J Plant Mol Breed (2017) 5(2): 60-67
DOI: 10.22058/JPMB.2018.78467.1154

RESEARCH ARTICLE

Analysis of genetic diversity, phylogenetic relationships and population structure of Arasbaran cornelian cherry (*Cornus mas* L.) genotypes using ISSR molecular markers

Karim Farmanpour Kalalagh, Mehdi Mohebodini*, Alireza Ghanbari

Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran

ABSTRACT: Cornelian cherry (*Cornus mas* L.), considered as the ancestor of cultivated trees in Arasbaran region, is a medicinally and economically plant species. However, little is known about genetic diversity, breeding programs, and population structure of this species in mentioned region. Keeping this in view, the main objectives of present study were to analysis the genetic diversity, phylogenetic relationships and population structure of cornelian cherry genotypes from Arasbaran region using Inter Simple Sequence Repeat molecular markers. Utilized primers amplified 153 bands, of which 98 bands were polymorphic (64% polymorphism). Highest Jaccard's similarity coefficient was obtained 0.777. Based on Unweighted Pair Group Method with Arithmetic Averages, genotypes were divided into seven major groups. On the other hand, Principal Coordinate Analysis (PCoA) as a complementary method to cluster analysis demonstrated genotypes grouping in phylogenetic dendrogram. Relatively low amount of three main components in Principal Component Analysis (PCA) (41.464%) indicated the scattering distribution of utilized primers' sequence in cornelian cherry genome. The mean values of polymorphism information content, marker index, resolving power, observed number of alleles, effective number of alleles, Nei's gene diversity, and Shannon's information index were 0.230, 1.769, 4.7, 1,642, 1.498, 0.271, and 0.392 respectively. Population structure analysis showed the seven groups or sub- populations (K= 7) when the amount of K value was set at K= 2 to K= 10, which demonstrated the results of phylogenetic dendrogram and Principal Coordinate Analysis (PCoA). Results of this study can be useful for planning future studies on cornelian cherry germplasm and breeding programs.

KEYWORDS: Primer, Polymorphism, Nei, Shannon, Dendrogram, Germplasm

INTRODUCTION

Arasbaran situated in the north of East Azerbaijan province and northwest of Iran. Since 1976, UNESCO has registered 72460 hectares of this region as biosphere reserve. Horticultural activities in this biosphere are mainly pomology, olericulture, and arboriculture. One of the most important small fruits in Arasbaran with many distributions is cornelian cherry (*Cornus mas* L.). Cornelian cherry belongs to the Cornaceae family, genus *Cornus* L. [16, 23], like most *Cornus* species is diploid (2n=18) [11] and, native to Central and Southern Europe and parts of western Asia [5, 10, 12]. In addition to economic and nutritional values, the stones and leaves of cornelian cherry are used as pharmaceutical raw materials. According to the principles of organic agriculture, high tolerance of cornelian cherry to disease and pests makes it suitable for production [1, 6, 14]. Genetic resources generally play an important role in the life of people in most countries at different levels (local, national, and international) [8]. Genetic structure of plant species can be considered as an interaction of

*Corresponding author (✉): Mohebodini@uma.ac.ir
Received: 31 Dec. 2017 / Revised: 15 Jan. 2018
Accepted: 22 Jan. 2018

evolutionary processes and mechanism including mutations, genetic erosion, crossing-system, gene flow, and selection [21, 24]. In addition, analysis of genetic diversity in natural species provides valuable information on the history of plant evolution that can be exploited in plant conservation and management [27]. Many aspects of environmental protection such as genetic erosion and regeneration of extinct plant populations can be investigated by carefully studying of genetic diversity and population structure. One of the main objectives of environmental protection is preserving genetic diversity in different populations. Decrease in genetic diversity levels reduces the ability of plant populations to environmental changes [13, 26]. Furthermore, genetic diversity is the basis of breeding programs and selecting process depend on genetic diversity existence. Evaluation of genetic diversity is done by morphological, biochemical and molecular markers. Molecular methods especially PCR-based markers are very important and powerful techniques for evaluating genetic relationships, evolution, selecting superior plants, and examining the similarities and differences of various genotypes. Among different molecular markers, Inter-simple sequence Repeat (ISSR) markers are used for several reasons; for example, unlike SSR, the ISSR technique does not require previous information from DNA sequence, and designed primers do not have the exclusive rights. Therefore, the initial cost for the ISSR primer sequencing is low [3], working with ISSR is easy, fast, and also show a high polymorphism [25], amplified bands are visible with agarose and acrylamide gel electrophoresis without requiring radioactive materials [2]. Due to use of long primers, ISSR repeatability is much higher than RAPD primers.

In view of the fact that Arasbaran region is rich with cornelian cherry genotypes, the population history of these genotypes is still in the dark and little is known about breeding, genetic diversity, and population structure. Therefore, understanding of the genetic diversity in Arasbaran cornelian cherry genotypes is vital for characterizing and conserving germplasm, breeding programs, and registering new cultivars. Keeping the above in view, the main objectives of the present study were (i) to select ISSR primers capable of detecting genetic polymorphism in Arasbaran cornelian cherry genotypes for studying related indices; (ii) to evaluate the amount of genetic distances and genetic relationships (phylogeny) among different genotypes and (iii) to determine the population structure of selected cornelian cherry genotypes.

MATERIALS AND METHODS

Sampling and genomic DNA extraction

Leaf samples of twenty genotypes were collected from different regions of Arasbaran including Yokhari-Baghlar, Zardvan-Chaei, Razoular, Khaneh-Khousro, Alkhoumlar, Kalalagh, Ashaghe-Baghlar, Kafshan-evi, Dashli, Chilakhaneh, Kouhneh-Baghlar, and Gale-Darasi (Table 1). All sampled trees were mature adults and apparently healthy. Young, fresh, and unblemished leaves were taken in May from each tree and were stored at -80˚C for DNA extraction [7]. Total genomic DNA was isolated from 200 mg of dried leaf samples using a modified Cetyl trimethylammonium bromide (CTAB) extraction technique [4]. Genomic DNA samples were run in 0.8% agarose gel electrophoresis in order to check their quantity and quality. Purity and concentration of DNA samples were further determined using spectrophotometer (6705 UV/Vis model). DNA samples were diluted with double-distilled water (DDW) to a final concentration and stored at -20˚C.

Table 1. Locations and geographical characteristics of selected cornelian cherry genotypes.

Genotype name	Locality	Latitude (N)	Longitude (E)	Altitude (ft)
Cm1	Yokhari-Baghlar	38°49'01.6"	47°03'29.5"	4048
Cm2	Zardvan-Chaei	38°49'36.5"	47°03'30.6"	3971
Cm3	Razoular	38°50'07.5"	47°03'17.6"	3875
Cm4	Khaneh-Khousro	38°52'42.2"	47°02'22.1"	3403
Cm5	Alkhoumlar	38°50'39.4"	47°03'00.1"	3800
Cm6	Kalalagh	38°49'11.5"	47°03'25.4"	4050
Cm7	Zardvan-Chaei	38°49'40.6"	47°03'29.2"	3976
Cm8	Ashaghe-Baghlar	38°49'49.0"	47°03'26.3"	3922
Cm9	Alkhoumlar	38°51'08.4"	47°02'47.7"	3686
Cm10	Kafshan-evi	38°51'32.7"	47°02'54.6"	3605
Cm11	Dashli	38°49'22.9"	47°03'33.5"	4011
Cm12	Yokhari-Baghlar	38°49'05.0"	47°03'30.6"	4039
Cm13	Razoular	38°50'04.5"	47°03'19.9"	3865
Cm14	Chilakhaneh	38°50'16.2"	47°03'20.2"	3827
Cm15	Gale-Darasi	38°51'34.2"	47°01'23.6"	4273
Cm16	Kalalagh	38°49'18.7"	47°03'25.6"	4386
Cm17	Kouhneh-Baghlar	38°49'28.6"	47°03'29.6"	3978
Cm18	Gale-Darasi	38°51'35.6"	47°01'38.2"	4174
Cm19	Chilakhaneh	38°50'25.9"	47°03'17.6"	3803
Cm20	Ashaghe-Baghlar	38°49'58.4"	47°03'24.1"	3913

Table 2. Primer names, Primer sequences, Annealing temperatures, and measured indices.

No.	Primer name	Primer sequence (5′-3′)	Tm (°C)	TL	PL	PPL (%)	PIC	MI	Rp	Na	Ne	h	I
1	UBC839	ACACACACACACACACGA	53	12	9	75	0.272	2.448	5.5	1.750	1.629	0.338	0.482
2	UBC811	GAGAGAGAGAGAGAGAT	52	12	9	75	0.242	2.178	5.5	1.750	1.617	0.335	0.478
3	UBC810	GAGAGAGAGAGAGAGAC	43	9	7	77.77	0.310	2.170	6	1.777	1.628	0.342	0.490
4	UBC819	ACACACACACACACACC	53	14	8	57.14	0.196	1.568	5	1.570	1.462	0.246	0.352
5	Al1	GAGAGAGAGAGACC	43.7	10	5	50	0.195	0.975	3.5	1.50	1.365	0.197	0.284
6	Al2	GAGGAGGAGGC	38	11	6	54.54	0.196	1.176	4	1.545	1.406	0.229	0.332
7	UBC818	AGAGAGAGAGAGAGAGT	42	15	9	60	0.215	1.935	4.5	1.60	1.550	0.286	0.402
8	UBC815	CTCTCTCTCTCTCTCTG	44	13	10	76.92	0.303	3.030	5.5	1.769	1.535	0.304	0.446
9	UBC817	CACACACACACACACAT	49	8	5	62.5	0.215	1.075	3.5	1.625	1.430	0.247	0.364
10	UBC822	ACACACACACACACACT	49	10	7	70	0.242	1.694	4.5	1.70	1.550	0.301	0.433
11	UBC829	GACAGACAGACAGACA	49	13	9	69.23	0.236	2.124	5.5	1.692	1.461	0.274	0.405
12	UBC833	GGAGAGGAGAGGAGA	54.3	14	8	57.14	0.196	1.568	5	1.571	1.534	0.244	0.351
13	UBC834	GGGTGGGGTGGGGTG	54	12	6	50	0.177	1.062	4	1.50	1.306	0.181	0.271
Average	-	-	-	11.7	7.5	64	0.230	1.769	4.7	1.642	1.498	0.271	0.392

Note: Tm (Annealing temperature), TL (Total Loci), PL (Polymorphic Loci), PPL (Percentage of Polymorphic Loci), PIC (Polymorphism Information Content), MI (Marker Index), Rp (Resolving power), Na (Observed number of alleles), Ne (Effective number of alleles), h (Nei's gene diversity), I (Shannon's information index).

PCR amplification

A total of thirteen ISSR primers were used for genotyping. Primer sequences, annealing temperatures, and other information are given in Table 2. PCR amplification for all primers was conducted with a thermocycler machine (Qantarus model) in a 8 µl reaction mixture of 1 µl template DNA (20 ng), 3.75 µl 2 × Master Mix buffer (0.2 units/µl Amplicon Taq DNA Polymerase, 2 mM $MgCl_2$, 0.4 mM dNTPs), 1.2 µl primer (3 µM), and the final volume was made up with 2.05 µl DDW. The PCR program consisted of an initial denaturation for 4 min at 94˚C, then 35 cycles of a denaturation step at 92˚C for 40s, primer(s) annealing at 38-54.3˚C for 40s (touch-down starting at 2˚C up and down Tm), and 72˚C for 2 min, with a final extension at 72˚C for a period of 6 min. Amplified Bands were separated on 1.5% agarose gel pre-stained with Gel stain solution (dye) using 1x TBE (Tris-Borat⁄EDTA) buffer. The gels were run for 105 minute at 68 voltage and separated bands were visualized by photograph taking (Gel Doc SUV/SN: G089301).

Table 3. PIC, MI, and RP formulas

(1) $PIC= 2f_i\ (1-f_i)$

f_i: frequency of marker fragments that were present.
$(1-f_i)$: frequency of marker fragments that were absent.

(2) $MI= PIC\times PL$

PL : Polymorphic Loci

(3) $Rp=\sum I_b$
$[I_b= 1-(2\times \left| 0/5-p \right|)]$

I_b: represents band informativenss
P: fraction of the total genotypes in which the band is present.

Data analysis

The ISSR primers that showed clear bands in the genotypes were visually scored as binary matrix for absence (0) and presence (1) of bands. Accordingly, the banding patterns generated by primers were assessed to analyze the genetic relatedness among different cornelian cherry genotypes. For the purpose of studying phylogenetic relationships, evaluating of Cophenetic value (r) for Jaccard, Dice, and Simple Matching coefficients was done based on Unweighted Pair Group Method with Arithmetic Averages (UPGMA) clustering method using NTSYS-pc 1.02 software. Also, Principle Coordinate Analysis (PCoA) and Principle Component Analysis (PCA) were conducted using NTSYS-pc 2.02 and GenAlEx 6.41 softwares respectively. Indices of genetic diversity such as the observed number of alleles (Na), effective number of alleles (Ne) [15], Shannon's information index (I) [22], and Nei's gene diversity [17] were calculated using PopGene 32 software. Polymorphism information content (PIC) [20], Marker index (MI), and Resolving power (Rp) [18] for each primer were obtained using the formulas in Table 3. The model-based program STRUCTURE 2.3.4 [19] was applied to the ISSR data to infer the population structure in the dataset.

RESULTS and DISCUSSION

In order to study the related indices in cornelian cherry genotypes, utilized primers were amplified 153 bands, of which 98 bands were polymorphic. The sizes of all amplified bands in all primers were estimated to be within

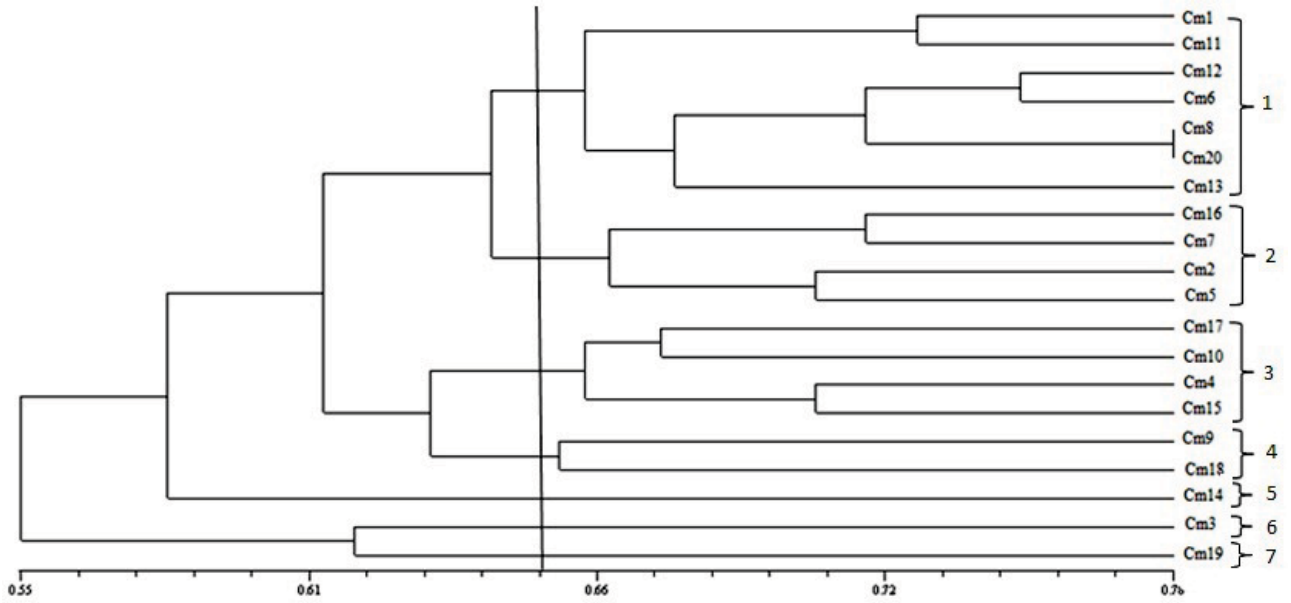

Figure 1. Phylogenetic dendrogram of selected Arasbaran cornelian cherry genotypes based on ISSR molecular markers

the range of 100 to 3000 base pairs. The number of amplified bands by each primer was different, so that the UBC818 amplified 15 bands, and UBC817 amplified 8 bands. The highest polymorphism (77.77%) belonged to the UBC810 and the lowest (50%) was related to Al1 and UBC834. Total polymorphism in all primers was 64% (Table 2). Analyzing Cophenetic value for different coefficients showed that the Jaccard's similarity coefficient had the highest Cophenetic value (r = 0.86), which indicates well fit and high correlation between the similarity matrix and final dendrogram. According to Jaccard's similarity coefficient matrix, the genetic similarity coefficient between genotypes varied from 0.444 to 0.777, with the highest genetic similarity (0.777) between Cm8 and Cm20 genotypes and the lowest (0.444) between Cm10 and Cm19 genotypes (Table 4). In phylogenetic dendrogram based on similarity matrix, the genotypes were classified into seven groups at the coefficient of 0.65 (Figure 1).

Bootstrapping test with 10000 replications was used to determine the location of the cutting line and to confirm the accuracy of the grouping. Another distinct method, called discriminant analysis, was also used and confirmed the grouping with 100% probability. Principal Component Analysis (PCA) and Principal Coordinate Analysis (PCoA) were performed to studying of primers' sequence distribution in cornelian cherry genome and as a complementary method to distance-based clustering dendrogram respectively (Table 5) (Figure 2). The results of PCA showed that the first three components explained

Table 5. Principal component analysis (PCA)

Component	Eigen values (%)	Cumulative (%)	Variance (%)
1	10.227	10.227	17.988
2	10.140	20.367	12.988
3	8.290	28.657	10.488
Total	28.657	59.251	41.464

41.464% of the total variance, with the contribution of the first component being 17.988%. The relatively low proportion of the first three components in justifying the total variance indicates that the sequences of utilized primers throughout the cornelian cherry genome are scattered; in other words, they are not concentrated in specific regions of genome. In fact, these findings are contrary to the studies and results of the statistical analyzes on morphological traits. In investigation of morphological traits, the researcher intended to identify the components and traits that justify the highest variance, while in molecular studies, the high variance obtained from the first components indicates that most of the

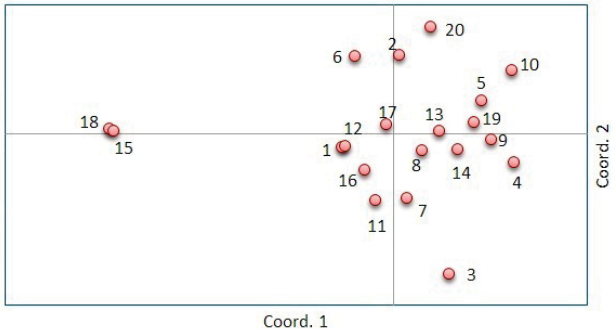

Figure 2. Principal Coordinate Analysis (PCoA)

Table 4. Similarity coefficients between selected Arasbaran cornelian cherry genotypes

	Cm1	Cm12	Cm6	Cm16	Cm11	Cm17	Cm2	Cm7	Cm8	Cm20	Cm13	Cm3	Cm14	Cm19	Cm5	Cm9	Cm10	Cm4	Cm18	Cm15
Cm1	1																			
Cm12	0.688	1																		
Cm6	0.676	0.747	1																	
Cm16	0.575	0.626	0.656	1																
Cm11	0.727	0.676	0.646	0.606	1															
Cm17	0.686	0.696	0.686	0.585	0.656	1														
Cm2	0.656	0.707	0.616	0.656	0.626	0.646	1													
Cm7	0.616	0.646	0.696	0.717	0.585	0.707	0.717	1												
Cm8	0.717	0.747	0.696	0.595	0.626	0.646	0.656	0.656	1											
Cm20	0.696	0.747	0.676	0.656	0.606	0.666	0.656	0.595	0.777	1										
Cm13	0.616	0.686	0.616	0.676	0.666	0.666	0.656	0.636	0.717	0.696	1									
Cm3	0.616	0.525	0.575	0.575	0.606	0.585	0.515	0.616	0.555	0.575	0.616	1								
Cm14	0.676	0.606	0.555	0.515	0.505	0.666	0.595	0.515	0.656	0.656	0.555	0.515	1							
Cm19	0.595	0.545	0.515	0.515	0.606	0.606	0.474	0.575	0.575	0.535	0.474	0.616	0.515	1						
Cm5	0.686	0.656	0.646	0.646	0.616	0.595	0.707	0.646	0.646	0.686	0.666	0.606	0.606	0.464	1					
Cm9	0.646	0.616	0.606	0.525	0.575	0.636	0.545	0.545	0.585	0.585	0.545	0.545	0.505	0.525	0.595	1				
Cm10	0.646	0.636	0.646	0.626	0.595	0.676	0.646	0.626	0.626	0.626	0.666	0.545	0.545	0.444	0.616	0.656	1			
Cm4	0.717	0.565	0.595	0.575	0.565	0.666	0.636	0.636	0.656	0.595	0.676	0.575	0.575	0.515	0.666	0.565	0.666	1		
Cm18	0.565	0.555	0.484	0.505	0.575	0.595	0.606	0.484	0.565	0.606	0.585	0.545	0.505	0.525	0.616	0.656	0.636	0.626	1	
Cm15	0.646	0.555	0.606	0.565	0.616	0.636	0.565	0.606	0.585	0.626	0.606	0.606	0.606	0.505	0.676	0.636	0.676	0.707	0.696	1

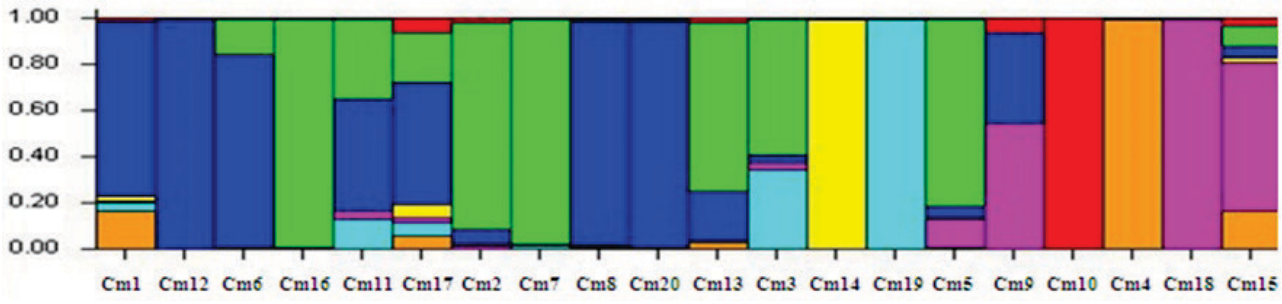

Figure 4. Inferred population structure of selected Arasbaran cornelian cherry genotypes based on ISSR molecular markers

amplified molecules are concentrated in certain parts of the genome; therefore, this is not desirable for more comprehensive examination of variation in all parts of the genome.

Observed number of alleles ranged from 1.50 (Al1 and UBC834) to 1.777 (UBC810) with a mean of 1.642; and, effective number of alleles varied between 1.629 (UBC839) and 1.306 (UBC834) with a mean of 1.498. Highest values of Shannon's information index and Nei's gene diversity in thirteen primers were related to UBC810 (0.490 and 0.342, respectively), demonstrating that this primer has been able to show the high diversity among studied genotypes. Using the number of alleles alone as a statistic for assessing the efficiency of the primer makes it possible to estimate inaccurate estimation of the primer value. Therefore, criteria such as polymorphism information content (PIC), and Resolving power (Rp) which are based on the allele frequency, can better reflect the efficiency of utilized primers better. PIC represents the amount of polymorphism created by a primer that varies from zero to one. According to the results, PIC varied between 0.177 and 0.310. UBC810 with the highest polymorphism had higher PIC. On the other hand, both AL1 and UBC834 with the lowest polymorphisms had lower PIC. According to the Table 2, there is a direct relationship between the PIC value and polymorphism; thus the PIC also increases with increasing polymorphism. On the other hand, primers with highest PIC can better determine the genetic distance of genotypes. Resolving power ranged from 3.5 (Al1) to 6 (UBC810). The highest and the lowest marker indexes were related to UBC815 (3.030) and AL1 (0.975) respectively. Model-based clustering method, based on Bayesian statistical index, was performed in STRUCTURE 2.3.4 software to analyze the population structure, assuming the ancestry and allele frequency models to be "admixture" and "continuous" respectively. The actual number of sub-populations were determined

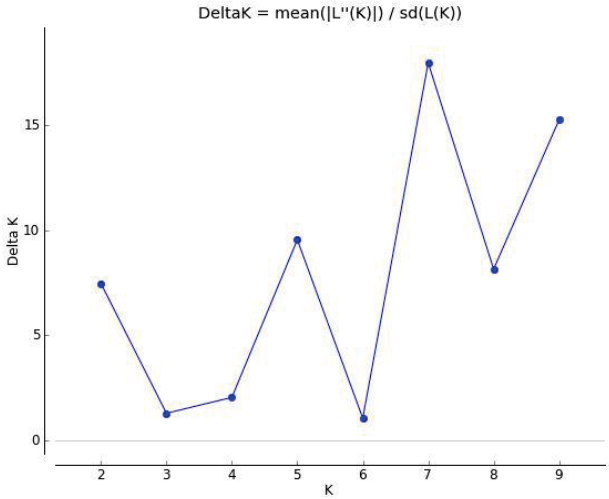

Figure 3. Estimation of DeltaK from calculated K and LnP(K) using the web-based STRCTURE HARVESTER program

by Evanno et al., method [9] assuming K = 1 to K = 10 (K represents the number of sub- population). Therefore, maximum Δk value was obtained at K = 7 (Figure 3).

According figure 4, genotypes were separated into seven sub-populations in theory which were partitioned into different colored segments. Each genotype is represented by a single vertical bar. The greater the proportion of the color, the greater the possibility of the represented population to be distributed into the corresponding group. The relationship of all genotypes from the seven sub-populations based on the ISSR molecular markers was also presented in cluster dendrogram, which was consistent with the Principal Coordinates Analysis (PCoA).

REFERENCES

[1] Bijelic, S.M., Golosin, B.R., Ninic Todorovic, J.I., Cerovic, S.B. and Popovic, B.M. 2011. Physicochemical fruit characteristics of cornelian cherry (*Cornus mas* L.) genotypes from Serbia. HORTSCIENCE 46: 849–853.

[2] Bornet, B. and Branchard, B. 2001. Nonanchored Inter Simple Sequence Repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Molecular Biology Reporter 19: 209-215.

[3] Chennaoui-Kourda, H., Marghali, S., Marrakchi, M. and Trifi-Farah, N. 2007. Genetic diversity of *Sulla* genus (Hedysarea) and related species using Inter Simple Sequence Repeat (ISSR) markers. Biochemical Systematics and Ecology 35: 682-688.

[4] Doyle, J.J and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

[5] Ercisli, S. 2004. Cornelian cherry germplasm resources of Turkey. Journal of Fruit and Ornamental Plant Research 12: 87-92.

[6] Ercisli, S., Orhan, E. and Esitken, A. 2006. Genetic diversity in fruit quality traits in cornelian cherry (*Cornus mas* L.). Asian Journal of Chemistry 18: 650-654.

[7] Ercisli, S., Orhan, E., Esitken, A., Yildirim, N. and Agar, G. 2008. Relationships among some cornelian cherry genotypes (*Cornus mas* L.) based on RAPD analysis. Genet Resour Crop Evol 55: 613-618.

[8] Ercisli, S., Yilmaz, S.O., Gadze, J., Dzubur, A., Hadziabulic, S. and Aliman, J. 2011. Some Fruit Characteristics of Cornelian Cherries (*Cornus mas* L.). Not Bot Hort Agrobot Cluj 39: 255-259.

[9] Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611–2620.

[10] Farmanpour Kalalagh, K., Mohebodini, M., Ghanbari, A., Chamani, E. and Erfani, M. 2016. Determination of genetic diversity among Arasbaran cornelian cherry (*Cornus mas* L.) genotypes based on quantitative and qualitative traits. IRANIAN JOURNAL of GENETICS and PLANT BREEDING 5: 32-40.

[11] Ghanavati, F. 2011. Cytogenetical survey of *Cornus mas* populations in Iran. New Cell Moll Biotech J 1: 71-79. (Article in Persian with an abstract in English)

[12] Hassanpour, H., Hamidoghli, Y. and Samizadeh, H. 2012. Some fruit characteristics of Iranian cornelian cherries (*Cornus mas* L.). Not Bot Horti Agrobo 40: 247-252.

[13] Hogbin, P.M. and Peakall, R. 1999. Evaluation of the contribution of genetic research to the management of the endangered plant *Zieria prostrata*. Conser Biol 13:514-522.

[14] Jacimovic. V., Bozovic, D., Ercisli, S., Ognjanov, V. and Bosancic, B. 2015. Some Fruit Characteristics of Selected Cornelian Cherries (*Cornus mas* L.) from Montenegro. Erwerbs-Obstbau 57: 119-124.

[15] Kimura, M. and Crow, J.F. 1964. The number of alleles that can be maintained in a finite population. Genetics 49: 725-738.

[16] Naderi, B., Maghsoudlou, Y., Aminifar, M., Ghorbani, M. and Rashidi, L. 2015. Investigation on the changes in color parameters and turbidity of cornelian Cherry (*Cornus mas* L.) produced by microwave and conventional heating. Nutrition and Food Sciences Research 2: 39-46.

[17] Nei, M. 1973. Analysis of gene diversity in subdivided populations (population structure/ genetic variability/heterozygosity/gene differentiation). Proc. Nat. Acad. Sci 70: 3321-3323.

[18] Prevost, A. and Wilkinson, M.J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor Appl Genet 98: 107-112.

[19] Pritchard, J.K., Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

[20] Roldan-Ruiz, I., Dendauw, J., Van Bockstaele, E., Depicker, A. and De Loose, M. 2000. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Molecular Breeding 6: 125–134.

[21] Schaal, B.A., Hayworth, D.A., Olsen, K.M., Rauscher, J.T. and Smith, W.A. 1998. Phylogeographic studies in plants: problems and prospects. Molecular Ecology 7: 465-474.

[22] Shannon, C.E. and Weaver, W. 1949. The mathematical theory of communication, University of Illinois Press, Urbana, Illinois, USA.

[23] Stankovic, M.S., Zia-ui-haq, M., Bojovic, B.M. and Topuzovic, M.D. 2014. Total phenolics, flavonoid content and antioxidant power of leaf, flower and fruits from cornelian cherry (*Cornus mas* L.). Bulgarian Journal of Agricultural Science 20: 358-363.

[24] White, T.L., Adams, W.T. and Neale, D.B. 2007. Forest Genetics, CABI Publishing, Cambridge, Massachusetts, USA.

[25] Wunsch, A. and Hormaza, J.I. 2002. Cultivar identification and genetic fingerprinting of temperate fruit tree species using DNA markers. Euphytica 125: 59–67.

[26] Yamagishi, M., Nishioka, M. and Kondo, T. 2010. Phenetic diversity in the *Fritillaria camschatcensis* population grown on the Sapporo campus of Hokkaido university. Landscape Ecol Eng 6: 75–79.

[27] Zhang, D.Q., Gao, L.M. and Yang, Y.P. 2010. Genetic diversity and structure of a traditional Chinese medicinal plant species, *Fritillaria cirrhosa* (Liliaceae) in southwest China and implications for its conservation. Biochemical Systematics and Ecology 38: 236-242.

تجزیه و تحلیل تنوع ژنتیکی، روابط فیلوژنتیکی و ساختار جمعیت ژنوتیپ های زغال اخته (*Cornus mas* L.) ارسباران با استفاده از نشانگرهای مولکولی ISSR

کریم فرمان پور کلالق، مهدی محب الدینی*، علیرضا قنبری

گروه علوم باغبانی، دانشکده کشاورزی و منابع طبیعی، دانشگاه محقق اردبیلی، اردبیل، ایران

* نویسنده مسئول: Mohebodini@uma.ac.ir

چکیده

زغال اخته (*Cornus mas* L.) از درختان کشت شده قدیمی در منطقه ی ارسباران بوده که دارای ارزش اقتصادی و دارویی است. با این حال اطلاعات کمتری در مورد تنوع ژنتیکی، برنامه های اصلاحی و ساختار جمعیت این گونه وجود دارد. با در نظر گرفتن این عوامل، هدف اصلی مطالعه ی حاضر تجزیه و تحلیل تنوع ژنتیکی، روابط فیلوژنتیکی و ساختار جمعیت ژنوتیپ های زغال اخته ارسباران با استفاده از نشانگرهای مولکولی ISSR می باشد. آغازگرهای استفاده شده در مجموع ۱۵۳ باند تولید کردند که ۹۸ باند چند شکل بودند (۶۴٪ چند شکلی). بالاترین ضریب تشابه جاکارد ۰/۷۷۷ به‌دست آمد. بر پایه ی UPGMA، ژنوتیپ ها به هفت گروه اصلی تقسیم بندی شدند. از طرف دیگر، تجزیه به مختصات اصلی به عنوان روشی مکمل بر تجزیه ی خوشه ای، گروه بندی ژنوتیپ ها در نمودار فیلوژنی را تایید نمود. مقدار نسبتا پایین سه مولفه ی اصلی (۴۱/۴۶۴٪)، حالت پراکنده بودن توالی آغازگرهای استفاده شده در ژنوم زغال اخته را نشان داد. میانگین شاخص اطلاعات چند شکلی، شاخص نشانگری، قدرت تفکیک، تعداد آلل های مشاهده شده، تعداد آلل های موثر، شاخص تنوع نی، و شاخص اطلاعات شانون به ترتیب ۰/۲۳۰، ۱/۷۶۹، ۴/۷، ۱/۶۴۲، ۱/۴۹۸، ۰/۲۷۱ و ۰/۳۹۲ بودند. در بررسی ساختار جمعیت، زمانی که مقدار K از ۲ تا ۱۰ تنظیم شد، هفت گروه یا زیر جمعیت نمایان شدند که نتایج حاصل از نمودار فیلوژنتیکی و تجزیه به مختصات اصلی را تایید نمود. نتایج حاصل از این مطالعه می تواند در برنامه ریزی مطالعات آینده بر ژرم پلاسم و برنامه های اصلاحی زغال اخته مفید و موثر باشد.

کلمات کلیدی: آغازگر، چند شکلی، شاخص تنوع نی، شانون، دندروگرام، ژرم پلاسم