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ORIGINAL ARTICLE

# Genetic Diversity in Apple Accessions Belong to Different Species Collected from Natural Populations of Tianshan Mountains, South-West Kyrgyzstan

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**Abstract**
There isn't any concrete consensus about the origin of domesticated apple. However, it is generally thought that the origin of this apple primarily comes from *M. sieversii,* also known as Central Asia wild apple. The second significant contribution is thought to be supplied by *M. sylvestris*. Central Asia is an important origin region for apples. Within this region, mountainous and forestlands of Kyrgyzstan constitute a significant source for wild apple species. The present study was conducted to put forth the genetic relationships between 65 accessions belonging to *M. kirgishorum, M. domestica, M. niedzwetzkyana*, and *M. sieversii* apple species collected from different regions of Kyrgyzstan and 12 accessions belonging to *M. baccata, M. prunifolia, M. sylvestris* and *M. domestica* species collected from different regions of Turkey. A combination of ISSR and SRAP markers were used in this study. About 71.1% polymorphism was obtained with ISSR primers and 83.5% with SRAP primers. Similarity level of accessions studied was found between 0.74 and 0.95. All materials used in this study were genetically separated from each other. The apple accessions used in this study were generally placed in a mixed fashion with the other materials in the dendrogram. Apart from some exceptions, standard apple accessions of *M. domestica* species taken from Kyrgyzstan and Turkey were grouped separately from standard apple cultivars. While the genotypes of wild species were mostly placed in the other group of the dendrogram, small number of genotypes of *M. domestica* species was placed in this group. Present outcomes revealed significant information for the preservation, assessment and breeding of wild apple species of Kyrgyzstan.

**Keywords** Central Asia · Endangered species · Genetic resources · *Malus* spp · Malus niedzwetzkyana

## Genetische Diversität von Apfel-Akzessionen innerhalb verschiedener Arten, die in Wildbeständen des Tianshan-Gebirges im Südwesten Kirgisistans erfasst wurden

**Schlüsselwörter** Zentralasien · Gefährdete Spezies · Genetische Ressourcen · *Malus* spp · Malus niedzwetzkyana

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## Introduction

Apple is one of the most produced fruit crops in the world with over 86 million tons of production (FAO 2015). Today, domesticated apple (*Malus domestica* Borkh.) is cultured over a large geography from cold sections of Siberia to temperate sections of Ecuador. The origin centers of apple worldwide are known as Eastern Asia, Central Asia, Western Asia-Europe and North America (Janick et al. 1996).

The origin of domesticated apple probably comes from *Malus sieversii*, also known as Central Asia domesticated apple (Harris et al. 2002; Coart et al. 2006). Besides, *Malus sylvestris,* also known as Western Europe domesticated apple, may have slight parental contributions to gene pool of domesticated apples (Robinson et al. 2001). Similarly, Cornille et al. (2012) pointed out *M. sieversii* as the main species and *M. sylvestris* as the second important species for evolution of domesticated apples. It was also reported that *M. sieversii* was the primary progenitor for domesticated apples and there was a great interest on this species by botanists for genetic diversity and population structure studies (Zhang et al. 2015). However, there are still findings in literature about complexity of the origins of domesticated apples. The high-level cpDNA diversity identified in *Malus* species may explain this complex structure (Coart et al. 2006).

Wild apple genetic resources in Central Asia are quite significant since selections for desired characteristics in commercial apple species narrowed the genetic diversity. Apple populations in Central Asia may constitute a significant gene pool to overcome various problems such as resistance to biotic and abiotic stress conditions, fruit quality characteristics, tree growth forms and some other unsolved problems (Forsline et al. 2010). Within Central Asia, Kyrgyzstan is an important apple origin center. Besides walnut, pistachio and almond species, there are important apple populations and forests in different sections of Kyrgyzstan. However, there are serious anthropogenic losses in these materials and they may be lost totally in near future (Dzunusova et al. 2008). There are three apple species naturally existing in Kyrgyzstan. They (*Malus sieversii* (Ledeb.) M. Roem, (*Malus kirghisorum*) and (*Malus niedzwetzkyana* (Dieck). C.K. Schneid)) are also known as the origin of domesticated apple. *Malus sieversii* has natural spread over dry and mountainous sections of Kazakhstan, Kyrgyzstan, China, Tajikistan, Uzbekistan and Turkmenistan. *Malus kirghisorum* is genetically close to *Malus sieversii*, but different in fruit shape, color and other characteristics. In *Malus niedzwetzkyana* species, there are pink-purple pigmentations over the leaf, flower and fruits. These two species can exist in the same regions, but *Malus sieversii* is more common (Volk et al. 2009). Omasheva et al. (2015) reported that some researchers indicated wild apple species of Kazakhstan as *Malus sieversii, Malus kirghisorum* and *Malus niedzwetzkyana* and some others indicated as *M. sieversii* and *M. niedzwetzkyana*.

There are several endangered plant species in Central Asia and urgent measures should be taken to prevent these species from extinction. *M. sieversii* and *M. niedzwetzkyana* were also reported among endangered species and placed in red-books. These species were endangered through habitat loss and degradation, land clearing and genetic erosion, grafting of commercial varieties and hybridization. Because of narrow genetic bases of domesticated apples, these endangered species should be preserved at *ex situ* and *in situ* levels (Eastwood et al. 2009). It was also pointed out that *M. niedzwetzkyana* was placed in *Red Book of Threatened Species of Kazakhstan* (Omasheva et al. 2015) and red list of threatened species (IUCN 2016).

Different marker systems are employed to put forth the genetic diversity in plants. Among them, inter-simple sequence repeat (ISSR) markers involve amplification of DNA segment between two identical microsatellite repeat regions. ISSRs have high reproducibility possibly due to the use of longer primers (16–25 mers) as compared to RAPD primers (10-mers) which permits the sub-sequential use of high annealing temperature (45–60 °C), leading to higher stringency (Zietkiewicz et al. 1994). This system was successfully used in apples before (Goulao and Oliveira 2001; He et al. 2011; Uzun et al. 2016). On the other hand, SRAP is a polymerase chain reaction (PCR)-based marker system as described by Li and Quiros (2001). The SRAP is a simple and efficient marker system that can be adapted for a variety of purposes in different crops. It has reasonable through put rate, discloses numerous codominant markers, targets open reading frames, and allows easy isolation of bands for sequencing. This marker system was also successfully used in *M. sieversii* apple species before (Zhang et al. 2009).

The present study was conducted to put forth apple genetic diversity of Kirgizstan and to determine genetic relationships between these apple species. With this study, the relationships among the genotypes belonging to *Malus sieversii, Malus kirghisorum* and *Malus niedzwetzkyana* species collected from forestlands of southwestern Kirgizstan, some domesticated apples of *M. domestica* species, some standard apple cultivars collected from Turkey and wild apple species were investigated.

## Materials and Methods

### Plant Material and DNA Isolation

A total of 77 apple accessions belonging to 7 different apple species were used as the plant material in this study. Of



**Fig. 1** Some endangered old apple trees in southwestern Kyrgyzstan (A. Uzun©)

these accessions, 65 (34 for *M. kirghisorum*, 23 for *M. domestica*, 4 for *M. niedzwetzkyana*, 4 for *M. sieversii*) were collected from different sections of Southwestern Kyrgzystan with intensive apple populations. Majority of these materials exist in mixed form with forest trees in mountainous sections of the country and some standard cultivars exist in old orchards. The wild apple trees especially in forestlands are quite old trees and some are under the danger of extinction (Fig. 1). The apple materials used in this study exhibit significant variations with regard to fruit morphology (Fig. 2). In present study, 12 apple species (9 for *M. domestica*, 1 for *M. baccata* Borkh., 1 for *M. prunifolia*, 1 for *M. sylvestris* (L.) Mill.) supplied from the collections of Egirdir Fruit Research Institute of Turkey were also included (Table 1). Genomic DNA was extracted from young leaves with CTAB method as described by Doyle and Doyle (1990). DNA concentration was measured with a NanoDrop, ND 100 spectrophotometer (NanoDrop Technologies Inc. Wilmington, DE, USA), and 10 ng/μL DNA templates were made using TE (10-mM Tris–HCl, 0.1-mM ethylene diamine tetra acetic acid (EDTA), and pH 8.0).

## ISSR and SRAP Analysis

A total of 12 ISSR primers, previously experimented by Fang and Roose (1997), Gulsen et al. (2010) and Uzun et al. (2016) were used in this study (Table 2). PCR reaction components and PCR cycles were applied as described by Uzun et al. (2009a). For SRAP analyses, all apple samples were worked with 16 primer combinations (Table 2). In SRAP analyses, PCR components and cycles were arranged as described by Uzun et al. (2009b).

PCR products were run 2% agarose gel including 1 X TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) at 110 V for 2.5–3 h. Resultant gels were imaged under UV light. A 100 bp standard DNA ladder was used for estimating the size of ISSR and SRAP fragments.

## Data Analysis

All gel bands of ISSR and SRAP markers were scored as either present (1) or absent (0) and resultant data was analyzed with Numerical Taxonomy Multivariate Analysis System (NTSYS-pc version 2.1) software package (Rohlf 2000). Similarity index was created in accordance with Dice's coefficient (Dice 1945). The similarity index was then used to create the dendrogram presenting the genetic relationships among the investigated materials. To create similarity matrix and dendrogram, ISSR and SRAP data were assessed together. Goodness of fit test, also called Mantel test, was performed by using ultrametric distance matrix obtained from the dendrogram and similarity matrix (Mantel 1967). The result of this test is a cophenetic correlation coefficient, *r*, indicating how well the dendrogram represents similarity data. Polymorphic Information Content (PIC) for dominant markers was calculated as:



**Fig. 2** Differences observed in fruit morphologies of some apple accessions used in this study (A. Uzun©)

PIC = 1 – [f 2 + (1 – f)2], where 'f' is the frequency of the marker in the data set. PIC for dominant markers is maximum of 0.5 for 'f' = 0.5 (De Riek et al. 2001). The GenAlEx ver. 6.5 program was run to determine allele frequency (*p* and q), number of effective alleles (Ne), Shannon's information index (I), expected (He) and unbiased expected heterozygosity (uHe) (Peakall and Smouse 2012).

## Results and Discussion

A total of 71 bands were obtained from 12 ISSR primers and 57 of them were polymorphic. The polymorphism ratio was identified as 71.1% (Table 2). In a previous study carried out with ISSR markers on apple gene sources of Turkey, polymorphism ratio was identified as 68.5% (Uzun et al. 2016). $(CA)_6AC$ primer yielded the greatest number of total and polymorphic bands. The least number of bands was obtained from $(AG)_8T$, $(GACA)_4$ and $BDB(CA)_7C$ primers. The PIC values obtained from ISSR primers varied between 0.00 ($(AG)_8T$, $BDB(CA)_7C$) and 0.34 ($(CAC)_3GC$). For ISSR primers, effective alleles (Ne) varied between 1.00 ($(AG)_8T$ and $BDB(CA)_7C$) and 1.66 (average 1.26), Shannon's information index (I) between 0.00 and 0.54 (average 0.25), expected heterozygosity and unbiased expected heterozygosity between 0.00 and 0.37 (average 0.16). The lowest values of these parameters were observed in $(AG)_8T$ and $BDB(CA)_7C$ primers and the highest values in $VHV(GT)_8G$ primer (Table 3).

A total of 68 bands were obtained from 16 SRAP primer combinations and polymorphism ratio was identified as 83.5%. SRAP primers yielded higher polymorphism ratio than ISSR primers. PIC values for SRAP primers varied between 0.11 (Em12/Me8) and 0.38 (Em2/Me4). Values for effective alleles (Ne) ranged from 1.21 (Em12/Me8) to 1.74 Em11/Me2) (average 1.50), for Shannon's information index (I) from 0.21 (Em8/Me4) to 0.56 (Em2/Me4) (average 0.44), for expected heterozygosity from 0.13 (Em8/Me4) to 0.41 (Em11/Me2) and for unbiased expected heterozygosity (uHe) from 0.14 (Em8/Me4) to 0.42 (Em11/Me2) (average 0.29) (Table 3).

The similarity levels among 77 apple accessions belonging to 7 species varied between 0.74–0.95 (Fig. 3). Current findings were similar with the results of Goulao and Oliveira (2001) (~0.75–1.00) and Uzun et al. (2016) (0.79–0.98) obtained with ISSR markers. On the other hand,

**Table 1** List of apple materials studied and their number, species, altitude collected and region

| No | Species/Cultivar | Altitute | Region | No | Species (Cultivar) | Altitute | Region |
|---|---|---|---|---|---|---|---|
| 1 | *M. kirghisorum* | 1528 | K. Alma | 40 | *M. domestica 'UC'* | 1280 | S. Chelek |
| 2 | *M. kirghisorum* | 1529 | K. Alma | 41 | *M. niedzwetzkyana* | 1276 | S. Chelek |
| 3 | *M. kirghisorum* | 1566 | K. Alma | 42 | *M. kirghisorum* | 1259 | S. Chelek |
| 4 | *M. kirghisorum* | 1567 | K. Alma | 43 | *M. kirghisorum* | 1803 | S. Chelek |
| 5 | *M. kirghisorum* | 1569 | K. Alma | 44 | *M. kirghisorum* | 1804 | S. Chelek |
| 6 | *M. kirghisorum* | 1574 | K. Alma | 45 | *M. kirghisorum* | 1823 | S. Chelek |
| 7 | *M. niedzwetzkyana* | 1580 | K. Alma | 46 | *M. kirghisorum* | 1822 | S. Chelek |
| 8 | *M. kirghisorum* | 1578 | K. Alma | 47 | *M. kirghisorum* | 1860 | S. Chelek |
| 9 | *M. niedzwetzkyana* | 1583 | K. Alma | 48 | *M. kirghisorum* | 1859 | S. Chelek |
| 10 | *M. kirghisorum* | 1591 | K. Alma | 49 | *M. domestica 'UC'* | 1861 | S. Chelek |
| 11 | *M. kirghisorum* | 1594 | K. Alma | 50 | *M. kirghisorum* | 1860 | S. Chelek |
| 12 | *M. kirghisorum* | 1598 | K. Alma | 51 | *M. domestica* 'Sary Chelek' | 1288 | S. Chelek |
| 13 | *M. kirghisorum* | 1604 | K. Alma | 52 | *M. domestica* 'Zimny' | 1143 | C. Gangag |
| 14 | *M. kirghisorum* | 1610 | K. Alma | 54 | *M. domestica* 'K. Zimny' | 1143 | C. Gangag |
| 15 | *M. kirghisorum* | 1606 | K. Alma | 56 | *M. domestica* 'Suzak Sarisi' | 1143 | C. Gangag |
| 16 | *M. kirghisorum* | 1146 | C. Gangag | 57 | *M. domestica* 'Rashida' | 1143 | C. Gangag |
| 17 | *M. kirghisorum* | 1147 | C. Gangag | 58 | *M. domestica* 'Biskek Kanat' | 1144 | C. Gangag |
| 18 | *M. kirghisorum* | 1145 | C. Gangag | 59 | *M. domestica* 'Biskek' | 1144 | C. Gangag |
| 19 | *M. kirghisorum* | 1148 | C. Gangag | 60 | *M. domestica* 'Colpanbay' | 1145 | C. Gangag |
| 20 | *M. kirghisorum* | 1150 | C. Gangag | 61 | *M. domestica* 'Rozmarin' | 1150 | C. Gangag |
| 21 | *M. kirghisorum* | 1371 | Arslanbob | 62 | *M. domestica* 'Smirenko' | 1151 | C. Gangag |
| 22 | *M. kirghisorum* | 1380 | Arslanbob | 63 | *M. domestica* 'Aygul' | 1144 | C. Gangag |
| 23 | *M. kirghisorum* | 1382 | Arslanbob | 64 | *M. domestica* 'Sinap' | 1265 | S. Chelek |
| 24 | *M. domestica 'UC'* | 1384 | Arslanbob | 65 | *M. domestica* 'A.A. Aport' | 1285 | S. Chelek |
| 25 | *M. domestica 'UC'* | 1399 | Arslanbob | 68 | *M. domestica* 'Akalma' | 1398 | Arslanbob |
| 26 | *M. kirghisorum* | 1390 | Arslanbob | 69 | *M. domestica* 'Sabran' | 1398 | Arslanbob |
| 27 | *M. kirghisorum* | 1443 | Arslanbob | 71 | *M. domestica* 'Daldabir' | 921 | FRI-Egirdir |
| 28 | *M. kirghisorum* | 1435 | Arslanbob | 72 | *M. domestica* 'Huryemez' | 921 | FRI-Egirdir |
| 29 | *M. kirghisorum* | 1438 | Arslanbob | 73 | *M. domestica* 'Orak' | 921 | FRI-Egirdir |
| 30 | *M. sieversii* | 1261 | S. Chelek | 74 | *M. sylvetris* | 921 | FRI-Egirdir |
| 31 | *M. sieversii* | 1260 | S. Chelek | 75 | *M. domestica* 'Tavsanbasi' | 921 | FRI-Egirdir |
| 32 | *M. niedzwetzkyana* | 1266 | S. Chelek | 76 | *M. domestica* 'Fuji' | 921 | FRI-Egirdir |
| 33 | *M. sieversii* | 1268 | S. Chelek | 77 | *M. domestica* 'G. Delicious' | 921 | FRI-Egirdir |
| 34 | *M. kirghisorum* | 1269 | S. Chelek | 78 | *M. prunifolia* | 921 | FRI-Egirdir |
| 35 | *M. domestica 'UC'* | 1270 | S. Chelek | 79 | *M. domestica* 'Yenice' | 921 | FRI-Egirdir |
| 36 | *M. domestica 'UC'* | 1271 | S. Chelek | 80 | *M. domestica* 'Demir' | 921 | FRI-Egirdir |
| 37 | *M. sieversii* | 1274 | S. Chelek | 81 | *M. domestica* 'Red Chief' | 921 | FRI-Egirdir |
| 38 | *M. domestica 'UC'* | 1274 | S. Chelek | 82 | *M. baccata* | 921 | FRI-Egirdir |
| 39 | *M. domestica 'UC'* | 1277 | S. Chelek | – | – | – | – |

1-69: Collected from Kyrgyzstan; 71-82: Collected from FRI, Egirdir, Turkey
*UC* Unknown cultivar; *C. Cangag* Galgiz Cangag; *K. Alma* Kara Alma; *S. Chelek* Sary Chelek; *FRI* Fruit Research Institute

a higher variation was observed in apples with POGP (peroxidase gene-based polymorphism) markers (Gulsen et al. 2010). All materials used in this study were genetically separated from each other. The apple species used were generally placed in a mixed fashion with the other materials in the dendrogram. Apart from some exceptions, standard *M. domestica* species supplied from Kyrgyzstan and Turkey were placed in a different group from the wild apple species (Group A). While mostly the genotypes of wild species were placed in group B of the dendrogram, small number of *M. domestica* species was placed in this group.

In group A of the dendrogram, three *M. domestica* species ('Aygul' (63), 'Kyrgysky Zimny' (54) and one unknown (24) cultivar) supplied from Kyrgyzstan form a sub-group with a similarity level of 0.74 and placed the farthest from the others. *M. baccata* (82) and *M. pruni-*

**Table 2** List of ISSR and SRAP primers and their total fragment number (TFN), polymorphic fragent number (PFN), polymorphism ratio (PR) and PIC value

| *ISSR Primers* | *TFN* | *PFN* | *PR (%)* | *PIC* |
|---|---|---|---|---|
| (CAC)3GC | 7 | 7 | 100 | 0.34 |
| (AG)8T | 3 | 0 | 0 | 0.00 |
| (TCC)5RY | 2 | 1 | 50 | 0.01 |
| (GACA)4 | 3 | 3 | 100 | 0.04 |
| BDB(CA)7C | 3 | 0 | 0 | 0.00 |
| HVH(CA)7T | 8 | 5 | 63 | 0.05 |
| (GA)8YG | 5 | 4 | 80 | 0.15 |
| VHV(GT)8G | 7 | 7 | 100 | 0.33 |
| (GAA)6 | 8 | 8 | 100 | 0.23 |
| (CT)8TG | 7 | 6 | 86 | 0.20 |
| (CA)6AC | 12 | 11 | 92 | 0.23 |
| (AGC)6G | 6 | 5 | 83 | 0.15 |
| *Mean* | 5.9 | 4.8 | 71.1 | 0.15 |
| *Total* | 71 | 57 | – | – |
| *SRAP Primers* | *TFN* | *PFN* | *PR (%)* | *PIC* |
| Em10/Me8 | 10 | 9 | 90 | 0.22 |
| Em11/Me2 | 4 | 4 | 100 | 0.27 |
| Em11/Me6 | 5 | 5 | 100 | 0.25 |
| Em12/Me3 | 3 | 2 | 67 | 0.04 |
| Em12/Me8 | 2 | 1 | 50 | 0.11 |
| Em2/Me4 | 5 | 5 | 100 | 0.38 |
| Em2/Me5 | 3 | 2 | 67 | 0.18 |
| Em2/Me6 | 3 | 3 | 100 | 0.21 |
| Em3/Me4 | 3 | 2 | 67 | 0.04 |
| Em4/Me5 | 4 | 4 | 100 | 0.23 |
| Em6/Me4 | 5 | 5 | 100 | 0.23 |
| Em6/Me6 | 4 | 3 | 75 | 0.35 |
| Em7/Me2 | 5 | 5 | 100 | 0.13 |
| Em8/Me4 | 4 | 3 | 75 | 0.18 |
| Em9/Me4 | 5 | 4 | 80 | 0.23 |
| Em9/Me4 | 3 | 2 | 67 | 0.19 |
| *Mean* | 4.3 | 3.7 | 83.5 | 0.2 |
| *Total* | 68 | 59 | – | – |

*folia* (78) species were distinctively separated from the other materials and grouped together. Zhou and Li (2000) recommended that *M. prunifolia* was a hybrid between *M. sieversii* and *M. baccata.* Savelyeva and Kudryavtsev (2015) carried out an AFLP study with several apple species but without *M. prunifolia* and identified *M. baccata* in a different group from *M. domestica, M. sieversii, M. niedzwetzkyana* and *M. sylvestris* species. It was also reported that *M. baccata* and *M. prunifolia* had a common haplotype and shared the same maternal generation (Volk et al. 2015 cited in Gao et al. 2015). Two *M. kirghisorum* genotypes and one *M. niedzwetzkyana* genotype together formed a small cluster. These three genotypes were taken from Karaalma section of Kyrgyzstan and trees were close to each other. Of 34 *M. kirghisorum* genotypes, only 3 were placed in group A and 31 were placed in group B. Similarly, 3 of 4 *M. niedzwetzkyana* genotypes were also placed in group B. In group A, 5 Kyrgyz cultivars 'Biskek Kanat' (58), 'Colpanbay' (60), 'Biskek' (59), 'Suzak Sarisi' (56) and 'Rashida' (57) and the international cultivar 'Redchief' (81) supplied from Fruit Research Institute-Egirdir, Turkey formed the same sub-group. The last sub-group of group A was formed with Turkish ('Orak' (73), 'Huryemez' (72), 'Tavsanbasi' (75)) and international cultivars 'G. Delicious' (77), 'Fuji' (76), two Kyrgyz cultivars ('Zimny' (52) and one unknown (39) cultivar) and one *M. kirghisorum* (12) genotype. Herein, Turkish, Kyrgyz and other international apple cultivars were not grouped separately from each other and generally grouped in a mixed fashion. Gharghani et al. (2009) carried out a study with SSR markers and identified old apple cultivars from different regions of the world in the same group. Similarly, Uzun et al. (2016) grouped Turkish and international cultivars in a mixed fashion. On the other hand, Pereira Lorenzo et al. (2008) identified differences between Spanish and non-Spanish apple cultivars. Gasi et al. (2010) also indicated that except for some species, Bosnia and Herzegovinian cultivars separated from the foreign cultivars.

Several *M. kirghisorum* genotypes, 4 *M. sieversii* genotype, 3 *M. niedzwetzkyana* and some *M. domestica* species were placed in group B of the dendrogram. The materials in this group formed several sub-groups. Two *M. kirghisorum* genotypes (14, 47) were placed in a separate subgroup. 'Alma Ata Aport' (65) (*M. domestica*) cultivar and *M. sylvestris* (74) were placed in the same small cluster. In a previous study, a high level haplotype sharing was identified between *M. sylvestris* and *M. domestica* and an interspecific gene flow was assumed between them (Coart et al. 2006). In an AFLP study of Savelyeva and Kudryavtsev (2015), 4 *M. sylvestris* genotype shared the same sub-group with *M. domestica* species. The researchers pointed out that *M. sylvestris* had contribution for the formation of several Russian apple cultivars. The other sub-group in group B was composed of 3 Turkish cultivars 'Demir' (80), 'Yenice' (79) and 'Daldabir' (71), Kyrgyzstan-originated 5 cultivars 'Akalma' (68), 'Rozmarin' (61), 'Smirenko' (62), 'Sabran' (69) and one unknown (40) cultivar, 3 *M. kirghisorum* (19, 20, 42) genotypes and one *M. niedzwetzkyana (*41) genotype. In group B, two *M. kirghisorum* (18, 27) genotypes and one *M. kirghisorum* (17) genotype were grouped separately. Another sub-group of group B was composed of 3 *M. domestica* cultivars 'Sinap' (64), and two unknown (36, 38) cultivars and 2 *M. kirghisorum* genotypes (43, 44). All of the materials in this sub-group were taken from Sary Chelek region.

In group B, 4 *M. sieversii* genotypes (30, 31, 33, 37), one *M. niedzwetzkyana* (32) and one *M. kirghisorum* (11)

**Table 3** ISSR and SRAP primers studied, their estimated allele frequency (*p* and q), number of effective alleles (Ne), Shannon's information index (I), expected (He) and unbiased expected heterozygosity (uHe)

| *ISSR Primers* | *p* | *q* | *Ne* | *I* | *He* | *uHe* |
|---|---|---|---|---|---|---|
| (CAC)3GC | 0.29 | 0.71 | 1.57 | 0.49 | 0.33 | 0.33 |
| (AG)8T | 1.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| (TCC)5RY | 0.50 | 0.50 | 1.01 | 0.02 | 0.01 | 0.01 |
| (GACA)4 | 0.34 | 0.66 | 1.02 | 0.05 | 0.02 | 0.02 |
| BDB(CA)7C | 1.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| HVH(CA)7T | 0.80 | 0.20 | 1.16 | 0.14 | 0.09 | 0.09 |
| (GA)8YG | 0.73 | 0.27 | 1.44 | 0.42 | 0.27 | 0.28 |
| VHV(GT)8G | 0.43 | 0.57 | 1.66 | 0.54 | 0.37 | 0.37 |
| (GAA)6 | 0.34 | 0.66 | 1.42 | 0.40 | 0.26 | 0.26 |
| (CT)8TG | 0.18 | 0.82 | 1.20 | 0.28 | 0.15 | 0.16 |
| (CA)6AC | 0.27 | 0.73 | 1.35 | 0.32 | 0.21 | 0.21 |
| (AGC)6G | 0.56 | 0.44 | 1.32 | 0.33 | 0.21 | 0.21 |
| Mean | 0.54 | 0.46 | 1.26 | 0.25 | 0.16 | 0.16 |
| *SRAP Primers* | *p* | *q* | *Ne* | *I* | *He* | *uHe* |
| Em6/Me4 | 0.40 | 0.60 | 1.48 | 0.43 | 0.28 | 0.28 |
| Em9/Me4 | 0.57 | 0.43 | 1.52 | 0.44 | 0.30 | 0.30 |
| Em4/Me5 | 0.48 | 0.52 | 1.70 | 0.54 | 0.38 | 0.38 |
| Em2/Me5 | 0.55 | 0.45 | 1.36 | 0.34 | 0.22 | 0.23 |
| Em8/Me4 | 0.51 | 0.49 | 1.24 | 0.21 | 0.13 | 0.14 |
| Em/2/Me4 | 0.34 | 0.66 | 1.69 | 0.56 | 0.38 | 0.39 |
| Em9/Me4 | 0.53 | 0.47 | 1.61 | 0.48 | 0.33 | 0.33 |
| Em11/Me2 | 0.54 | 0.46 | 1.74 | 0.60 | 0.41 | 0.42 |
| Em3/Me4 | 0.55 | 0.45 | 1.30 | 0.31 | 0.19 | 0.20 |
| Em2/Me6 | 0.55 | 0.45 | 1.36 | 0.34 | 0.22 | 0.23 |
| Em6/Me6 | 0.48 | 0.52 | 1.65 | 0.51 | 0.36 | 0.36 |
| Em7/Me2 | 0.75 | 0.25 | 1.36 | 0.42 | 0.25 | 0.26 |
| Em10/Me8 | 0.35 | 0.65 | 1.60 | 0.50 | 0.34 | 0.34 |
| Em11/Me6 | 0.44 | 0.56 | 1.65 | 0.53 | 0.36 | 0.37 |
| EM12/Me3 | 0.70 | 0.30 | 1.50 | 0.49 | 0.31 | 0.32 |
| Em12/Me8 | 0.73 | 0.27 | 1.21 | 0.28 | 0.16 | 0.16 |
| Mean | 0.53 | 0.47 | 1.50 | 0.44 | 0.29 | 0.29 |

genotype were placed in the same cluster. *M. sieversii* was reported as the primary progenitor for domesticated apples (Robinson et al. 2001; Harris et al. 2002; Zhang et al. 2009). Savelyeva and Kudryavtsev (2015) in an AFLP study, identified *M. sieversii* genotypes within the same sub-group. On the other hand, Omesheva et al. (2015) identified genetic differences among *M. sieversii* populations collected from five different geographical regions of Kazakhstan. Current *M. sieversii* genotypes were taken from Sary Chelek regions of Kyrgyzstan. Probably the genotypes from different regions may present different groupings. While one of 4 *M. niedzwetzkyana* genotypes (32) shared the same group with *M. sieversii,* the others were placed in different sub-groups of the dendrogram. Some researchers assess *M. niedzwetzkyana* as a separate species (Omesheva et al. 2015) and some others indicate it as a sub-species of *M. sieversii* (Wang et al. 2015). Harris et al. (2002) in a nuclear ribosomal internal transcribed spacer gene (ITS) study, grouped some of *M. niedzwetzkyana* and *M. sieversii* materials together in the same group and placed some others in different groups. It was stated that *M. niedzwetzkyana* and *M. sieversii* were closely related species, but not the same species (Omesheva et al. 2015). Current findings also support this idea.

Another sub-group of that main group included 6 *M. kirghisorum* (6, 13, 45, 46, 48, 50) and 2 *M. domestica* (49, 51) genotypes. On the other hand, 11 *M. kirghisorum* (15, 16, 21, 22, 23, 26, 28, 29, 34, 4, 5) genotypes collected from different regions, 2 unknown *M. domestica* genotypes (25, 35) and one *M. niedzwetzkyana* (9) genotype formed a separate cluster. The last sub-group of the dendrogram was composed of 3 *M. kirghisorum* (1, 2, 3) genotypes. In the dendrogram, *M. kirghisorum* and *M. domestica* genotypes were placed in mixed fashion in small groups. Again, majority

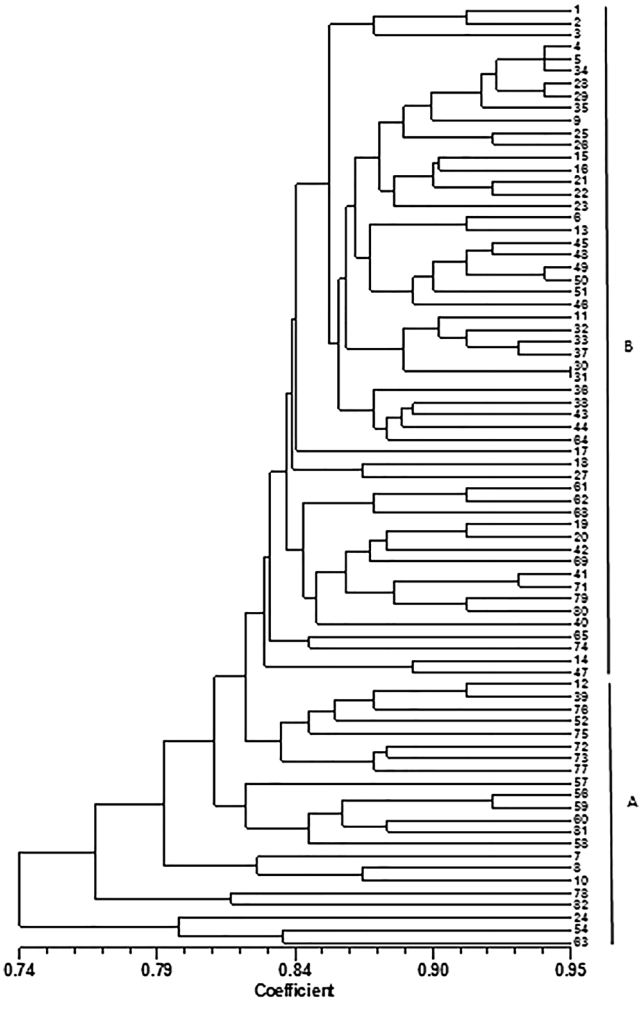



**Fig. 3** UPGMA cluster obtained from ISSR and SRAP data showing the relationships in apple species (Names of the accessions are in Table 1)

of them were placed in close sub-groups with *M. sieversii*, a genotype of *M. kirghisorum*. Some researchers assessed *M. kirghisorum* as a separate species but closely related with *M. sieversii*. It was reported that habitat of these two species coincided and heterogenetic populations existed in close fields (Dzhangaliev 2003; Volk et al. 2009). These two species do not exist in nature as isolated from each other and mostly exist in mixed fashion and therefore there may be natural hybrids of these two species in nature (Volk et al. 2009). Current findings also revealed these two species as closely related species. Such a case may be better explained by using more *M. sieversii* genotypes collected from different regions.

## Conclusions

In present study, the relationships of three important apple species of Kyrgyzstan (*M. sieversii, M. kirghisorum, M. niedzwetzkyana)* with each other and with domesticated apples (*M. domestica)* were investigated. A significant diversity was observed among investigated materials. A genetic diversity was also observed even among the genotypes of the same region. These apples also exhibited phenotypic (fruit shape, color, tree form etc.) variations (data not shown). Similarly, significant morphological differences were reported within *M. sieversii* genotypes of Kyrgyzstan (Dzhangaliev 2003) and within *M. orientalis* genotypes of northern Caucasia region (Hofer et al. 2013). In this case, preservation and development strategies should be created especially for endangered species included in red list like *M. niedzwetzkyana*. In present study, a significant variation was observed within *M. niedzwetzkyana*. This diversity should be preserved and used in improving the diversity and breeding of the other species. The *M. kirghisorum*, also known as Kyrgyz apple, also harbors a significant diversity in itself. In other countries with natural spread of apple species like Kyrgyzstan, urgent measures should be taken for the preservation of this rich diversity. Wild species are lost either naturally or with anthropogenically (Eastwood et al. 2009; Zhang et al. 2009; Wang et al. 2015). Besides diversity preservation (*ex situ*/*in situ*), detailed characterization and breeding programs should also be focused on in future researches.

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**Conflict of interest** A. Uzun, K. Turgunbaev, A. Abdullaev, H. Pınar, S. Ozongun, A. Muratbekkızı, M. Badyrov, A.İ. Ilbas, K. Gürcan and S. Kaymak declare that they have no competing interests.

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