Subpop. B | Subpop. C |
|---|---|---|---|
| Subpop. A | - | | |
| Subpop. B | 0.0495 | - | |
| Subpop. C | 0.0615 | 0.0384 | - |
| Subpop. D | 0.0525 | 0.0401 | 0.0439 |

## 4. Conclusion

*Adonis* L. is mainly considered a medicinal plant. Because of its marked effects as a cardiotonic agent in treating heart diseases, some species of the genus *Adonis* L. and their extracts have been widely used clinically in countries such as Russia and China. Breeding programs are necessary to develop cultivars having higher metabolite yields, and genetic diversity estimation of plant genetic materials is an important prebreeding activity in plant breeding. This study emphasized that iPBS marker system can be successfully used in exploring genetic diversity of *Adonis* genetic resources, which will enhance breeding efficiency of *Adonis* genotypes.

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