THE MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF BARBERRY GENOTYPES FROM TURKEY AND KYRGYZSTAN

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MSc. Thesis

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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF BARBERRY GENOTYPES FROM KYRGYZSTAN AND TURKEY

Prof. Dr. Sezai ERCİŞLİ danışmanlığında, Haıder Natiq YAHYA tarafından hazırlanan bu çalışma 23/03/2017 tarihinde aşağıdaki jüri tarafından Bahçe Bitkileri Anabilim Dalı, Meyve Yetiştirme ve Islahı Bilim Dalı'nda Yüksek Lisans tezi olarak **oybirliği/~~oy çokluğu~~ (3./3.)** ile kabul edilmiştir.

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ÖZET

YÜKSEK LİSANS TEZİ

TÜRKİYE VE KIRGIZİSTAN'DAN ALINAN KADIN TUZLUĞU GENOTİPLERİNİN MOLEKÜLER VE MORFOLOJİK KARAKTERİZASYONU

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Bu çalışma Türkiye'deki Kayseri ilinden toplanan 10 adet kadıntuzluğu *(Berberis crataegina)* genotipi ve Inter Simple Sequence Repeat Markers (ISSR) Molecular Markers kullanan Kırgızistan'dan toplanan 22 adet kadıntuzluğu *(Berberis sphaerocarpaKar. Et Kit)* genotipini içeren iki popülasyon arasındaki ilişkiyi ve genetic çeşitliliği araştırmak amacıyla yürütülmüştür. Karakterizasyon işlemi 32 çalı formlu kadın tuzluğu genotipi üzerinde 20 (ISSR) primer kullanılarak gerçekleştirilmiş ve 150 banttan 111'inin polimorfik olduğu elde edilmiştir. Bant boyutu 190 bp ile 1400 bp arasında değişirken, üretilen her bir primer için bant numarası 5 ila 14 arasında değişmiştir ve 20 (ISSR) primerden üretilen ortalama allel sayısı 7.5'tur. Analiz, NTSYS Pc-2.11X yazılımı kullanarak yapılmıştır ve UPGMA tekniğine dayanan bir dendrogram oluşturulmuştur. 2 primer olan (AG)7YC ve (TCC)5RY monomorfikiken,diğer 3 primer (TAA)8, (CA)6AC ve (CAC)6 hiç bir sonuç vermemiştir. Öteyandan, primerlerin geri kalanı (%28.6-%100) ve % 74 ortalama arasında sıralanan polimorfizm oranlarıyla polimorfiktir. Ayrıca, bu çalışma aynı genotipi kapsayan meyveler arasındaki morfolojik bir karşılaştırmayı içermektedir, bu karşılaştırma bazı morfolojik karakterlerin (ağırlık, uzunluk ve genişlik) temeline dayanmaktadır. Bu çalışmada yapılan tüm gözlemler (ISSR) moleküler işaretleyicileri kullanmanın türleri belirli bir cins içerisinde ayırmada güvenilir bir yöntem olduğunu, gelecekteki yapılacak çalışmalar, genetik kaynakların karakterizasyonu için işaretleyicileri seçmede karar vermenin değerli bir kanıtını sağlayacaktır ve kadın tuzluğu bitkisi ıslah programlarının geliştirilmesine katkıda buluacaktır.

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Anahtar Kelimeler: Morfolojik ve moleküler karakterizasyon, kadın tuzluğu, *Berberis sphaerocarpa* Kar. et Kit, *Berberis crataegina*.

ABSTRACT

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This study was conducted to investigate the genetic diversity and the relationship between 2 populations including 10 genotypes of *Berberis crataegina* collected from the province of Kayseri in Turkey and 22 genotypes of *Berberis sphaerocarpa* Kar. et Kit collected from Kyrgyzstan using Inter Simple Sequence Repeat (ISSR) Molecular Markers. The characterization was performed using 20 ISSR primers on 32 Barberry genotypes and obtained 150 bands of which 111 bands were polymorphic.Band size varied between 190 bp and 1400 bp, band numbers for each primer produced varied between 5 to 14 and the average of alleles produced from 20 (ISSR) primers was 7.5.The analysis was performed using NTSYS Pc-2.11X software, and a dendrogram was constructed based on UPGMA technique.3 primers $(TAA)_8$, $(CA)_6AC$, and $(CAC)_6$ did not show any results whereas, 2 primers $(AG)_7YC$ and $(TCC)_5RY$ were monomorphic. On the other hand, the rest of primers were polymorphic with polymorphism percentages ranged between (28.6%-100%) and an average of 74%.Also, this study included a morphological comparison between fruits of same involved genotypes, this comparison is based on some morphological characteristics (weight, length, and width). All observations made in this study provide that using (ISSR) molecular markers is a reliable method for separating types within a specific genus, and a valuable evidence for decision making in choosing markers for future works, characterization of germplasm, and for contributing of developing Barberry improvement programs.

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Keywords: Morphological and molecular characterization,Barberry,*Berberis sphaerocarpa* Kar. et Kit, *Berberis crataegina*.

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Haider N. YAHYA
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CONTENTS

LIST OF SYMBOLS and ABBREVIATIONS

°C	**:** Degree Celsius
bp	**:** Base pair
cm	**:** Centimetre
CTAB	**:** Cetyltrimethylammonium Bromide
dNTP	**:** Deoxynucleotide triphosphate
EDTA	**:** Ethylene diamine tetraacetic acid
g	**:** gram
ISSR	**:** Inter Simple Sequence Repeat
K2O	**:** potassium oxide
kb	**:** Kilo base
kg/he	**:** (kilogram / hectare)
m	**:** Metre
M	**:** Molar
mg	**:** Miligram
MgCl2	**:** Magnesium chloride
ml	**:** Milliliter
mm	**:** millimeter
mM	**:** Millimolar
N	**:** Nitrogen
ng	**:** Nanogram
P2O5	**:**Phosphorus pentoxide
PCR	**:** Polymerase Chain Reaction
RAPD	**:** Random Amplified Polymorphic DNA
RFLP	**:** Restriction Fragment Length Polymorphism
RPM	**:** Revolutions Per Minute
SSR	**:** Simple Sequence Repeat
TBE	**:** Tris, Boric Acid, EDTA
TE	**:** Tris – EDTA
U	**:** Unit

UV	**:** Ultraviolet
V	**:** Volts
w/v	**:** Weight / Volume
w/w	**:** Weight / Weight
μ**g**	**:** Microgram
μ**l**	**:** Microliter
μ**M**	**:** Micromolar

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

1.1. Barberry Classification

The taxonomic information of Barberry bush is given in the table 1.1 (Anonymous 2017).

Table 1.1. The taxonomic information of Barberry bush (*Berberis* L.)

RANK	**SCIENTIFIC NAME**	**COMMON NAME**
Kingdom	Plantae	Plant
Subkingdom	Tracheobionta	Vascular plants
Super division	Spermatophyta	Seed plants
Division	Magnoliophyta	Flowering plants
Class	Magnoliopsida	Dicotyledons
Subclass	Magnoliidae	
Order	Ranunculales	
Family	Berberidaceae	Barberry family
Genus	*Berberis* L.	Barberry

1.1.1. Preface

Barberry has been a valued fruit since it was first identified 2500 years ago not only for human consumption, but also for its medicinal properties. Berberis is the Arabic name of the fruit that signifies a shell and many authors believe that the name is derived from this word because the leaves are shiny like the inside of an oyster shell. The name Berbery seems to have been first applied to this fruit by Averroes (an Arabic writer on medicines) (Ağaoğlu and Gerçekcioğlu 2013).

Because of the shrub contains spines, it was considered as a holy plant by the ancient Italians; whereas the plant considered as a religious symbol by the local inhabitants in America due to its wide and multi therapeutic benefits and usages (Saeed Arayne *et al*. 2007; Ağaoğlu and Gerçekcioğlu 2013).

Also, Barberry was used by the ancient indigenous in Iran, Turkey, Bulgaria, and America for treating a lot of common diseases by using different plant parts including leaves, barks, fruits and roots (Imanshahid and Hosseinzadeh 2008). In Iran and Georgia dried Barberry is used as a food additive in some local dishes and food products such as sauces, jellies, candies, juices and jams, etc.(Hassanpour and Alizadeh 2013). From the root there is a yellow dye that can be extracted and used for various purposes.

In the south Khorasan province of Iran, the Barberry production reaches approximately 8540 tons yearly and the cultivated areas, estimated 8000 hectares. Khorasan province, located in the northeastern of Iran, produce 4500 tons of Barberry which comprise about 90% of Iran's total production of Barberry (Javadzadeh 2013).

Barberry is also considered as ornamental plant because shrubs have beautiful foliage and attractive flowers and fruits. Shrubs are also used as a good cover for the soil to protect it from hard continuously erosions (Bonner and Karrfalt 2008).

Nowadays, the genus *Berberis* takes attractions of researchers and plant breeders because it was found that the plant having the ability for the treatment of significant types of common diseases like kidney diseases, rheumatic diseases, diarrhea, liver diseases, scarlet fever, brain disorders, heat, thirst, nausea, periodic neuralgia, fevers, vomiting in pregnancy, blood purifier, gastric and duodenal ulcers and sores. Also, it could be used to treat diabetes, abdominal, pelvic congestion and used as anti-tumors (Arayne *et al*. 2007).

Barberries medicinal importance comes from high alkaloids contents, where 22 types of alkaloids have been extracted in various ratios from different plant parts (bark, fruit, leaf and root). These alkaloids include Anthocyanin, Hydroxycanthine, Berbamine, Berberine, Chlorogenic acid, etc. (Arayne *et al*. 2007; Javad-Mousavi *et al*. 2016).

Existence of different morphological characteristics from different *Berberis* species genotypes suggests that there is a difference in quality and quantity in terms of Barberry contents of medicinal substances. Also morphological identification and classification of Barberry bush (*Berberis* L.) is extremely limited and not fully well-conducted. The availability of an extensive germplasm collection of Barberry bush (*Berberis* L.) from different geographical regions around the world has made it possible to evaluate their genetic relationships.

The taxonomy of the genus *Berberis* is still somewhat uncertain, and the occurrence of hybridization and, perhaps, also some degree of introgression in transitional areas has produced intermediate forms that cause difficulties in *Berberis* taxonomy (Bottini *et al*. 2007). Therefore, molecular characterization and classification of Barberry bush (*Berberis* L.) is very necessary.

Many of *Berberis* species have economic and environmental devastating impacts, and due to its dense existence may cause changing in the soil chemistry, preventing the water runoff from reaching other plants and can also replace the indigenous vegetation and alters the whole ecosystem as a result.

Beside what are mentioned above, *Berberis* species also have negative effects on the wheat production and other field crops because *Berberis* is considered as an alternative host and perfect place to regenerate new and virulent strains for many fungal diseases like stem rust and black rust (Keet *et al*. 2016).

1.1.2. The origin of the barberry

Barberis species grow usually in the northern hemisphere (Tanker *et al*. 2007). In addition, it is a large genus containing 15 genera and about 650 species. *Berberis* is native to Asia, Europe, North Africa and America (Ebadi *et al*. 2010).

Also the plant spread widely in British and Scandinavian islands. There is a lot of evidence referred to the natural existence of *Berberis* in Iran (Horasan province) and central of Anatolia which was helpful for local residents of these lands by utilizing the different parts of the plants (Bark, fruits, roots and leaves) as a therapy for many kinds of common diseases for more than 2500 years ago.

Due to the *Berberis* importance as a therapeutic plant and due to its various benefits, the plant is considered as a religious symbol or as a holy plant according to some ancient civilizations (Ağaoğlu and Gerçekcioğlu 2013).

1.1.3. Barberry characterizations and growth habits

Barberry is deciduous or evergreen bush with spiny or non-spiny branches and 0.5-3 m in high. Some botanists consider the genus *Mahonia* comprising of nearly 100 species that somehow close to the Barberry should be a type of *Berberis*, whereas others consider *Mahonia* as a various genus (Bonner and Karrfalt 2008).

Berberis species are often diploid (2n=28), and some reports showing that there are some tetraploid species growing in regions with low rain rates, while those growing in regions with high rain rates are commonly diploid.

Generally, the tetraploid species seems to be more tolerant to drought conditions from that with diploid species (Alemardan *et al*. 2013).

1.1.4. The Leaf

Leaves of Barberries species are small, oval, 2-5 cm long and 1-2 cm broad (Moghaddam *et al.* 2007), with slightly serrated margins and non-lobed surface. Each 2-5 of leaves together form a cluster, and some of these leaves can be modified into 1 spine like *Berberis crataegina* or 3 branched spines like *Berberis vulgaris* in the axils of nodes.

Depending on the different species of the Barberry, the color of leaves can be transformed from green, yellow purple and red with change of seasons given plant's structure a beautiful appearance which is associated with utilizing the plant in ornamental purposes like *Berberis thunbergii* or Japanese Barberry (Lehrer *et al*. 2008).

1.1.5. The flowering

Barberries flowers are yellow, usually 4-6 mm, depending on the region they are blooming in May or June; the flower is perfect (hermaphrodite); which means it contains male and female organs, as well as the flower is self pollinated, pollens can also be transferred by insects or animals.

Generally, flowers in 1-3 clusters, each cluster contains more than 50 single flowers of which approximately 80% are converted into fruits. The flower clusters are arranged spirally around the floral stem.

Typically, the flower consists of 6 sepals and 6 petals and secreting nectar from petal bases which make flowers attractive to pollinators like bees, wasps, flies, ants and beetles.

The flower's anthers produce a small amount of sticky pollens; moreover, Barberries flowers are self-pollinated as well as cross-pollinated. Commonly, it takes 170 days

from entire blooming to the full fruit ripening time in Barberry and also depends on region and cultivated conditions (Alemardan *et al*. 2013).

1.1.6. The Fruit

Barberries fruit's colors can be graded from being red to dark purple and they are small berry, oval and oblong in shape. Berries' weight differs depending on the species or varieties and can be ranged between 0.2-0.7 grams.

Seeds somehow are sold, depend on the species and varieties, and also each berry contains from 1-2 seed (Ağaoğlu and Gerçekcioğlu 2013). Fruit maturation differs depending on cultivated regions. Fruit ripening in summer or autumn and when the fruit's color converts into dark red indicate that the fruit contains a high percentage of Anthocyanin and low rates of sourness and berberine, whereas the rate of sweetness increases (Alemardan *et al*. 2013). Fruits are edible but the taste is very sour because of the fruit's high contents of vitamin C (Ghareeb *et al*. 2013).

1.1.7. Irrigation and Fertilization

Generally, water deficiency may cause reduction in yield of Barberries, however, Barberry is considered to have drought tolerant and the shrub has the ability to form fruits whether regular irrigation is available or not.

Farmers prefer to irrigate new cultivated plants once a week throughout the first year after cultivation and they also thought that it is necessary to reduce irrigation in fruits formation period to avoid the fruit dropping and to reduce the yield as a result.

It is advisable to irrigate shrubs during one week before the harvest in the petal fall stage. Depending on climatic and soil nature, if cultivated plants enter fruit production

stage it is preferable to irrigate shrubs once within 12-15 days. There are no obvious studies in regards to the process of the fertilization.

However, Barberry is low fertilizers demanding plant, but utilizing a fairly fertilization program may lead to better yield. Generally it is recommended to add 35-70 tons of animal fertilizers to the soil and 100 kg/he of N, 100kg/he of P2O5, and 50 kg/he of K2O before planting (Alemardan *et al*. 2013).

1.1.8. Barberries benefits

Barberry was well known as a therapeutic plant by ancient civilizations in Italy, India, and played a prominent role as a folk medicine with Bulgarians, Egyptians and native Americans.

The main uses of the Barberry as medicinal plant were unlimited, hence it was widely used with fennel seeds to prevent plague by ancient Egyptians and to recover dysentery by Indians.

Also Barberry is used to reinforce liver and heart, treating gallbladder diseases, inflammations and in processes of bladder stone fragmentations, other medicinal usages of Barberry are to treat blood pressure and stomach.

Researches in Barberries as therapeutic plant are still in progress and there are 22 types of alkaloids that have been extracted from the plant as well as the fruit is rich with vitamin C, organic acids, Phenol compounds such anthocyanin, carotenoid pigments ,phenolaes, polyphenolase and enzymes. These compounds significantly have therapeutic effects and also can be extracted from different plant parts (leaves, fruits, roots, and stems).

Recently, many researchers have revealed additional therapeutic uses of Barberry such as the ability to fight cancer cells and determined its expansion, as well as Barberries extracts associated to treat many diseases and disorders like scurvy, arthalgia, jaundice, diarrhea, sore throat, varicose, splenalgia, gastric ulcer, edema, constipation and to stanches the flow of hemorrhoid blood.

Barberries fruit's juice or tea which has been extracted from dry leaves have many remarkable effects as a tonic, calmative of stomach heat, laxative, or appetizer, gargled, and can promote delight sense (Aghbashlo *et al*. 2008; Javadzadeh and Fallah 2012). Recently, it has been found that extracted berberine plays a great role in terms of resisting cancer cells (Tang and Lee 2015).

1.1.9. Barberry side effects

Incorrect or irregular usage of Barberry may cause many side effects on human like nausea, vomiting, dizziness, nasal bleeding and some dermatological inflammations.

Furthermore, dried Barberry should be kept away from children and not be used by pregnant women because misusing of these substances may show toxicity symptoms like diarrhea, vomiting and nausea (Javadzadeh and Fallah 2012).

1.2. Inter-Simple Sequence Repeat (ISSR) Marker

DNA Molecular Marker technique can be utilized to investigate the variation in the DNA level and has been proved to be more effective in characterizing between closely related species (Ayele *et al*. 1999).

ISSR markers are also known as Random Amplified Microsatellites (RAMs). ISSR marker was discovered by some research task force at the beginning of 1990.

A microsatellite, Short Tandem Repeats (STRs), and Simple Sequence Repeats (SSRs), is a part of genome comprise of short DNA motifs (commonly 2-6 nucleotide residues) repeated multiple times in a row.

SSRs is required a flanked sequence to be known in order to design 5'-end for PCR-Primers. Inter-Simple Sequence Repeat (ISSRs) marker are fragments of the DNA that are flanked at both ends (5'-end or 3'-end) by such microsatellite sequences.

ISSRs fingerprinting was designed such that no prior DNA sequence information required. ISSR-PCR based molecular marker technique involves amplification of (DNA) fragments between 2 identical microsatellites repeat sites oriented in opposite direction utilizing single primers designed from microsatellite essential sites.

ISSR primers are designed to contain repetitive sequences complementary to microsatellite sites in the genome. Generally, there are 3 types of ISSR primers designed:

(1) Unanchored (primer comprises only of a repeated motif, e.g. 5'–(AC) 8–3').

(2) 5'-Anchored (primer comprises of a repeated motif with one or several non-motif nucleotides at the 5'-end, e.g. 5'– GA (AC) 8–3').

(3) 3'-anchored (primer comprises of a repeated motif with one or several non-motif nucleotides at the 3'-end, e.g. 5'–(AC) 8AG–3').Both 3'and 5'-anchored ISSR primers are suitable for detecting genetic diversity (Ng and Tan 2015).

ISSR molecular markers relatively demand a small amount of (DNA), also ISSR markers appear to be more polymorphism and show much highly polymorphic bands than RAPD markers (Ansari *et al*. 2012).

ISSR molecular marker has many advantages over other molecular markers and it seems to be quick, simple and have lower costs in comparison with RAPD molecular marker. In contrast (ISSR) markers are dominant markers and seem to have the reproducibility issue because of longer length of primers (16-25 bp).

ISSR markers are commonly used to evaluate the genetic variation and population structure, and they are very useful for DNA profiling, phylogenetic identification, taxonomic studies for inter-species and intra-species, genomic fingerprinting and gene mapping study (Abuduli 2015).

The main aim of this study is to characterize of genetic diversity and phylogenetic relationships between 22 genotypes of *Berberis sphaerocarpa* Kar. et Kit brought up from Kyrgyzstan and 10 genotypes of *Berberis crataegina* brought up from Turkey. It was carried out by using 20 (ISSR) primers. Also, this study includes a fruit morphological comparison in terms of the fruit's length, width, and weight. As a result, the final information on genetic diversity and phylogenetic relationship among these genotypes are an important contribution for classification, utilization of Barberry bush (*Berberis* L.) germplasm resources and improving breeding programs. In addition, it is very useful in Barberry bush conservation strategy in the future. In order to submit this work 5 chapters have been developed; Chapter 2 includes the literature review which summarizes other's works. Chapter 3 shows materials and methods which are conducted to serve the main objective of this work. Chapter 4 presents analysis and results. Chapter 5 introduces conclusions and recommendations.

2. LITRETURE REVIEW

There are a few genetic studies that have been conducted using molecular markers in order to evaluate the variation and genetic structure among Barberry species, and most of the classification studies have focused on morphological characteristics or medicinal properties of Barberry.

Hence, in present days, many researchers and students around the world have became more interested in Barberry due to its significant therapeutic impacts and in assessing the genetic structure using molecular marker technique. In this chapter, we will review some of these studies.

Bottini *et al*. 2002, 7 AFLP primers have been used to assess the genomic DNA isolated from 22 populations of 13 different *Berberis* species. These populations were taken from naturally growing plants in different localities of the Patagonia district in Argentina. The aim of this work is to assess the intra-interspecific relations among the tested Patagonian species of the genus *Berberis*, also this study encompasses the similarity between AFLP data, evaluating seed protein contents, morphological traits, and the relationship among individuals belonging to homoploid and polyploid collections in addition to revealing the hybridization between the tested species. 7 (AFLP) primers give rise to 231 bands. 199 bands from the given bands were polymorphic. Dendogram data exhibited that, in general populations of the same species formed closely related groups with high coefficient of similarity. Principal co-ordinate analysis showed 2 separates sub-groups;

1- *Berberis bidentata* and their putative ancestors, *Berberis darwinii* and *Berberis linearifolia* which form homogamic group.
2- *Berberis boxifolia, Berberis heterophylla,* and *Berberis parodii* which could form a polyploid hybrid complex.

Rob and Durka 2006, 10 loci microsatellite were isolated from invasive *Mahonia aquifolium* for the purpose of discovering the hybridization and the genetic variability within and amongst the invasive pattern and the three American native patterns of *M. aquifolium*, *M. repenes*, and *M. pinnata*, 8 individuals in each population and 9 individuals in 5 populations of *Berberis vulgaris* a closely related to *Mahonia* and native to Europe. This study was the first attempt of the development of *Mahonia* Microsatellite Markers and these markers should be applicable for the future genetic studies that focused on genetic variability and hybridization within and between the invasive and the native *Mahonia* populations.

Coˆte´and Leduc 2007, 64 AFLP primers with PCR amplification were employed to generate cultivar reference AFLP fingerprinting among 1-6 plant representative of 22 cultivars of Japanese Barberry (*Berberis thunbergii*) and one cultivar of *Mahonia aquifolium* which represents a control specimen. These plants are collected from two different places and represent an imported one by the plant breeders. The main drawback for the discrimination between common Barberry (*Berberis Vulgaris*) and Japanese Barberry is the depending on the morphological criteria which does not necessarily give correct results, especially if the plant misses some fundamental morphological criteria or when the plant is dormant. The Canadian government established a program allowing importation of 11 confirmed Japanese Barberry cultivars depending on the genetic identification of the approved cultivars utilizing AFLP molecular marker fingerprinting, also this method allowed the identification of plants that were confirmed to be mislabeled by exporters. Results showed that the loci seem to be conserved within *Mahonia* and partly also in *Berberis*. However, homozygous null alleles were found in 2, 1 and 3 loci, respectively, in *Mahonia aquifolium*, *Mahonia repens* and *Berberis vulgaris*. 3 markers (CA18, CA40, GA36) did not reveal any fragments in *Berberis vulgaris*.

Bottini *et al*. 2007, 13 of 17 Patagonian *Berberis* species representing both trans-Andean and endemic taxa, in addition to *Berberis trifoliolata* Moric., a species growing in southern and central Texas, USA was chosen as the out-group. Material was collected from wild populations from Argentina was sampled for this study.
Sequence analysis of the internal transcribed spacer (ITS) of the 18S (ITS1)-5.8S-26S (ITS2) rDNA region was performed in order to investigate the phylogenetic relationships between the involved Patagonian species of the genus *Berberis* of Berberidaceae family. This study was carried out to review the phylogenetic relationships of the 13 taxa of the genus *Berberis* by comparing the sequences of the ITS regions. Moreover, the obtained results were compared with those that using other sources of data, such as AFLP, isozymes, cytogenetics, seed proteins and morphology.
The divergence values between the pair wise sequence in the studied Patagonian species were in the range 2.9–22.9%. The lengths of the ITS1 and ITS2 sequences were in the range 227–231 bp and 220–224 bp, respectively, and the 5.8S sequence was 159 bp throughout all species. *Berberis microphylla sensu* Landrum does not appear to be monophyletic based on current sampling. Indeed, they suggest that *Berberis microphylla* should be distinguished from *Berberis buxifolia*, *Berberis parodii*, and *Berberis heterophylla*. ITS sequences, together with data obtained from morphological, biochemical, AFLP and cytological characterizations support the existence of diploid and polyploid hybrid speciation in the genus.

Heidary *et al*. 2009, Amplification Fragment Length Polymorphism (AFLP) marker based on 4 primers combinations was used to evaluate genetic variation and phylogenetic relationship among 1 population of cultivated seedless barberry (*Berberis vulgaris* L. var. asperma) and 7 populations of wild barberry (*Berberis integerrima*) which they grow in eastern and northeastern of Iran also 2 samples of ornamental species (*Berberis gagnepaini* , *Berberis thunbergii*) and 1 sample of *Mahonia aquifolium* have been used in this study. The obtained results at the molecular level revealed that *Berberis* and *Mahonia* belong to different genera whereas many morphological and systematic previous studies considered them within the same family. This study showed high variability among the wild Barberry populations also as

expected the distinct variation within the seedless Barberry population was very low and close to zero.

Lubell *et al*. 2009, extracted genomic DNA from leaves of 46 samples of Japanese Barberry (*Berberis thunbergii*) for Amplified Fragment Length Polymorphism (AFLP) analysis. Cultivated samples of *Berberis thunbergii* var. *atropurpurea* (purple foliage) and with feral plants of unknown parentage in the surrounding area, all plants (45 in total) within a 92-m radius of the specimen plant (putative originator plant (POP)) were included in this analysis. Also, the height, width, depth, foliage color, number of shoots, reproductive status and the distance to POP for every individual in the sample have been recorded. Shoots were counted at the base of the plant (12 cm up from the ground). On the other hand plant height, width and depth have been used to calculate an index of plant size (plant height · plant width · plant depth). Plant age was estimated using plant size, number of shoots and reproductive status. 70 AFLP markers used for this parentage investigation, 43 had allele frequencies in the range of 0.1 to 0.4. Number of markers with frequencies of 0.1 to 0.4 was used in this study was similar to the number which was used in other studies of woody plant species. 14% of the 43 feral plants in these samples had purple foliage and 30% were found growing within 16.5 m of the focal individual. Parentage analysis identified 7 plants (5 purple-leaved and 2 green-leaved) as descendants of the focal individual. 5 of these descendants are likely first-generation offspring and 2 are likely second-generation seedlings. In addition, 1 plant was identified as a backcross between the focal plant and 1 of its offspring. This results show that purple-leaved Japanese Barberry used in residential landscapes can contribute to plant invasions, at least under some circumstances.

Rezaei *et al*. 2011, characterized of 47 genotypes of Barberry, 45 of Iranian 6 years old shrubs collected from the east and northeast of Iran, these including both wild Barberry and seedless Barberry and 2 other genotypes were brought up from Germany as a reference genotype. 10 pairs of SSR molecular markers in addition to the morphological traits have been utilized to identify the related species and to distinguish the genetic and morphological relatedness between them.7 of these primers were found to be efficient

for producing polymorphic bands. A close relationship was found between cultivated Iranian seedless Barberries and *Berberis integerrima* by utilizing 3 SSR markers. Obtained results showed that there is a high genetic variance amongst collected genotypes with *Berberis integerrima* and SSR marker is a reliable tool for genetic diversity characterization.

Tripathi and Goswami 2011, used 80 Random Amplified Polymorphic DNA (RAPD) to assess the genetic diversity among 50 accessions of *Berberis lycium* Royle complex collected from various locations of India. Total of 11,683 amplicon generated 50 accessions of *Berberis lycium* complex with 28 primers. 332 amplification products scored, 284 (85%) were polymorphic and 48 monomorphic. Maximum numbers of 21 amplification products were obtained with primers OPAP-3 and 20 products with OPB-4. Average number of 11.5 bands obtained per primer and amplificon size ranged from 100 to 4, 500 bp and after study, no primer gave single band among all accessions. Polymorphic Information Contents (PIC) ranged from 0.013 to 0.52 with an average of 0.12. Dendrogram grouped all the accessions into 5 major groups. Principle Component Analysis (PCA) was also supporting the result obtained by dendogram. Present study is not supporting previous taxonomic classification of *Berberis lycium* Royle complex (based on morphological characters) but showed large diversity among them.

Allen *et al*. 2012, used microsatellite markers to characterize 24 specimens of *Berberis thunbergii* which include 1 specimen in the invasive range, 2 specimens in the native range and 21 specimens from horticultural cultivars were collected from the eastern United States to evaluate the genetic diversity among them and to identify the horticultural cultivars. 48 primer pairs were used to amplify high-quality PCR products, a total of 12 loci were identified, 8 polymorphic microsatellite markers were identified and utilized for screening the collected of 24 specimens, while the remaining were monomorphic. In addition to the importance of this study as assessing the *Berberis thunbergii* genetic relatedness and the population structure, it is also noted that regulating the invasiveness and species potential to host black stem rust, supporting study of the genetic basis for differences in the ecology of native and invasive

populations, phylogeographic analysis, and the assessment of cultivar identification using microsatellite markers.

Iqbal *et al*. 2013, used 12 Randomly Amplified Polymorphic DNA (RAPD) Primer to estimate the phylogenetic relation and the genetic diversity among 24 genotypes of *Berberis* were collected from different places of Khunar river catchment in Pakistan. The obtained data from the dendogram classified 24 collections into 2 main groups; group A (red fruits) and group B (black fruits). Molecular characterization was done to elaborate the genetic polymorphism. Primers based amplification in collections revealed high level of genetic polymorphism (6-86%). The genetic diversity estimated as genetic distances computed into dendrogram, separated the collections into 5 distinct groups A, B, C, D and E, disseminated as *Berberis parkeriana, Berberis lycium, Berberis pachyacantha, Berberis kunwarensis and Berberis orthobotrys*, respectively. However the collection B-8, which closely resembles *Berberis parkeriana* morphology was out grouped and needs future elaboration with more markers assorted discrimination.

Ahmed *et al.* 2013, by using biochemical and morphological made a comparison on 30 accessions of Barberry (*Berberis aristata* DC.). The accessions were collected from 3 different locations in Pakistan. The obtained results showed that there is a significant variability in terms of morphological characteristics and nutritional composition between the involved accessions which proposed that not only genotype, but its growing and location also are the main factors that determine the growing habit. The present study was carried out in order to increase our knowledge about the future utilization of Barberry (*Berberis aristata* DC.).

Obae et al. 2013, 274 specimens represent 59 different cultivars of Japanese Barberry (*Berberis thunbergii* DC.) belong to Beberidaceae family were investigated using 7 Amplified Fragment Length Polymorphism (AFLP) primers and with PCR technique to develop a key to identify cultivars approved for importation in Canada. This key could be used in a cultivar verification program to facilitate international trade of Japanese Barberry (*Berberis thunbergii* DC.) cultivars where wheat rust is a concern. Also, this

technique was found to be reliable for identifying cultivars and to authenticate the trueness-to-name of Japanese Barberry (*Berberis thunbergii* DC.) in production and in the market, and to control intra-cultivar genetic variants. Polymorphic markers from 7 primer combinations were able to clearly differentiate 57 of 59 cultivars evaluated. 2 cultivars, Aurea and Aurea Nana, could not be differentiated because they had identical marker profiles. Among the 274 plants tested, 263 were confirmed to be true-to-name and correctly labeled, whereas 11 plants could not be confirmed true-to-name. 7 of 20 cultivars evaluated exhibited detectable intra-cultivar genetic variation. Overall, nursery producers and retailers do not appear to be mixing or mislabeling Japanese Barberry cultivars.

Varas *et al*. 2013, used 18 Simple Sequence Repeat (SSRs) markers to assess the genetic diversity on calafate (*Berberis microphylla* G. Forst.) and on any species belonging to Barberidaceae family, to explore reproductive and propagative form of the SSRs markers that have been developed, and as a result it may be useful in developing breeding programs. Total genomic DNA was extracted from young stems, green fruits, and seeds of 3 accessions belonging to *Berberis microphylla* – Beberidaceae family growing naturally in (Chilean and Argentinean Patagonia) in South America, for the purpose of isolation (SSRs) microsatellites also, these isolated microsatellites were characterized on:1) 66 accessions of *Berberis microphylla*, 2) 15 accessions of other *Berberis* species to assess the interspecific transferability of these markers. 3) Additional accessions of *Berberis microphylla* utilized for the preliminary evaluation of microsatellite informativeness. The results confirm that (SSR) markers are very polymorphic and potentially useful in genetic studies in any species of the genus *Berberis*.

Jing and Wang 2013, Inter-Simple Sequence Repeat (ISSR) molecular marker was employed for the identification of 70 accessions of grape (*Vitis vinifera* L.) including 52 clones of 17 Chinese wild grape species, 10 (*vitis vinifera* L, cultivars), 7 interspecific hybrids, and 1 strain of *vitis rioaria* L. 96 ISSR primers were designed for this study, and the genomic DNA was extracted from the young leaves. In previous studies, RAPD

molecular marker was used to detect the genetic diversity among wild grape. However, using ISSR molecular marker seems to be more powerful than RAPD, because it is fast, simple, cost effective, reproducible and revels a much higher number of polymorphic bands than RAPD. It is essential for breeders to use Chinese grape as parents and to take the advantages of heterosis, because Chinese wild types are renowned by ease of cross-hybridization, and the main aim of the breeders is to raise fruits yield and quality. The main aim of this study is to detect the genetic diversity among Chinese wild grape and American and European grape using ISSR fingerprinting to clarify the interspecific and intraspecific relationships among the tested grape types.

Tao *et al*. 2014, extracted the genomic DNA from trend stem tissues of 50 accessions of Dragon Fruit (*Hylocereus* spp.) growing in China. 16 (ISSR) primers with 15 morphological traits have been employed to generate 111-Inter Simple Sequence Repeat markers to evaluate the genetic relatedness among Dragon Fruit (*Hylocereus* spp.) accessions. The main aim of this work is to unveil the genetic relatedness of the dragon fruit germplasms by both morphological traits and ISSR markers, to fingerprint the accessions using ISSR markers, and to investigate the coincidences and divergences of the distances between two marker systems.

Singh *et al*. 2015, *Berberis petiolaris* mainly contain a different class of alkaloids which have a significant importance of treating a large scale of diseases. Through this study a bioactive compounds including (magnoflorine, berberine, thalifendine, jatrorrhizine, berberrubine, demethyleneberberine, 8-oxoberberine, reticuline, N methyltetrahydroberberine, tetrahydropalmatine, and tetrahydroberberi, and palmatine) have been detected directly from different plant parts of stem, root, fruit and leaf utilizing direct analysis in real time-mass spectrometry method-Time-of-flight mass spectrometry (DART–TOF–MS). The main aim of this study is to develop an appropriate techniquefor identification of biologically active compounds in *Berberis petiolaris*directly from intact plant parts. Different parts of the plant such as leaf, stem, root and fruit were screened to study the quantitative and qualitative distribution of these bio- active compounds through plant parts.

Ganopoulos *et al*. 2015, used 22 genotypes of Barbary Fig (*Opuntia ficus-indica*) selected from 9 different localities in Greece have been used in this study, and the genomic DNA was isolated from the outer layer of mature leaves. ISSR molecular marker and 6 primers were employed to assess the genetic diversity among the tested genotypes. The high genetic diversity existing in the Greek germplasm proposed that it would be useful to utilize ISSR marker technique in Barbary fig breeding programs and germplasm management activities.

Giordani*et al*. 2016, used Random Amplified Polymorphic DNA (RAPD) molecular marker on 82 accessions of *Berberis microphylla* G. Forst. which were collected from southern Tierra del Fuego in Argentina. Taking into account the genetic profiles, geographical and environmental growing conditions, and morphological traits of *Berberis microphylla* G. Forst. shrubs, the primary interest of this work is to analyze the putative natural population in order to clarify the population structure and in relation of propagation strategy of the species. The genomic DNA was isolated from the leaves of 82 of adult shrubs of *Berberis microphylla* G. Forst. plant. Representing 23 putative populations, the number of plants for each population ranged from 1 to 7 among the 82 accessions. A subset of 39 plants representing 12 of the 23 putative populations was randomly defined in order to carry out a morphological characterization. Results of this work and with the comparison of phenotypic characteristics showed a wide diversity between and within the putative populations, also there is no geographic structure observed in the population, nevertheless some sub-populations were absolutely uniform, indicating a sexual propagation. Also results show a significant correlation among some morphological characters with environmental factors, and *Berberis microphylla* G. Forst. shrubs could exhibit a remarkable phenotypic plasticity.

Lambert and Obea 2016, Genomic DNA were extracted from young leaves of 51 different Barberry cultivars collected from germplasm collection located at the University of Connecticut, Storrs, CT, USA. In this study, SSR technique was used to assess the genetic diversity and to develop genotypic profiles to characterize and to differentiate cultivars in Barberry. Using molecular marker technique is remarkable

method to differentiate Barberry cultivars especially when the cultivars are dormant and the morphological characterization was somewhat difficult. Cultivars of Japanese barberry (*Berberis thunbergii* DC) are popular shrubs used in landscaping across the USA and Canada, In addition, plants in the genus *Berberis* are known to be alternate hosts for fungus that causes the black stem rust disease in cereal crops. Therefore, the USA and Canadian governments enacted legislation that restricting the importing of some *Berberis* species that may cause black stem rust disease. Results show seven of the 12 SSR loci were polymorphic in the 51 barberry cultivars tested. A total of 43 alleles were generated at the seven loci and the number of alleles at each locus ranged from three to nine. Allele sizes ranged from 110 to 245 bp. Jaccard's coefficient of similarity ranged from 0.10 to 1.00. The (UPGMA) dendogram cluster analysis of the Jaccard's coefficient of similarity matrix showed that 84.3% of the 51 cultivars tested were distinct from each other. 10 of the 11 cultivars approved for import into Canada were distinguishable based on their genotypic profiles. The results indicated that microsatellite-based genotyping could be used by regulatory agencies to identify and offer 'true-to-type' guarantees to cultivars destined for BSR 'quarantined' regions, and to verify the uniqueness of new cultivars when issuing Plant Patents. The 51 cultivars clustered mostly according to their leaf color, suggesting that a strong association existed between leaf color and some of the alleles at the seven polymorphic SSR loci.

Keet *et al.* 2016, used some Barberries species *(Berberis julianae* and *Berberis aristata*) have invasive impacts like altering the soil chemistry, preventing access to watercourses when reproducing as dense thickets, and also serve as alternate host for stem rust of wheat which may be harmful to the ecosystem; as a result, in many locations in south Africa. Hence, this study has been conducted to develop a practical method to reduce their environmental threats to the ecosystem by determining the geographical distribution of these species, assessing, determining seed germinability and to provide recommendation to control its natural occurrence. This study summarized by making a site survey utilizing GBS system to determine the natural distribution of aforementioned plants as well as many procedures should be regarded like assessing the plant habit and growth determines its flowering and seed formation periods, as well as performing a

convenient software program to identify places in which the eradication process will be performed. Finally, it's recommended to exclude manual eradication and to use herbicides to control the invasive impact of these shrubs.

3. MATERIALS and METHODS

3.1. Plant Material

In order to conduct this study, 22 different genotypes of *Berberis sphaerocarpa* Kar. et Kit and 10 genotypes of *Berberis crataegina* fresh leaves were randomly collected from bushes grown under natural conditions of 2141 m elevation and 42^{o} 20′28.36”N- 78^{o} 14’14.79”E the coordinates around Jeti-Oguz district and near to Issyk-Kul lake in Northeastern of Kyrgyzstan and from Ali Dağı district near to the Kayseri province (Kayseri located in central of Turkey with elevation of 1050 m) and 38°44′00″N 35°29′00″E the coordinates respectively.

According to geographic coordinates, 32 genotypes of naturally growing Barberry bushes were used in this study and from 2 different locations as shown in Figure 3.1 and Figure 3.2.

Figure 3.1. Sampling locations of 22 genotypes of *Berberis sphaerocarpa* Kar. et Kit collected from Kyrgyzstan.

Figure 3.2. Sampling locations of 10 genotypes of *Berberis crataegina* collected from Ali Dağı district- Kayseri province in Turkey

3.2. Genomic DNA Isolation

Total of 32 *Berberis sphaerocarpa* Kar. et Kit and *Berberis crataegina* fresh leaves collected from the Jeti-Oguz district in Kyrgyzstan as shown in figure 3.3 and figure 3.4 and from Ali dağı district-Kayseri province in Turkey as shown in figure 3.5 and figure 3.6 respectively.

22 specimens of *Berberis sphaerocarpa* Kar. et Kit were frozen in liquid nitrogen at -196°C and stored at -80°C until these specimens were sent to laboratories of the Erciyes University-Kayseri province in Turkey then DNA extraction steps are started. Genomic DNA extraction was conducted according to the modified CTAB (total DNA extraction protocol for plants) by Doyle and Doyle (1990).

100 mg of frozen Barberry leaf tissues directly were crushed using mortar and pestle. Leaves crushing process have been accompanied by adding a few drops of DNA Extraction Buffer (CTAB buffer) to each mortar.

CTAB buffer includes 100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2% (W/v) CTAB (Cetyl Trimethyl Ammonium Bromide), β-Mercaptoethanol and stir to dissolve before starting extractions steps. The mix of powdered leaf tissue and CTAB buffer was quickly transferred to 2ml Microcentrifuge tubes and was incubated at 65°C in water bath for 60 minutes with intermittent shaking in every 10 minutes.

After water bath process, 300 µl of Chloroform:Octanol alcohol (24:1) was added to the incubated tubes and centrifuged at 14000 rpm speed for 15 minutes, moreover, this process was repeated for additional 5 minutes after manual shaking for tubes to separate phases.

The aqueous phases of the centrifuged mix (top) were drawn off and placed into new labeled 2ml Microcentrefuge tubes. 300 µl of Chloroform:Octanol alcohol (24:1) was re-added and centrifuged again for 5 minutes at 14000 rpm speed. Once more aqueous phases were drawn into new and labeled Microcentrifuge tubes.

550 µl (2/3 volume) of chilled Isopropanol (-20°C) were added to precipitate the DNA in the bottom of tubes (white precipitate DNA and other compounds can be seen with eyes). Tubes were gently reversed upside-down for few times to mix components then they are placed inside the freezer (-20°C) for 30 minutes or a little more. Next step tubes were centrifuged for 2 minutes at 14000 rpm speed, and then the supernatant liquid was removed gently without disturbing the resultant DNA pellet.

The DNA pellet was washed with 500 µl of washing solution (10mM ammonium acetate and 76% Ethanol) to remove phenolic compounds and other components and tubes subsequently were left for 10 minutes at room temperature.

After washing, tubes were centrifuged for 3 minutes at 14000 rpm, then the supernatant liquid was removed from them then tubes were placed in the incubator for 2 hours to ensure perfect dryness for the DNA pellet. After that the dried DNA pellet was washed by double distilled water (the amount of water used to dissolve the DNA can vary depending on how much DNA isolated).

300 µl of TE solution (10 mM Tris and 0.1 mM EDTA at 7.4 PH) was added upon the DNA pellet, then tubes were kept in the incubator throughout the night at room temperature to ensure the DNA pellet dissolved completely .

DNA samples should be diluted with(100-200 µl) TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4) to obtain 10 ng/µl DNA templates for long term storage, whereas, should diluted with double distilled water (1:9 volume) in new and labeled PCR-tubes and kept in fridge at 4°C for short term storage.

Chemical Solutions and buffers used in this protocol are shown in Table 3.1.

Table 3.1. Solutions and buffers used in this study

Buffers	Components	Concentration	Preparation
Extraction Buffer (CTAB)	CTAB NaCl Tris-HCl pH(8.0) Na EDTA pH(8.0)	2% (w/v) 1.4 M 100 mM 20 mM	All ingredients were mixed then the volume were completed to 1000 ml of pure water with continuous mixing
Chloroform: Octanol	Chloroform Octanol	24:1 (w/w)	Chloroform and Octanol mixed with 24:1 ratio of weight
Isopropanol	Isopropanol(100%)	-	Pouring Isopropanol in one small bottle and putting in -20°C freezer
Washing solution	Ammonium acetat Ethanol	10mM 76%	Ammonium acetate (10 mM) mixed with Ethanol (76%) and put in +4℃.
TE Buffer	Tris pH(8.0) EDTA pH(8.0)	10 mM 0.1 mM	All ingredients were mixed then 495 ml of sterile water were added to complete the volume

Figure 3.3. Naturally grown *Berberis sphaerocarpa* Kar. et Kit bushes of Jeti-Oguz in Kyrgyzstan

Figure 3.4. Naturally grown *Berberis sphaerocarpa* Kar. et Kit bushes near to the Pinewood forest in Kyrgyzstan

Figure 3.5. Naturally grown *Berberis crataegina* bushes near the Kayseri province in Turkey

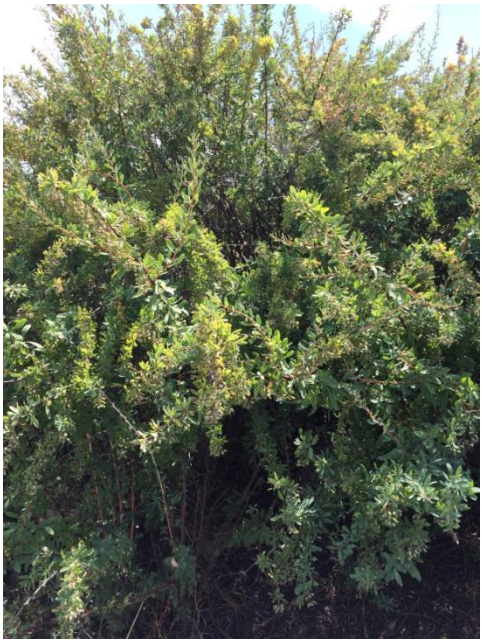

Figure 3.6. Naturally grown *Berberis crataegina* bushes around Ali Dağı district, near to the Kayseri province in Turkey

The summary of DNA isolation step by step shown in the following figure (Figure 3.7).

Figure 3.7. Image (1-6)Steps of genomic DNA isolation, Image (7) production of amplified DNA templates, using (PCR) instrument.Image (8) transferring the amplified DNA by 2% agarose gel electrophoresis methods.Image (9) recording and modifying obtained Images by UV (MS.Major Scince) visualizing system

3.3. Genomic DNA Quantity and Quality Confirmation

Genomic DNA concentration of Barberry leaves was measured with 1% agarose gel followed gel electrophoresis. 5 µl genomic DNA sample + 5 µl loading dye in addition to 2 µl DNA ladder (1 kb) were injected into the gel wells.

Then the DNA was transferred for 45 minutes at 150 volts by gel electrophoresis following this step exposing the gel into UV light and saving images.

The presence of a highly resolved high molecular weight bands indicate good quality DNA, but the presence of a smeared band indicates DNA degradation and the DNA isolation steps should be repeated.

3.4. ISSR (Inter Simple Sequence Repeat) Markers

In this study a total 20 ISSR Molecular Markers were used to characterize genetic diversity and for phylogenetic analyses of 32 Barberry bush genotypes. The primer lists used in this study are given in the table 3.2.

Table 3.2. List of 20 ISSR primers used in this study.

ISSR Marker	5' --> 3' Sequence
1	HVH $(TCC)_7$
2	$(CT)_8$TG
3	$(GACA)_4$
4	DBDA$(CA)_7$
5	$(AG)_8$T
6	$(TAA)_8$
7	$(GT)_8$YA
8	$(AGC)_6$G
9	$(AG)_7$YC
10	$(CA)_6$AC
11	$(GT)_6$GG
12	HVH $(CA)_7$T
13	VHV $(GTG)_7$
14	BDB $(CA)_7$C
15	$(CAC)_3$GC
16	$(CAC)_6$
17	$(CA)_8$R
18	$(GAA)_6$
19	$(GA)_8$YG
20	$(TCC)_5$RY

3.5. PCR (Polymerase Chain Reaction) Amplification And Agarose Gel Electrophoresis Analysis of Molecular Markers

3.5.1. PCR (Polymerase Chain Reaction) amplification

For amplification of 20 ISSR Markers Polymerase Chain Reaction (PCR) was carried out within 0.2 ml PCR tubes at 15 µl volumes. For the 15 µl PCR reaction containing

9.27 µl double distilled water, 1.5 µl PCR buffer, 0.7 µl MgCl2, 0.33 µl dNTPs, 1 µl of primers, 0.2 µl 16 Unit Taq polymerase, and 2 µl template DNA.

Finally, mineral oil was added upon the mix to prevent evaporation produced by thermal cycles from PCR process (Table 3.3).

PCR Amplification was performed using (Sense Quest) Lab Cycler (Figure 3.8) following the (ISSR-PCR) protocol: Initial denaturation at 94°C for 3 minutes, 35 cycles at 94°C for 1 minutes, 35 cycles at 53°C for 50 second, 35 cycles at 72°C for 2 minutes, and final extension step at 72°C for 7 minutes, whereas the reaction was kept at 4°C (Table 3.4).

Table 3.3. Components of the 15 µl PCR reaction

PCR Components	volume
Double distilled water	9.27 µl
PCR buffer	1.5 µl
MgCl2	0.7 µl
dNTPs	0.33 µl
primer	1 µl
Taq polymerase(16 U)	0.2 µl
DNA	2 µl
Meneral oil	1 Drop

Figure 3.8. (Sense Quest) Lab Cycler used in this study

Table 3.4. Polymerase Chain Reaction **(**PCR) program for amplification of 20 ISSR markers used in this study

Reaction Steps	Number of Cycle	Temperature (°C)	Time
Initial Denaturation	1	94	3:00
Denaturation	35	94	1:00
Annealing Temperature	35	53	50
Extension	35	72	2:00
Final Extension	1	72	7:00
Hold	1	4	∞

3.5.2. Agarose gel electrophoresis

20 ISSR markers were separated on 2% agarose gel in order to visualize the DNA samples used DNA loading dye. Each PCR products was loaded on agarose gel by mixing with DNA loading dye.

2% Agarose gel was prepared by mixing 600 ml 0.5 X TBE buffer +12 g agarose then the mixture was placed in the microwave for 8 minutes and when the solution temperature reached 70°C, the mixture was stained with 30 µl of Ethidium bromide.Subsequently, the mixture was poured into electrophoresis tray when the temperature reached 50°C. GENESTATM 100 bp DNA ladder was used to estimate the amplicon sizes. Electrophoresis was performed with 0.5 X TBE buffer for 3 hours at 110 V.

MS.Major Scince UV visualizing system was used to visualize band patterns on agarose gel (Figure 3.9). Also, images were recorded and modified by smart view pro 1100 Imager system version 1.1.2.0 copyright© 2014 Major scince. Solution and buffers used for agarose gel electrophoresis are given in Table 3.5.

Figure 3.9. UV MS.Major Scince visualizing system used in this study

Table 3.5. Solution and buffers used for agarose gel electrophoresis used in this study

Buffer or Solution	**component**	**quantity**	**Preparation**
1 liter TBE (10 X)	Trizma	108 g	The ingredients were mixed together and the total volume were completed to 1 liter pure water with 1 hour of continuous mixing
	Boric acid	55 g	
	Na_2EDTA	20 ml from 0.5 M EDTA	

Buffer or Solution	**component**	**concentration**
Loading Dye (5X)	Tris-Hcl	10 mM
	EDTA, (pH7.6)	5mM
	Bromophenol blue	0.03%
	Xylene cyanol	0.03%
	Glycerol	30%

3.6. Data Evaluation and Analysis

Obtained results after displaying images of agarose gel electrophoresis. And when reading amplification bands contained in these images, only strong bands were taken

into consideration. According to their presence and absence, it was evaluated as "1" or "0" respectively (Sokal and Sneath 1963).

To evaluate the genetic diversity among the two Barberry populations NTSYS-pc (Version 2.11X, Rohlf 2000) software (Numerical Taxonomy and Multi-variation Analysis System) was used to constitute UPGMA (Unweighted Pair Group Method with Arithmetic Mean of Cluster analysis) dendogram (Sneath and Sokal 1973).

3.7. Fruit Morphological Comparison

Previously, the morphological characteristics were widely used to distinguish between related species. *Berberis* L. (Barberry) is the largest genus in Berberidaceae family, which consists of approximately 15 genera and 650 species. Due to the inter and intra-specific hybridization within *Berberis* genus which resulted in species with very close morphological characteristics. Therefore, the morphological characterization alone is not sufficient in distinguishing between closely related species like *Berberis*.

This study includes fruit morphological comparisons between the same sampled plants of *Berberis crataegina* fruits and collected from the same bushes used for ISSR characterization, whereas *Berberis sphaerocarpa* Kar. et Kit fruits morphological characterization results were obtained from a previous study of Yilmaz, *et al*. (2015), which is under approval for publication (in press). Also the morphological comparison was between involved samples of same species.

Fruit morphological characterizations include weight, length, and width were considered in this study. Beside the genetic characterization, also morphological characterization has been dependent in this study to support obtained results. It should be noted that, morphological characteristics are still the essential bases used to conduct germplasm programs and for contributing of developing Barberry improvement programs.

4. RESULTS and DISCUSSION

4.1. Genomic DNA Isolation

Genomic DNA isolation of 32 Barberry bush genotypes was carried out according to the method of Dolye and Dolye (1990). Samples were diluted and visualized by agarose gel electrophoresis running on 2% agarose gel about 3 hours at 110 volts.

DNA quantity and quality were measured by visualizing the isolated DNA samples using agaraose gel electrophoresis running on 1% agarose gel for 45 minutes at 150 volts. Concentrations of our Barberry genomic DNAs were significant for PCR reaction performance. The stock of Genomic DNA concentration was diluted into (10 ng/ µl) for further PCR analysis.

4.2. Polymerase Chain Reaction (PCR)

A premixed, (ready to use) solution PCR-mix which contains (Double-Distilled water, DNA polymerase, MgCl2, dNTP's, and PCR buffers) was prepared (Table 4.1) and used for the final PCR reaction with the addition of DNA samples and ISSR primers (Table 4.2). The optimal concentrations for PCR reactions and reaction conditions (Table 4.3) were determined by optimal studies.

Table 4.1. PCR-mix reagent and volume

PCR-mix reagent	volume
Double -Distilled water	9.27 µl
PCR buffer	1.5 µl
MgCl2	0.7 µl
dNTPs	0.33 µl
Taq polymerase(500 U)	0.2 µl

Table 4.2. Final PCR-mix solution concentration

Reagent	volume
PCR-mix reagent	12 µl
Primer	1µl
DNA	2µl
Mineral oil	1 Drop

Table 4.3. Optimized PCR-Reaction condition for (ISSR) Markers

Reaction Steps	Number of Cycle	Temperature (°C)	Time
Initial Denaturation	1	94	3:00
Denaturation	35	94	1:00
Annealing Temperature	35	53	50
Extension	35	72	2:00
Final Extension	1	72	7:00
Hold	1	4	∞

4.3. Inter Simple Sequence Repeat (ISSR) Marker Analysis

In this study 20 Inter Simple Sequence Repeat (ISSR) primers were used for characterizing the genetic variation of 32 Barberry genotypes in addition to clarifying the genetic diversity and relationships between them. The reproducible and bright bands were scored according to the binary matrix of allelic presence (1) or absence (0) for further analysis, on the other hand, primers that did not show any bands recorded as (9).

Total of 20 ISSR marker fragments were separated by agarose gel electrophoresis method on 2% agarose gel. As a result of screening 32 Barberry genotypes with 20 ISSR Markers by Polymerase Chain Reaction (PCR), a total of 150 alleles were scored

and 111 of them were polymorphic with an average of 7.5 alleles per primer. The number of alleles per locus generated by each marker varied from 5 to 14 alleles.

The highest number of alleles (14) were detected in primers $(AGC)_6G$ and $(GA)_8YG$ as shown in figure 4.1, and the lowest number of alleles (5) was detected in the primer $(GT)_8YA$.

3 primers $(TAA)_8$, $(CA)_6AC$, and $(CAC)_6$ did not work, whereas 2 primers $(AG)_7YC$ and $(TCC)_5RY$ were monomorphic (Figure 4.2). The polymorphism rate ranged between 28.6% and 100% with an average of 74% as shown in Table 4.4, Figure 4.3, Figure 4.4 and Figure 4.5.

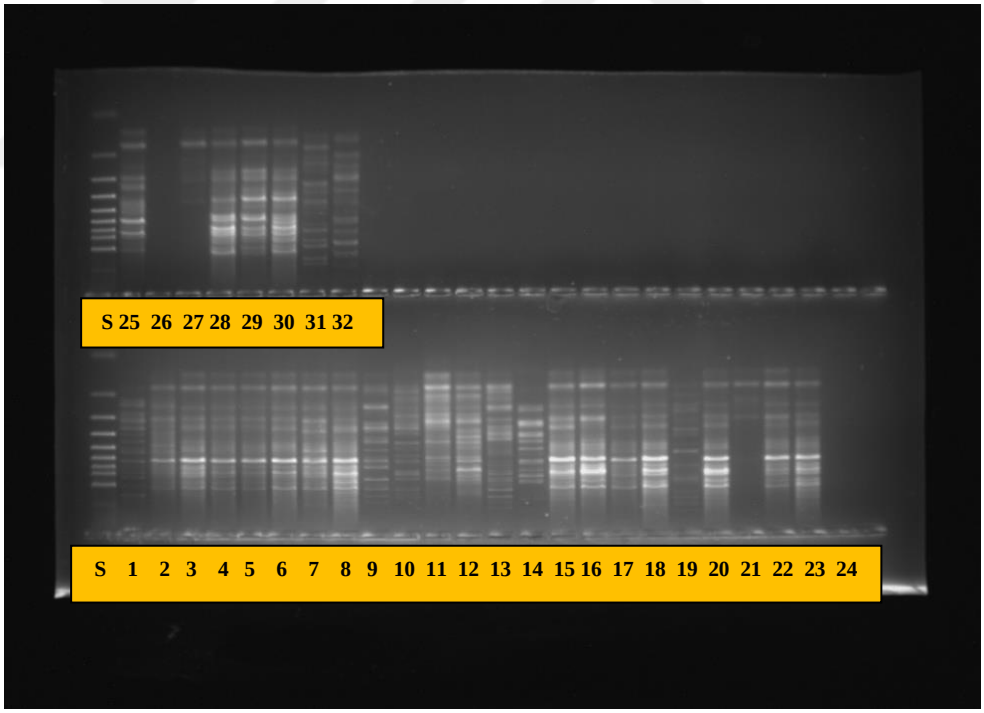

Figure 4.1. Highest numbers of alleles (14) obtained by $(GA)_8YG$ primer at 2% agarose gel, (S) refers to the standard DNA

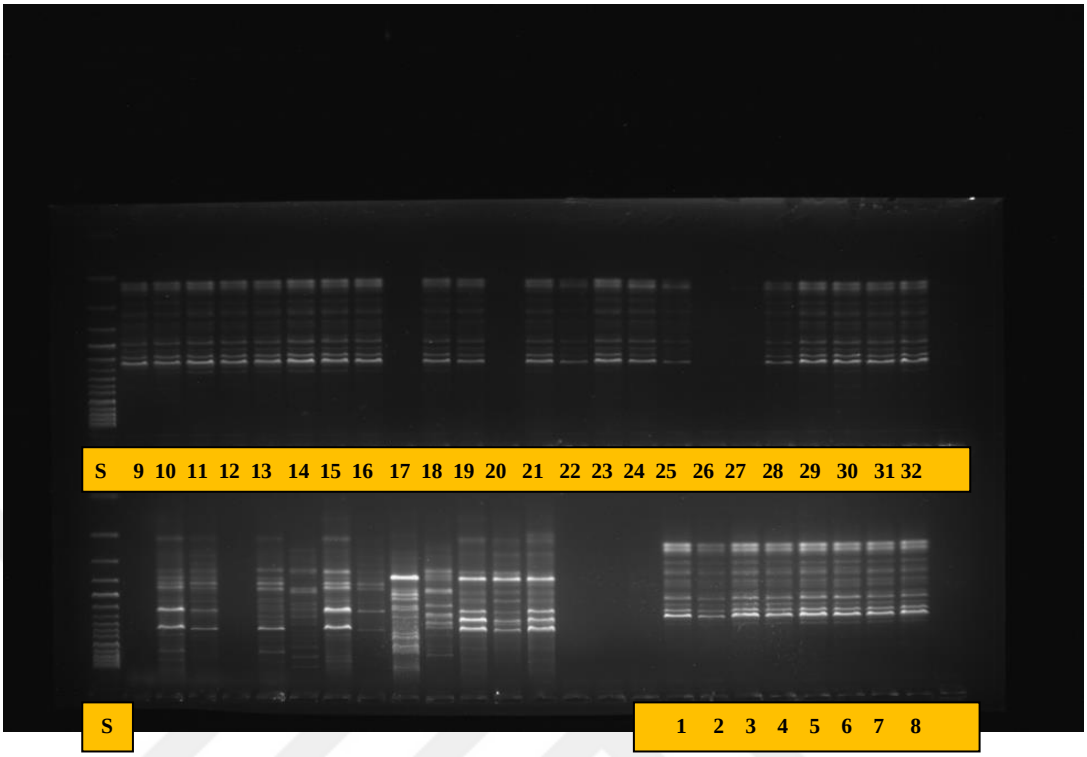

Figure 4.2. Monomorphic band profile obtained by $(AG)_7YC$ primer at 2% agarose gel

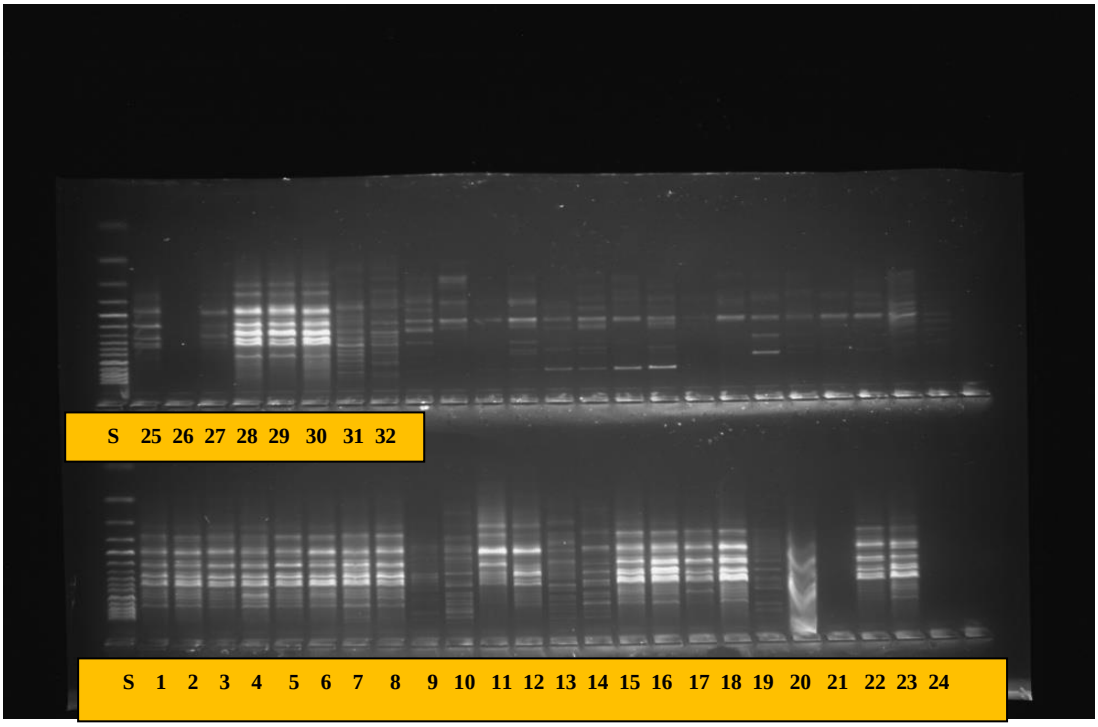

Figure 4.3. Polymorphic band profile obtained by HVH $(TCC)_7$ primer at 2% agarose gel

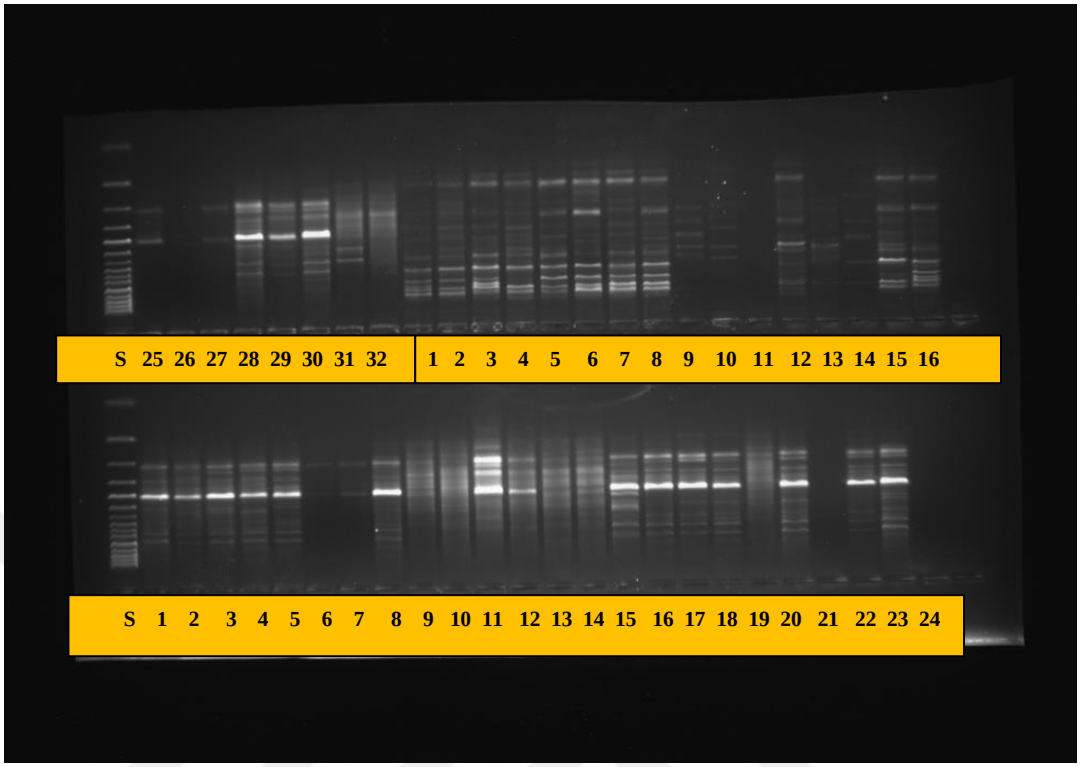

Figure 4.4. Polymorphic band profile obtained by DBDA(CA)7 and (AG)8T primer at 2% agarose gel

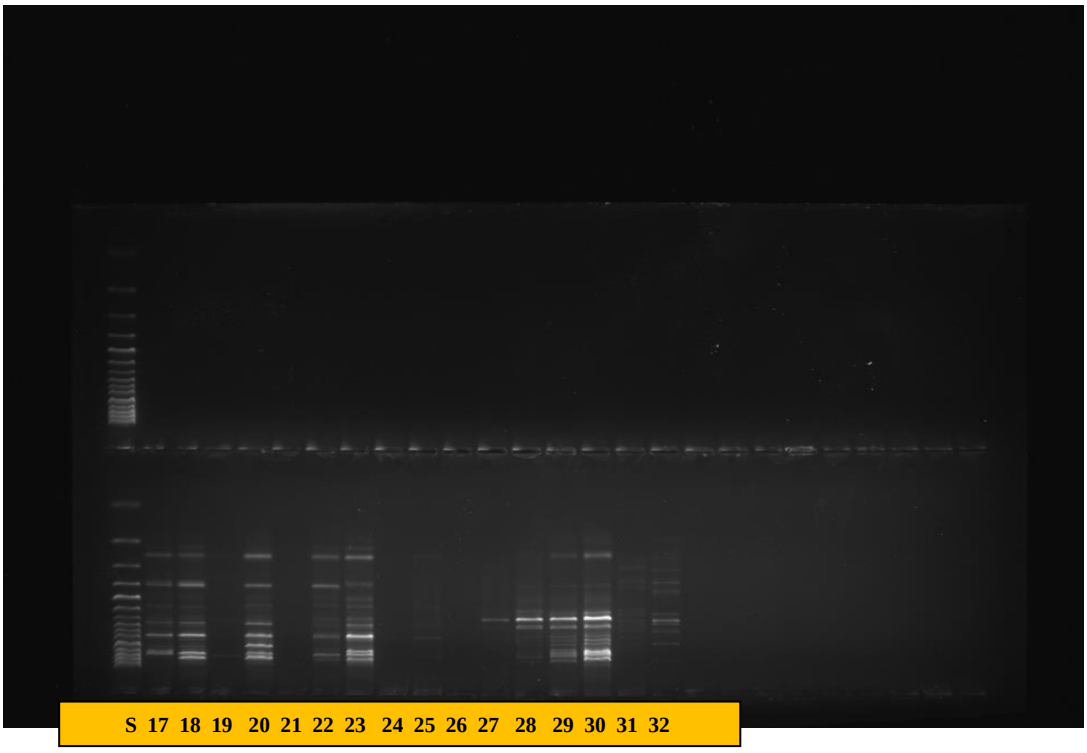

Figure 4.5. Polymorphic band profile obtained by (AG)8T primer at 2% agarose gel

Table 4.4. List show allele's numbers and polymorphic alleles in addition to the polymorphism percentage of each ISSR marker used in this study.

NO	ISSR Primers	Bands Numbers	Polymorphic alleles	PolymorphismRate %
1	HVH (TCC)7	9	9	100
2	(CT)8TG	8	7	87.5
3	(GACA)4	6	5	83.3
4	DBDA(CA)7	10	10	100
5	(AG)8T	13	13	100
6	(TAA)8	0	-	-
7	(GT)8YA	5	3	60
8	(AGC)6G	14	14	100
9	(AG)7YC	10	0	0
10	(CA)6AC	0	-	-
11	(GT)6GG	8	5	62.5
12	HVH (CA)7T	8	6	75
13	VHV (GTG)7	9	8	88
14	BDB (CA)7C	7	7	100
15	(CAC)3GC	6	6	100
16	(CAC)6	0	-	-
17	(CA)8R	7	2	28.6
18	(GAA)6	6	2	33.3
19	(GA)8YG	14	14	100
20	(TCC)5RY	20	0	0
Total		150	111	74

The minimum and maximum length of marker fragments ranged from 190 to 1400 base pairs (Table 4.5). The maximum length 1400 base pair was detected from primer (AG)8T, minimum length 190 base pair was detected from primer (AGC)6G.

Table 4.5. Total number of alleles, and fragment size

NO	Marker Name	Total number of alleles	Fragment sizes
1	HVH $(TCC)_7$	9	320-1200
2	$(CT)_8TG$	8	300-900
3	$(GACA)_4$	6	300-1000
4	DBDA$(CA)_7$	10	320-1100
5	$(AG)_8T$	13	220-1400
6	$(TAA)_8$	00	00
7	$(GT)_8YA$	5	290-1050
8	$(AGC)_6G$	14	190-1100
9	$(AG)_7YC$	10	220-700
10	$(CA)_6AC$	00	00
11	$(GT)_6GG$	8	320-1000
12	HVH $(CA)_7T$	8	375-1000
13	VHV $(GTG)_7$	9	300-1000
14	BDB $(CA)_7C$	7	200-1000
15	$(CAC)_3GC$	6	300-1000
16	$(CAC)_6$	00	00
17	$(CA)_8R$	7	380-1200
18	$(GAA)_6$	6	250-900
19	$(GA)_8YG$	14	250-1100
20	$(TCC)_5RY$	10	400-1100

As it is clear from results we can say that ISSR molecular markers are reliably effective and informative in the assessment of genetic diversity on Barberry genotypes.

According to obtained results we recommend to utilize the ISSR primers that were tested in this study because they were highly polymorphic except for $(AG)_7YC$ and $(TCC)_5RY$ because they were monomorphic and could not be useful in future investigations on Barberry's characterization using ISSR Molecular Marker technique.

Also, it is recommended to exclude $(TAA)_8$, $(CA)_6AC$, and $(CAC)_6$ in future investigations because they will not work and these types of DNA segments are not available on the genomic DNA of *Berberis* species.

4.4. Inter Simple Sequence Repeat (ISSR) Markers Phylogenetic Relationship Analysis

To investigate the relationship among 32 Barberry genotypes on the basis of 20 ISSR marker data, UPGMA (Unweighted Pair Group Method of cluster Analysis) dendrogram was generated using NTSYS- pc (Numerical Taxonomy and Multi-variation Analysis SystemVersion 2.11X) software (Sneath and Sokal 1973) as shown in Figure 4.6.

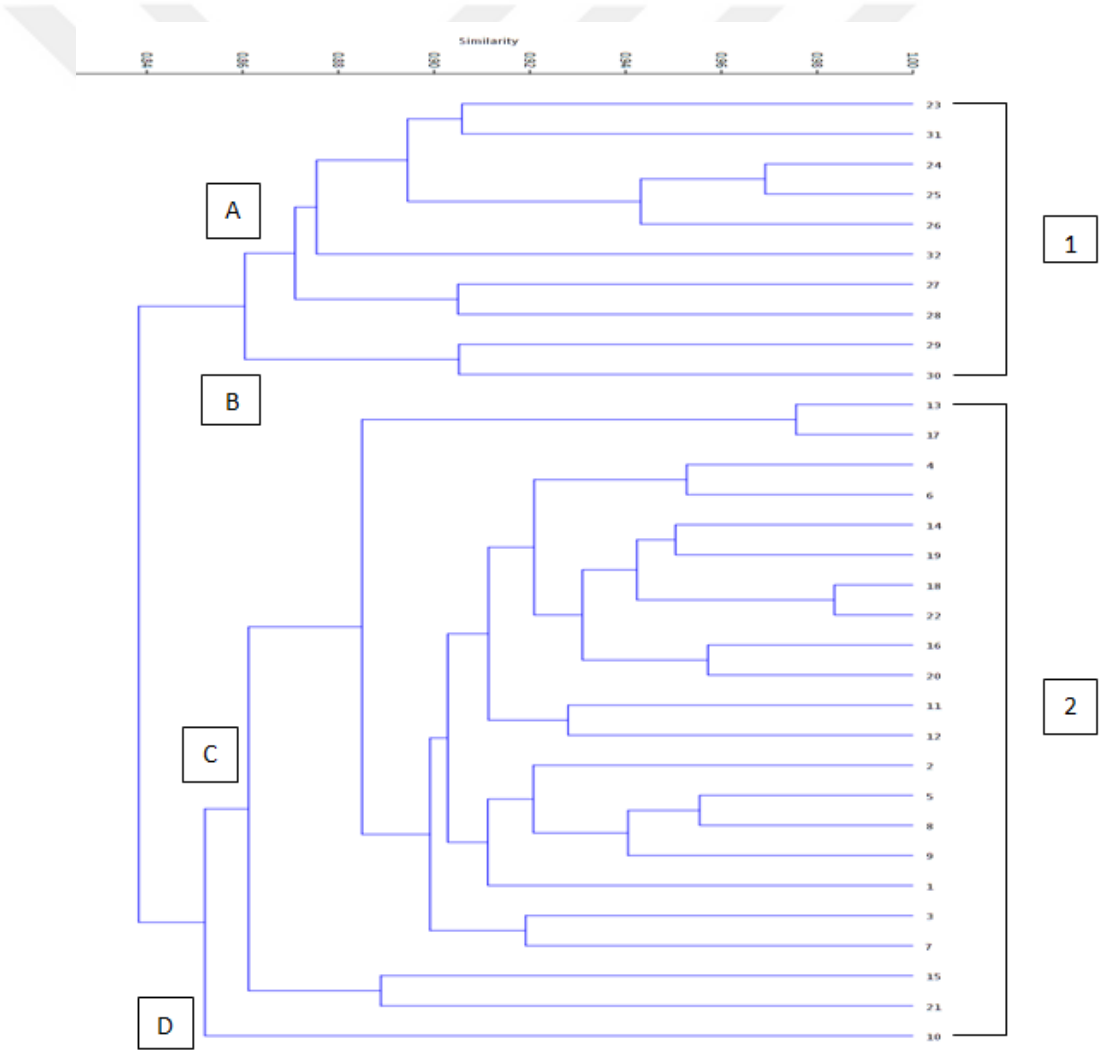

Figure 4.6. UPGMA dendogram generated for 32 Barberry genotypes based on 20 ISSR markers data

The UPGMA dendogram constituted based on 20 ISSR markers data indicated that the dendogram consisted of 2 major clusters with the coefficient similarity ranged between 0.84 and 1.00.

The first major cluster (1) which represents the Turkey genotypes is further split into 2 sub-groups, the first sub-group (A) includes 23, 31, 24, 25, 26, 32, 27, and 28 genotypes, whereas the second sub-group (B) includes 29 and 30 genotypes.

The second major cluster, which represents the Kyrgyzstan genotypes, also splits into 2 sub-groups. The first sub-group (C) includes 4, 6, 14, 19, 18, 22, 16, 20, 11, 12, 2, 5, 8, 9, 1, 3, 7, 15, and 21 genotypes, whereas the second sub-group (D) includes 10 genotype.

Results indicate that 29 and 30 genotypes which were taken from Turkey are too close to each other and they are well separated from other Turkey sub-group genotypes. On the other hand, the genotype 10 which is taken from Kyrgyzstan shows us it falls into same main cluster with the first sub-group but appears that the relationship between them are somewhat distant.

4.5. Similarity Index

A similarity index of 32 Barberry genotypes were constructed using NTSYS-pc (Numerical Taxonomy and Multi-variation Analysis SystemVersion 2.11X) software (Table 3.6).

Table 4.6. Similarity Index for 32 Barberry genotypes using NTSYS-pc (Version 2.11X) software.(1-22) represent Kyrgyzstan genotypes, (23-32) represent Turkey genotypes

1 | 1.0
2 | 0.9 1.0
3 | 0.9 0.9 1.0
4 | 0.9 0.9 0.9 1.0
5 | 0.9 0.9 0.9 0.9 1.0
6 | 0.9 0.9 0.9 1.0 0.9 1.0
7 | 0.9 0.9 0.9 0.9 0.9 0.9 1.0
8 | 0.9 0.9 0.9 0.9 0.9 0.9 0.9 1.0
9 | 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 1.0
10 | 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 1.0
11 | 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.7 0.8 1.0
12 | 0.8 0.8 0.8 0.9 0.8 0.9 0.8 0.8 0.7 0.7 0.9 1.0
13 | 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.7 0.7 0.7 1.0
14 | 0.8 0.8 0.7 0.8 0.8 0.8 0.8 0.7 0.7 0.8 0.8 0.8 0.7 1.0
15 | 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.7 0.8 0.8 0.9 0.8 0.7 1.0
16 | 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.7 0.8 0.8 0.8 0.8 0.7 0.9 1.0
17 | 0.9 0.8 0.9 0.9 0.9 0.9 0.8 0.9 0.6 0.7 0.8 0.8 0.7 0.6 0.9 0.9 1.0
18 | 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.8 0.8 0.8 0.8 0.8 0.7 0.9 0.9 0.9 1.0
19 | 0.9 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.7 0.9 1.0
20 | 0.9 0.9 0.9 0.9 1.0 0.9 0.9 0.9 0.7 0.7 0.8 0.8 0.7 0.7 0.9 0.9 0.9 1.0 0.8 1.0
21 | 0.8 0.9 0.8 0.9 0.8 0.9 0.8 0.8 0.8 0.8 0.9 0.8 0.7 0.7 0.8 0.8 0.5 0.9 0.8 0.7 1.0
22 | 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.7 0.8 0.8 0.8 0.8 0.7 0.9 0.9 0.9 0.9 0.8 0.9 0.8 1.0
23 | 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.8 0.8 0.8 0.8 0.8 0.7 0.9 0.9 0.9 1.0 0.8 1.0 0.9 0.9 1.0
24 | 0.9 0.9 0.8 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.9 0.8 0.8 0.8 0.9 9999.0 0.8 0.9 9999.0 0.9 0.8 0.9 1.0
25 | 0.7 0.8 0.8 0.8 0.8 0.8 0.8 0.7 0.7 0.7 0.7 0.7 0.7 0.6 0.8 0.7 0.7 0.7 0.7 0.8 0.7 0.8 0.7 0.7 1.0
26 | 0.7 0.7 0.8 0.7 0.7 0.7 0.7 0.7 0.7 0.8 0.6 0.6 0.6 0.5 0.6 0.6 9999.0 0.7 0.7 9999.0 0.7 0.7 0.6 0.6 0.7 1.0
27 | 0.7 0.6 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.6 0.6 0.6 0.6 0.6 0.7 0.7 0.6 0.8 0.6 0.7 0.6 0.7 0.8 1.0
28 | 0.8 0.8 0.9 0.9 0.9 0.9 0.8 0.9 0.7 0.8 0.8 0.8 0.8 0.8 0.9 0.8 0.8 0.9 0.8 0.9 0.9 0.9 0.9 0.8 0.8 0.7 0.8 1.0
29 | 0.8 0.8 0.9 0.9 0.9 0.9 0.9 0.9 0.8 0.8 0.8 0.8 0.7 0.8 0.9 0.8 0.8 0.9 0.8 0.9 0.8 0.9 0.9 0.8 0.8 0.7 0.8 0.9 1.0
30 | 0.9 0.8 0.9 0.9 0.9 0.9 0.9 0.9 0.7 0.8 0.8 0.8 0.8 0.8 0.9 0.9 0.8 0.9 0.8 0.9 0.8 0.9 0.9 1.0 0.7 9999.0 0.6 0.9 1.0 1.0
31 | 0.7 0.7 0.7 0.7 0.7 0.8 0.7 0.7 0.7 0.6 0.6 0.7 0.7 0.6 0.7 0.7 0.6 0.7 0.8 0.6 0.6 0.7 0.7 0.9 0.6 0.6 0.4 0.6 0.7 0.7 1.0
32 | 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.6 0.7 0.7 0.8 0.7 0.7 0.8 0.8 0.6 0.8 0.8 0.8 0.8 0.8 0.8 0.9 0.7 0.8 0.5 0.8 0.8 0.8 0.8 1.0

4.6. Morphological Fruit Comparison

In this study fruit morphological comparison were made considering the fruit morphological diversity within and between *Berberis sphaerocarpa* Kar. et Kit and *Berberis crataegina* fruits.

Fruit morphological characteristics, including fruits width, length, and weight of 32 Barberry bush genotypes were analyzed pomologically to determine fruit

characteristics using SPSS (Statistical package for social sciences version 15.0) software and Microsoft Office Excel software.

Berberis sphaerocarpa Kar. et Kit fruits morphological analyzing results were obtained from a previous study of Yilmaz *et al* (2015), which is under approval for publication (in press) (Figure 4.7), whereas fruits pomological analysis of *Berberis crataegina* was carried out in the laboratories of Erciyes university in Turkey (Figure 4.8) and (Figure 4.9).

Among of 32 *Berberis sphaerocarpa* Kar. et Kit and *Berberis crataegina* genotypes were investigated in this study, greatest fruit widths were observed in genotypes 14 (10.82 mm), 21 (10.74 mm), 16 (10.55 mm), and 8 (10.28 mm) which are taken from Kyrgyzstan; the lowest width value was observed in genotypes 31 (3.99 mm) and 23 (4.18 mm) which are taken from Turkey.

Considering the fruit lengths of genotypes, greatest fruit lengths were observed in genotypes 22 (13.96 mm), 11 (13.72 mm), and 13 (13.16 mm) which are taken from Kyrgyzstan, whereas the lowest value was investigated in genotype 31 (6.76 mm) which is taken from Turkey.

Regarding to fruits weight, the greatest fruit weight value was observed in genotypes 16 (0.54 g), 21 (0.53 g), and 17 (0.50 g) which were taken from Kyrgyzstan, and the lowest weight value was observed in genotypes 26 (0.06 g) and 31(0.07 g) (Table 4.7).

Obtained results from the UPGMA dendogram, and with comparison with the fruit morphological data it was found that there is no significant variation among *Berberis sphaerocarpa* Kar. et Kit genotypes in terms of fruit's (width, length, and weight) even among the sub-groups (C) and (D). Also, there is no significant variation among *Berberis crataegina* genotypes also among sub-groups (A) and (B) of same morphological characteristics involved in this study. On the other hand, observed variations were between cluster (1) which represents *Berberis crataegina* genotypes

(taken from Turkey) and cluster (2) which represents *Berberis sphaerocarpa* Kar. et Kit genotypes (taken from Kyrgyzstan) and fruits of the genotypes within cluster (2) were generally heavier, wider, and longer than fruits of the genotypes within cluster (1).

Obtained results from UPGMA dendogram indicated that the ISSR markers represent a powerful tool for investigation of closely related *Berberis* species such as *Berberis sphaerocarpa* Kar. et Kit and *Berberis crataegina.* In addition to, ISSR marker has the ability to distinguish between species that have different fruit morphological characteristics which will enable plant breeders to conduct Barberry development programs and to improve Barberry germplasm.

Table 4.7. List of Fruit morphological values (weight, length, and width) of 32 Barberry shrub genotypes

Genotypes	Width(mm) [min – max]	Length (mm) [min – max]	Weight(g) [min – max]
1	9.02 ± 0.581 ^{abcde} 8.23 – 10.08	11.22 ± 0.72 ^{abcd} 9.99 – 12.01	0.41 ± 0.05 ^{abcd} 0.35 – 0.50
2	7.49 ± 0.41 ^{e} 6.85 – 8.16	11.88 ± 0.46 ^{abc} 11.08 -12.54	0.28 ± 0.02 ^{cdefg} 0.26 – 0.33
3	7.98 ± 0.58 ^{bcde} 7.11 – 9.22	10.95 ± 0.91 ^{abcd} 9.59 - 12.93	0.29 ± 0.04 ^{bcdefg} 0.23 – 0.37
4	9.96 ± 0.35 ^{abcde} 9.55 – 10.48	8.98 ± 0.66 ^{cdefg} 7.88 -9.83	0.40 ± 0.05 ^{abcde} 0.28 – 0.44
5	9.50 ± 0.96 ^{abcde} 8.28 – 11.42	10.98 ± 0.33 ^{bcde} 10.45 – 11.45	0.44 ± 0.09 ^{abcd} 0.34 – 0.60
6	7.58 ± 0.43 ^{de} 7.08 – 8.54	10.95 ± 0.64 ^{abc} 10.35 – 12.39	0.23 ± 0.04 ^{defg} 0.16 – 0.29
7	8.86 ± 0.53 ^{abcde} 8.03 – 9.66	11.95 ± 0.54 ^{abc} 11.38 – 13.05	0.48 ± 0.05 ^{abcd} 0.39 – 0.54
8	10.28 ± 0.74 ^{abcd} 8.82 – 11.38	10.76 ± 0.53 ^{bcde} 10.08 – 11.42	0.47 ± 0.08 ^{abcd} 0.34 – 0.57
9	7.97 ± 0.56 ^{cde} 7.09 – 8.91	10.79 ± 0.90 ^{bcde} 9.42 – 12.51	0.31 ± 0.05 ^{abcdefg} 0.24 – 0.37
10	8.53 ± 0.59 ^{abcde} 7.53 – 9.31	11.86 ± 0.79 ^{abc} 10.38 – 12.59	0.30 ± 0.05 ^{bcdefg} 0.23 – 0.36
11	9.36 ± 0.87 ^{abcde} 8.16 – 10.88	13.72 ± 0.95 ^{ab} 12.43 – 15.57	0.46 ± 0.06 ^{abcd} 0.39 – 0.57
12	9.19 ± 0.46 ^{abcde} 8.46 – 9.82	11.00 ± 0.70 ^{abcd} 10.00 – 12.36	0.35 ± 0.06 ^{abcde} 0.27 – 0.47

Table 4.7. (Continue)

13	9.02 ± 0.74 ^{abcde} 8.15 – 10.18	13.16 ± 2.30 ^{a} 11.09 – 18.88	0.45 ± 0.04 ^{abcd} 0.37 – 0.50
14	10.82 ± 1.05 ^{a} 9.56 – 12.16	12.01 ± 0.69 ^{abc} 11.01 – 13.34	0.46 ± 0.12 ^{abcd} 0.33 – 0.65
15	8.49 ± 0.55 ^{abcde} 7.86 – 9.54	9.49 ± 0.48 ^{cdefg} 8.83 – 10.03	0.33 ± 0.04 ^{abcdef} 0.26 – 0.39
16	10.55 ± 0.62 ^{abc} 9.47 – 11.15	12.11 ± 0.66 ^{abc} 11.79 – 12.76	0.54 ± 0.07 ^{ab} 0.47 – 0.63
17	9.35 ± 1.02 ^{abcde} 7.53 – 10.54	12.47 ± 0.73 ^{abc} 11.12 – 13.53	0.50 ± 0.09 ^{abc} 0.38 – 0.68
18	8.50 ± 0.39 ^{abcde} 7.61 – 9.07	10.13 ± 0.67 ^{cdef} 9.05 – 11.00	0.33 ± 0.08 ^{abcdef} 0.21 – 0.45
19	8.82 ± 0.77 ^{abcde} 7.19 – 9.59	11.45 ± 0.66 ^{abc} 10.55 – 12.36	0.41 ± 0.07 ^{abcd} 0.30 – 0.54
20	9.81 ± 0.41 ^{abcde} 9.30 – 10.57	11.93 ± 0.75 ^{abc} 10.65 – 12.77	0.46 ± 0.06 ^{abcd} 0.37 – 0.58
21	10.74 ± 0.93 ^{ab} 9.18 – 12.17	11.34 ± 0.78 ^{abc} 10.40 – 12.55	0.53 ± 0.09 ^{a} 0.41 – 0.73
22	9.55 ± 0.63 ^{abcde} 8.86 – 10.83	13.96 ± 0.73 ^{ab} 12.78 – 15.25	0.44 ± 0.07 ^{abcd} 0.33 – 0.56
23	4.18±0.09 ^{f} 4.07-4.38	7.92±0.20 ^{defg} 7.67-8.32	0.08±0.004 ^{g} 0.08-0.09
24	4.51±0.19 ^{f} 4.12-4.72	8.97±0.20 ^{cdefg} 8.67-9.37	0.11±0.008 ^{fg} 0.10-0.12
25	5.09±0.16 ^{f} 4.85-5.40	9.70±0.11 ^{cdefg} 9.50-9.89	0.14±0.004 ^{efg} 0.14-0.16
26	4.17±0.45 ^{f} 3.52-5.4	7.81±0.03 ^{efg} 7.76-7.89	0.06±0.008 ^{g} 0.05-0.07
27	4.68±0.02 ^{f} 4.63-4.72	7.59±0.16 ^{fg} 7.36-7.90	0.10±0.006 ^{fg} 0.09-0.11
28	4.71±0.06 ^{f} 4.65-4.84	7.42±0.34 ^{fg} 7.08-8.12	0.09±0.001 ^{fg} 0.09-0.10
29	4.85±0.08 ^{f} 4.68-4.96	7.07±0.19 ^{fg} 6.86-7.46	0.09±0.001 ^{fg} 0.10-0.10
30	5.54±0.08 ^{f} 5.44-5.72	9.16±0.10 ^{cdefg} 8.99-9.35	0.17±0.007 ^{efg} 0.16-0.19
31	3.99±0.04 ^{f} 3.91- 4.06	6.76±0.03 ^{g} 6.69-6.81	0.07±0.006 ^{g} 0.06-0.08
32	4.86±0.21 ^{f} 4.51-5.24	9.21±0.21 ^{cdefg} 8.99-9.65	0.12±0.018 ^{fg} 0.10-0.17

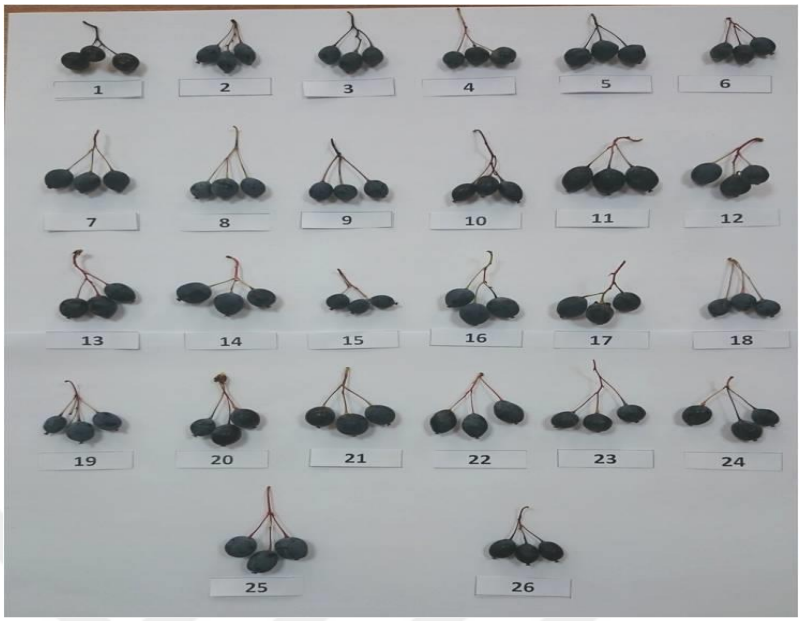

Figure 4.7. Samples of Barberry fruits (*Berberis sphaerocarpa* Kar. et Kit) investigated in this study

Figure 4.8. Samples' of Barrberry fruits (*Berberis crataegina*) investigated in this study

Figure 4.9. Samples' of Barrberry fruits (*Berberis crataegina*) investigated in this study

5. CONCLUSION

Barberry (Berberidaceae) is well known in many countries for its medical benefits, and it can be considered as a valuable source to extract many alkaloids from different plant parts (fruit, leaf, root, and bark), which can be helpful for treating various kinds of common diseases.

In addition to Barberries medical values, the plant has further uses in the local food industry and it can be used as ornamental plant, or for soil protection and prevention of soil erosions.

There are a few studies conducted on Barberry plant, furthermore, there is a requirement to conduct further genetic researches for effective conservation and improvement of existing germplasm on the wild Barbary plant. The morphological characterization of species in the genus *Berberis* based on morphological traits is still a matter of debates.

Nowadays ISSR Molecular Markers are reliable and powerful method for genetic investigation of related plant species and populations because they are not affected by environmental effects or plant growth stages. Also it can be used to investigate the variation in DNA level.

According to the obtained results, we recommend to utilize the (ISSR) primers that were tested in this study because they were highly polymorphic except for $(AG)_7YC$ and $(TCC)_5RY$ because they were monomorphic and could not be useful in the future investigations on Barberry's characterization using ISSR Molecular Marker technique. Also, it is recommended to exclude $(TAA)_8$, $(CA)_6AC$, and $(CAC)_6$ in future investigations because they will not work because these types of DNA segments are not available on the genomic DNA of *Berberis* species.

In this research, genetic and fruit morphological comparison was made by using 20 ISSR Molecular Markers to determine phylogenetic relations, and the genetic structure among 32 Barberry bush genotypes (22 genotypes of *Berberis sphaerocarpa* Kar. et Kit taken from Kyrgyzstan and 10 genotypes of *Berberis crataegina* taken from Turkey) to put the right bases that will enable researchers to make further genetic investigations upon Barberry species and populations, to improve barberries breeding programsand to develop effective germplasm conservation.

Regarding to phylogenetic relations, the UPGMA-Dendogram shows that the investigating individuals are divided evidently into two main groups; first group refers to Turkey genotypes which is divided into two sub-groups and genotypes (23, 31, 24, 25, 26, 32, 27, and 28) tend to be close to each others, whereas the second group refers to Kyrgyzstan genotypes which is also divided into two sub-groups and genotypes (4, 6, 14, 19, 18, 22, 16, 20, 11, 12, 2, 5, 8, 9, 1, 3, 7, 15, and 21) somewhat tend to be close to each other.

Population structure analysis, principal component analysis (PCA) and (PCA) graph were generated. Phylogenetic analyses were carried out at 20 ISSR molecular markers data results based on the entire DNA marker profiles show us that the most of 32 Barberry genotypes were clustered randomly into four main groubs; on the other hand, others are distributed separately.

The morphological comparison between fruits of *Berberis sphaerocarpa* Kar. et Kit which are taken from Kyrgyzstan and fruits of *Berberis crataegina* which are taken from Turkey using SPSS (Statistical package for social sciences version 15.0) software and Microsoft Office Excel software shows that fruits of *Berberis sphaerocarpa* Kar. et Kit are morphologically characterized by increasing in terms of length, width, and weight from *Berberis crataegina*'s fruits.

Pozharskiy and Chekalin 2015, Genomic DNA was extracted from the young leaves of (134) accessions represent 6 populations of *Berberis iliensis* and 4 populations of

Berberis sphaerocarpa as well as 3 populations of interspecific hybrids *Berberis iliensis*×*Berberissphaerocarpa* and 1 population of *Berberis oblonga*.

The tested plants were collected from Kazakhstan and analyzed with 5 primers using Inter-Simple Sequence Repeat (ISSR) molecular marker.

The main aim of this study is to lay the bases for more genetic investigations and evaluating the inter-specific genetic variation among *Berberis* species that grow naturally in Kazakhstan as well as making a clear taxonomic statue of local species to protect them from extinction.

101 bands in total were observed, whereas, 99 of them are polymorphic with average 20.4 per primer. 32.0±7.0 band presences per individual were observed. Also, the obtained results from the dendogram shows 3 distinct clusters, the first one represents the three populations of *Berberis iliensis* and the second cluster corresponds to *Berberis oblonga* population with *Berberis sphaerocarpa* populations whereas the third cluster represents the hybrid populations of the 3 aforementioned Barberry shrubs.

Also, structure analysis was performed, and results of structure diagram supports an assumption about inter-specific hybridization and also show similarity coefficient between the species involved.

Yilmaz *et al*. 2015, 26 wild genotypes of *Berberis sphaerocarpa* Kar. et Kit grow naturally in Jeti-Oguz district of Issyk-Kul city in northeastern of Kyrgyzstan.

In this study a pomological analysis was carried out to assess fruit characteristics.

Fruit morphological characteristics determined in this study includes fruit shape, plant spins and forms, fruit length, width, weight, length/width index, and water soluble dry matter content (WSDM) (%).

30 of black ripened fruits of each genotype were collected and assessed using Microsoft Office Excel software.

Obtained results from the pomological analysis refers that the fruit weights ranged between 0.23 g in the 01-JO-006 genotype and 0.61 g in the 01-JO-025 genotype. In terms of water soluble dry matter contents was between 16.67% in the 01-JO-014 genotype and 18.73% in the 01-JO-010 and 01-JO-018 genotypes.

Fruit shapes of genotypes were identified as long, long-spherical, spherical and oblate spherical.

The widths of fruits were ranged between 10.82 mm in 01-JO-014 genotype and 7.49 mm in 01-JO-002 genotype. The length of fruit was ranged between 13.96 mm in the 01-JO-022 genotype and 8.98 mm in 01-JO-004 genotype. In regards to the fruit weights of the genotypes, the values were ranged between 0.61 g in the 01-JO-025 and 0.23 g in the 01-JO-006 genotype.

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