International Journal of Agricultural and Natural Sciences
E-ISSN: 2651-3617 14(1): 7-15, 2021
Research Article

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF HYBRID INDIVIDUALS OBTAINED BY INTERSPECIES HYBRIDIZATION (*Prunus armeniaca* × *Prunus salicina*)

Mehmet Yaman and Aydın Uzun
[1]*Erciyes University, Faculty of Agriculture, Department of Horticulture, Kayseri/TURKEY*

**Corresponding Author:*
E-mail: mhmt.-07@hotmail.com

(Received 25th December 2020; accepted 12th April 2021)

ABSTRACT. The production of apricot increases annually worldwide. The biggest reason for this increase is the development of new varieties suitable for the market's demands as a result of apricot breeding studies besides the apricot fruit having different usage areas. Although the chance of success is very low, interspecies hybridization technique is one of the breeding methods used to create variation in apricot. In this study, the genetic variation of 18 hybrid individuals obtained as a result of the inter-species breeding study in apricot (*Prunus armeniaca*) and plum (*Prunus salicina*) species and their parents have been defined by SRAP marker technique and morphologic data. 101 scored bands were obtained from 15 different marker combinations used in the study. 79 of them were polymorphic and the average polymorphism value was determined as 75.97%. The similarity index in the dendrogram varied between 0.64 and 0.92. In terms of morphological parameters, no differences could be determined according to the parents of the hybrid individuals due to their young age. The results of this study may lead to new studies in the future of the use of new candidates' varieties and rootstock combined with new studies that include determining the fruit characteristics of these genotypes.

Keywords: *interspecies hybridization, SRAP, apricot, plum*

INTRODUCTION

Apricot world production is approximately 4 million tons according to 2019 statistics, and Turkey has the first place of world apricot production with 846 606 tons in the year [1]. Although late spring frosts create a risk for apricot [2, 3], world apricot production increases year by year [4]. The reasons of this increase are due to the development of new varieties obtained in relation to the market's demands and consumers, in addition to Apricot breeding studies carried out in different countries and different usage areas. [5]. The purpose of created breeding programs is to develop new varieties in Apricot and to bring the desired characters in a fast and reliable way together. Although there is a wide variation within the species in apricot, the breeding period is prolonged due to the high degree of heterozygosity. In recent years, apricot breeding programs have focused on earliness [6], incompatibility [7], and resistance to the shark virus [8]. Furthermore, since ecology has an effect on fruit quality and yield in most apricot species, it also affects the improvement adaptation to soil conditions, especially in late spring frosts.

In apricot breeding programs, hybridization and selection breeding methods are the most widely used to develop new species [9]. New apricot varieties with fruit size, early, high SSC content have been developed by hybridization breeding (especially intra-species hybridization) [10, 11]. Although the opportunity of success is low, interspecies hybridization studies are among the breeding methods used flesh and skin color of apricot in recent years. In this way, new interspecies hybrid varieties such as "Pluot" were developed [12, 13].

For years, apricot varieties have been evaluated by different researchers with molecular, morphological, biochemical markers and genetic variations are determined by these techniques [14, 15, 16]. Due to environmental conditions that affect the morphological and biochemical structure, molecular descriptions are the most reliable among these marker systems. Molecular marker systems breeding programs are very useful in determining genetic diversity and detecting differences between varieties. One of these markers, SRAP marker system, it is simple, efficient system that can be adapted for a variety of purposes in different crops, has reasonable throughput rate, discloses numerous co-dominant markers, targets open reading frames (ORFs), and allows easy isolation of bands for sequencing.

The main aim of this study is to determine the morphological and genetic diversity in hybrid individuals obtained via interspecies hybridization by using the SRAP marker system.

MATERIALS AND METHODS

Material

The study material consists of interspecies hybrid genotypes. These genotypes have been obtained from the breeding study made with hybridization interspecies in apricot varieties such as Ninfa, Palstein and Proce de Tyrinthe (mother parent), and plum varieties such as Black Splendor, Black Diamond and Black Amber (father parent), which are located in the Apricot Collection Garden of Alata Horticultural Research Institute in 2018 and 2019. Hybrid individuals are 1 and 2 years old and are located in the Fruit Collection Parcel of Erciyes University.

Method

Molecular Marker Analysis

DNA isolation was performed on young leaves and the CTAB method was used [17]. DNA concentrations of genotypes were measured with a spectrophotometer (BioTek Instruments, Inc., Winooski, VT, United States) and DNA samples were prepared in TE (10 mMTris-HCl, 0.1 mM EDTA, pH 8.0) solution and kept at -20 ° C until use.

PCR Analysis

The DNA samples were mixed (bulk), and pre-screening was performed with 216 SRAP primer combinations as 12 forward and 18 reverse. As a result of these tests, 15 SRAP marker combinations giving the most polymorphic bands were used (Table 2). PCR components and cycles were arranged in accordance with the method specified by [18]. PCR products were run in 2% agarose gel at 100 volts for 2-3 hours. To determine band widths, 100 bp DNA ladder was used. Resultant bands were imaged under UV light [19, 20].

Data Analysis

Number (1) is given when the bands obtained from the image of the gels after the imaging process, and (0) is given in the absence of the bands. If there was no

amplification, the scores were recorded by giving numbers (9). The data obtained were analyzed using NTSYSpc 2.1 computer package program [21]. Similarity index between individuals were identified [22]. Dendrogram of hybrid genotypes were created according to UPGMA method based on the DICE similarity matrix. The distance between the genotypes in the created DICE similarity matrix was determined by calculating the matrix values in the OUTPUT module. Also, total band number, polymorphic band number and polymorphism ratio were determined for each marker used in this study. While calculating the rate of polymorphism, the formula (Polymorphic Number of Bands * 100 / Total Number of Bands) was used.

Morphological Analysis

To determine some morphological characteristics of Apricot × Plum hybrid individuals obtained from 2018 and 2019 studies, the TG / 56/4 (proj.3) coded UPOV criteria, which renewed on 15 August 2011, and used. Although it is very difficult to evaluate some parameters in 1- and 2-year-old hybrid plants, these features were included in this study to determine the difference that emerged in the first two years.

RESULTS AND DISCUSSION

In the SRAP marker analysis performed to determine the genetic relationship of inter-species hybrid individuals and their parents, the base lengths of the markers varied between 90 bp and 1900 bp. A total of 101 scorable bands were obtained, 79 of these bands were identified as polymorphic. The highest scorable band number was obtained from Em11-Me12 primer combination with 13 bands in total, 11 of which were polymorphic. In this study, the average number of bands per marker was 6.73, and the average number of polymorphic bands per marker was 5.26. The average percentage of polymorphism was determined as 75.97% (**Table 1**).

The SRAP marker system was repeatability and highly polymorphic in Prunus armaniaca [23]. In a study conducted with the SRAP marker system on apricot species, the average number of bands per marker was 5.4, the number of polymorphic bands per marker was 3.9, and the average percentage of polymorphism was 73.0% [24]. In another related study, the genetic relationships of 196 different apricot varieties were determined. The average number of bands per marker was 13.1, the number of polymorphic bands per marker was 10.4, and the average polymorphism rate was 79.6% [25]. The current study in this aspect has similar to studies conducted by [25] and [26].

Table 1. *SRAP primer combinations used in Apricot × Plum hybrid plants, base length, total number of bands, number of polymorphic bands and polymorphism ratio*

Primer	Base Length	Total Number of Bands	Number of Polymorphic Bands	Polymorphism Rate %
Em1-Me2	90-200	4	3	75.00
Em1-Me4	100-1050	8	6	75.00
Em2-Me3	50-450	7	6	85.71
Em4me4	80-500	6	5	83.33
Em4-Me9	100-950	5	4	80.00
Em6-Me4	100-600	6	4	66.67
Em6-Me6	90-550	8	5	62.50
Em7-Me12	200-250	3	2	66.67
Em5-Me7	600-800	2	1	50.00
Em7-Me9	150-700	9	8	88.89
Em10-Me9	250-1500	9	7	77.78
Em11-Me12	130-1900	13	11	84.62
Em14-Me4	90-700	6	5	83.33
Em8-Me8	150-800	10	8	80.00
Em9-Me6	160-500	5	4	80.00
Mean	**90-1900**	**6.73**	**5.26**	**75.97**
Total	**-**	**101**	**79**	**-**

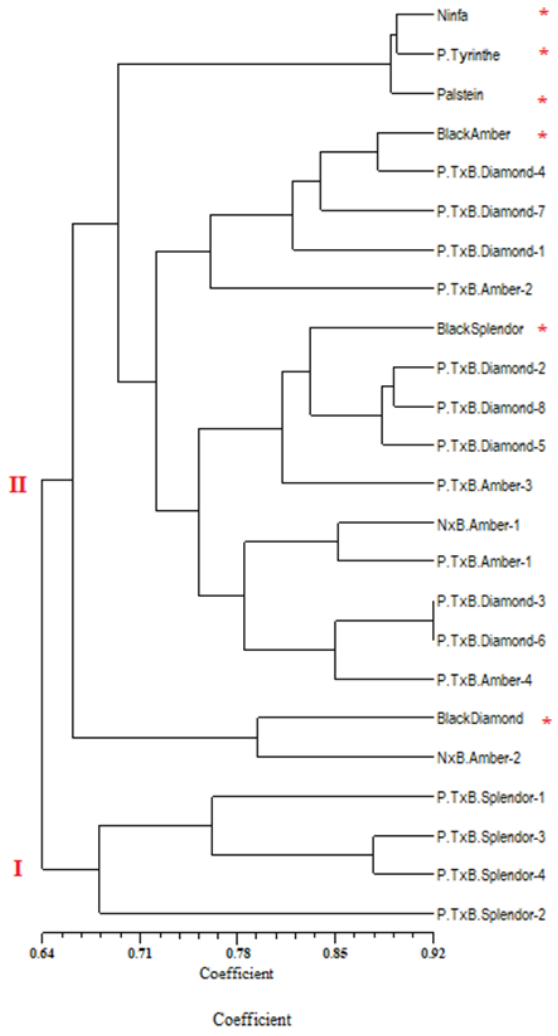

Fig. 1. *UPGMA dendrogram based on SRAP markers in hybrid plants*

In the dendrogram created according to the UPGMA method, the similarity index varied between 0.64 and 0.92 (**Fig 1**). A study conducted in Turkey aimed to determine with SRAP markers of genetic diversity in apricot genotypes. 19 different primer combinations were used in 57 apricot genotypes (presumed to be grown from seed) collected from Sakıt valley and the similarity index between the genotypes was between 0.75 and 0.94 [28]. In another study on apricot, the genetic similarity index varied between 0.62 and 0.83 among local apricot varieties grown in the region in Northern China [4] On the other hand, a study of SRAP, ISSR, and DAMD marker analyzes were conducted on some local and foreign varieties and their hybrid individuals. The similarity index varied between 0.65 and 0.87 [25]. In terms of the results obtained, our current study is similar to these studies mentioned in the literature.

In the current study, 2 main groups were formed in the dendrogram obtained with SRAP markers. Only Procede Tyrinthe x Black Splendor hybrids were included in the first main group. In the second main group, the parents used in the study and other hybrid individuals were included. Genetically closest genotypes have a similarity index of 0.92. and these genotypes were individuals numbered 3 and 6 obtained as a result of Proce de Tyrinte x Black Diamond hybridization. Also, In this study, the individuals (parents)

whose similar to each other were grouped. the used parents as both mother and father were grouped also in different places. It is thought that, in such cases may be caused by the differences that occur due to the crossing ovary occurred during fertilization.

It was determined as tree vigor (weak), tree habit(upright), tree: degree of branching(weak), leaf blade length (medium), leaf blade width(medium), leaf blade / leaf width(medium), leaf blade: intensity of green color of upper side(light), one-year-old shoot: size of bud(small), leaf blade: shape of base(truncate), leaf blade: length of tip(long), leaf: ratio length of blade /length of petiole(high), petiole: length(short), petiole: thickness(thin), petiole: anthocyanin coloration of upper side(weak), young shoot: anthocyanin coloration(medium) in individuals obtained by crossing different apricot and plum varieties (**Table 2**). These morphological measurements and observations in hybrid plants were made in plants at a very young age of 1 and 2 years, and these characteristics may change in the morphological parameters depending on the genetic structure and ecology in the following years.

A study was conducted to determine the plant characteristics of some apricot varieties and genotypes cultivated in Malatya region. According to the findings, it has been reported by researchers as tree: vigor (very weak, weak and medium) and tree: habit (drooping, upright to spreading, upright, right upright) [27]. Anthocyanin accumulation in leaves was identified as "weak", "Medium" and "Strong" [28].

The aim of this study was to show the SRAP marker system and morphological of genetic diversity in hybrid individuals obtained from inter-species hybridization using different apricot and plum varieties. In this study, a high level of polymorphism was obtained with SRAP molecular marker system in hybrid individuals. This result shows that the SRAP marker system can be useful in determining genetic variation among individuals. In some morphological parameters, significant morphological differences did not occur between hybrid individuals, this case is thought to arise depending on the age of the plants. In addition, it may be a guide for the use of these hybrid individuals for the development of new varieties or rootstock candidates by combining them with new studies including determining the fruit characteristics.

Table 1. *Some morphological properties of Apricot × Plum hybrid plants bands and polymorphism ratio*

Combination	Tree: vigor	Tree: Habit	Tree: degree of branching	Leaf blade length	Leaf blade width	Leaf blade / leaf width	Leaf blade: intensity of green color of upper side	One-year-old shoot: size of bud	Leaf blade: shape of base	Leaf blade: length of tip	Leaf: ratio length of blade /length of petiole	Petiole: length	Petiole: thickness	Petiole: anthocyanin coloration of upper side	Young shoot: anthocyanin coloration
Ninfa	Weak	Upright to spreading	Strong	Short	Narrow	Low	Medium	Medium	Truncate	Medium	Medium	Short	Medium	Weak	Weak
Proce de Tyrinthe	Medium	Upright to spreading	Strong	Short	Narrow	Low	Medium	Medium	Truncate	Medium	Medium	Medium	Medium	Weak	Medium
Palstein	Strong	Drooping	Strong	Short	Narrow	Low	Medium	Medium	Truncate	Medium	Medium	Medium	Medium	Weak	Strong
Black Splendor	Medium	Upright	Medium	Long	Medium	High	Dark	Medium	Truncate	Medium	High	Short	Medium	Medium	Medium
Black Diamond	Medium	Upright	Medium	Long	Medium	High	Dark	Medium	Truncate	Medium	High	Short	Medium	Medium	Medium
Black Amber	Medium	Upright	Medium	Long	Medium	High	Dark	Medium	Truncate	Medium	High	Short	Medium	Medium	Medium
NinfaxAmber-1	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
NinfaxAmber-2	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x Diamond-1	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x Diamond-2	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x Diamond-3	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x Diamond-4	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x Diamond-5	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x Diamond-6	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x Diamond-7	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x Diamond-8	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x B. Amber-1	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x B. Amber-2	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x B. Amber-3	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x B. Amber-4	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x B. Splendor-1	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x B. Splendor-2	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x B. Splendor-3	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium
P. Tyrinthe x B. Splendor-4	Weak	Upright	Weak	Medium	Medium	Medium	Light	Small	Truncate	Long	High	Short	Thin	Weak	Medium

CONCLUSION

The aim of this study was to show the SRAP marker system and morphological of genetic diversity in hybrid individuals obtained from inter-species hybridization using different apricot and plum varieties. In this study, a high level of polymorphism was obtained with SRAP molecular marker system in hybrid individuals. This result shows that the SRAP marker system can be useful in determining genetic variation among individuals. In some morphological parameters, significant morphological differences did not occur between hybrid individuals, this case is thought to arise depending on the age of the plants. In addition, it may be a guide for the use of these hybrid individuals for the development of new varieties or rootstock candidates by combining them with new studies including determining the fruit characteristics.

Acknowledgement. (This work has been supported by Erciyes University Scientific Research Projects Coordination Unit under Grant number of FDK-2018-8044)

REFERENCES

[1] FAO. (2017): Food and Agriculture Organization of the United Nations. "Crops data", http://www.fao.org/faostat/en/#data.

[2] Bartolini, S., Massai, R., Iacona, C., Guerriero, R., Viti, R. (2019): Forty-year investigations on apricot blooming: Evidence of climate change effects. Scientia Horticulturae 244: 399-405.

[3] Campoy, J. A., Audergon, J. M., Ruiz, D., & Martínez-Gómez, P. (2020): Genomic Designing for New Climate-Resilient Apricot Varieties in a Warming Context. In Genomic Designing of Climate-Smart Fruit Crops (pp. 73-89). Springer, Cham.

[4] Pinar H, Ercişli S, Bircan M, Ünlü M, Uzun A, Yılmaz, K.U., Yaman, M. (2017): Morphological, Molecular, and Self-(In) Compatibility Characteristics of New Promising Apricot Genotypes. Journal of Agricultural Science and Technology 19: 365-376.

[5] Pérez-Pastor, A., Ruiz-Sánchez, M. C., Domingo, R. (2014): Effects of timing and intensity of deficit irrigation on vegetative and fruit growth of apricot trees. Agricultural water management 134: 110-118.

[6] Ruiz, D., García-Gómez, B. E., Egea, J., Molina, A., Martínez-Gómez, P., Campoy, J. A. (2019): Phenotypical characterization and molecular fingerprinting of natural early-flowering mutants in apricot (*Prunus armeniaca* L.) and Japanese plum (*P. salicina* Lindl.). Scientia Horticulturae 254: 187-192.

[7] Tsering Dolkar, M. A., Slathia, D., Wani, A.W. (2018): Description of apricot flower and its implications in breeding. Journal of Pharmacognosy and Phytochemistry 7(2): 3721-3728.

[8] Mitrofanova, I. V., Mitrofanova, O. V., Lesnikova-Sedoshenko, N. P., Chelombit, S.V., Gorina, V. M., Chirkov, S. N. (2019): Some features of obtaining new breeding forms of apricot in vitro. In XVII International Symposium on Apricot Breeding and Culture 1290 (pp. 237-242).

[9] Yaman, M., Uzun, A. (2020): Evaluation of Superior Hybrid Individuals with Intra and Interspecific Hybridization Breeding in Apricot. International Journal of Fruit Science *20*(sup3), S2045-S2055.

[10] Ercisli, S. (2009): Apricot culture in Turkey. Sci. Res. Essays 4(8): 715-719.

[11] Bircan, M., Pinar, H., Yilmaz, C., Yildiz, A., Paydaş, S., Kaşka, N. (2010): The Apricot Breeding Program among Some Turkish and Foreign Cultivars. Acta Hort 862: 103-108.

[12] Urbanovich, O. Y., Kuzmitskaya, P. V., Kilchevsky, A. V. (2017): Identification and genetic diversity of plum cultivars grown in Belarus. Russian Journal of Genetics 53(7): 775-784.
[13] Numaguchi, K., Akagi, T., Kitamura, Y., Ishikawa, R., Ishii, T. (2020): Interspecific introgression and natural selection in the evolution of Japanese apricot (*Prunus mume*). The Plant Journal 104(6), 1551-1567.
[14] İmrak, B., Küden, A., Yurtkulu, V., Kafkas, E., Ercişli, S., Kafkas, S. (2017): Evaluation of some phenological and biochemical characteristics of selected new late flowering dried apricot cultivars. Biochemical Genetics 55(3): 234-243.
[15] Li, W., Liu, L., Wang, Y., Zhang, Q., Fan, G., Zhang, S., Liao, K. (2020): Genetic diversity, population structure, and relationships of apricot (*Prunus*) based on restriction site-associated DNA sequencing. Horticulture Research 7(1): 1-13.
[16] Ganji Moghadam, E. (2020): Comparison of Phenological, Morphological and Pomological Characteristics of 7 Apricot Promising Genotypes in Khorasan Razavi ProvThin. Journal of Horticultural Science 34(3): 0.
[17] Doyle, J. J., Doyle, J. L. (1990): Isolation of plant DNA from fresh tissue. Focus 12(13): 39-40.
[18] Uzun, A., Coşkun, Ö. F., Yaman, M., Pınar, H., Paris, K. (2017): Identification of Genetic Similarities among Walnut (*Juglans regia* L.) Genotypes Selected from Central Anatolia Region of Turkey with SRAP Markers. Alatarım 16(1): 26–34.
[19] Uzun, A., Yesiloglu, T., Aka-Kacar, Y., Tuzcu, O., & Gulsen, O. (2009). Genetic diversity and relationships within *Citrus* and related genera based on sequence related amplified polymorphism markers (SRAPs). Scientia horticulturae 121(3): 306-312.
[20] Uzun, A., Çil, A., Yaman, M., Coşkun, Ö. F. (2020): Genetic Diversity and Some Fruit Characteristics of Quins Genotypes Collected from Kayseri Region. Turkish Journal of Agriculture-Food Science and Technology 8(2): 318-323.
[21] Rohlf, F. J. (2000): NTSYS 2.1: numerical taxonomic and multivariate analysis system. New York, Exeter Software.
[22] Dice, L. R. (1945): Measures of the amount of ecologic association between species. Ecology 26(3): 297-302.
[23] Ming, L. I., Xia, H. U., Xing-jun, M. I. A. O., Zheng, X.U., Zhong, Z. H. A. O. (2016): Genetic diversity analysis of wild apricot (*Prunus armeniaca*) populations in the ili valley as revealed by SRAP markers. Acta Horticulturae Sinica 43(10): 1980.
[24] Uzun, A., Gulsen, O., Seday, U., Bircan, M., Yilmaz, K. U. (2010): SRAP based genetic analysis of some apricot cultivars. Rom. Biotech. Lett 15: 5396-5404.
[25] Li, M., Zhao, Z., Miao, X. (2014): Genetic diversity and relationships of apricot cultivars in North China revealed by ISSR and SRAP markers. Scientia Horticulturae 173: 20-28.
[26] Pinar, H., Unlu, M., Ercisli, S., Uzun, A., Bircan, M., Yilmaz, K. U., Agar, G. (2013). Determination of genetic diversity among wild grown apricots from Sakit valley in Turkey using SRAP markers. Journal of Applied Botany and Food Quality 86(1): 86:55–58.
[27] Yılmaz, K. U. (2008): Bazı yerli kayısı genotiplerinin fenolojik, morfolojik ve pomolojik özellikleri ile genetik ilişkilerinin ve kendine uyuşmazlık durumlarının moleküler yöntemlerle belirlenmesi. Phd thesis, Cukurova University, Institute of Natural Sciences, Adana. (in Turkish).
[28] Altan, H. (2019): Bazı melez kayısı genotiplerinin fenolojik, morfolojik ve meyve kalite özelliklerinin belirlenmesi. Master thesis. Mustafa Kemal University, Institute of Natural Sciences, Hatay. (in Turkish).