Turkish Journal of Botany
http://journals.tubitak.gov.tr/botany/
Research Article

Turk J Bot
(2023) 47: 331-341

doi:10.55730/1300-008X.2771

Nuclear DNA content and ploidy levels of living apple germplasm collection in Türkiye

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Received: 27.03.2023 • **Accepted/Published Online:** 02.08.2023 • **Final Version:** 26.09.2023

Abstract: One of the key uses of flow cytometry is the estimation of nuclear DNA content and ploidy level, which has proven a reliable and effective approach in many studies. The primary goal of this research is to estimate the nuclear DNA content and ploidy level of the apple genetic resource collection maintained in the Fruit Research Institute's living *Malus* collection for the first time using flow cytometry (for autochthonous varieties). Fresh apple leaf tissues were used for the flow cytometry analysis. For each genotype, nuclear DNA analysis was performed on three individual plants. Propidium iodide (PI) is used as a fluorochrome. Common vetch (3.65 pg/2C) was used as an internal standard. The 2C nuclear DNA content ranged from 1.46 pg to 2.45 pg. The variation in nuclear DNA content within the collection was statistically significant. Apple genotypes were split into two groups, diploid and triploid, according to their nuclear DNA content. Based on these results, 16.47% of apple genotypes were triploid, while 83.53% were diploid. The 2C nuclear DNA content in triploid genotypes varied from 2.04 to 2.45 pg and in diploids from 1.46 to 1.69 pg. The average nuclear DNA content in diploids was 1.56 pg, whereas it was 2.29 pg in triploids. The results of the study will be useful to determine the best strategies in breeding programs, as ploidy is one of the most important characteristics to consider in selecting parents for breeding purposes in addition to their agronomic characteristics.

Key words: *Malus x domestica*, genetic resources, chromosome number, flow cytometry, nuclear DNA content, ploidy

1. Introduction

Most of the commercially produced apple cultivars in the world belong to the *Malus x domestica* in the family Rosaceae. Rosaceae is subdivided into three subfamilies: Dryadoideae, Rosoideae, and Spiraeoideae (Potter et al., 2007). However, in a recent classification, Spiraeoideae was replaced by Amygdaloideae (USDA-ARS, 2019). Within the genus *Malus*, sections *Malus*, *Sorbomalus*, *Eriolobus*, *Docyneopsis*, and *Chloromeles* have been defined. The *Malus* section includes the series *Malus*, *Baccatae*, and *Siebolbianae* (Robinson et al., 2001). Although the actual number of species in the genus *Malus* is unknown, it is estimated that there are 30–78 species (Robinson et al., 2001; Zhou, 1999). It is believed that *M. x domestica* species, which are cultivated throughout the world, emerged as a result of interspecific hybridization (Way et al., 1991; Janick et al., 1996).

Two species of apple, *M. sylvestris* and *M. orientalis*, are widely distributed in Türkiye (Browicz, 1972). According to Büttner (2001), *M. orientalis* emerged in the northern Anatolia, Armenia, a mountain belt in the northern part of Iran, and the Caucasus. *M. sylvestris* var. *microphylla* is an endemic species to Türkiye, with a population that is restricted to Amasya Province (Browicz, 1972). *M. pumila* and *M. trilobata* are also among the local apple species of Türkiye (Way et al., 1991; FAO, 2018). It is possible to see many local varieties or genotypes along with different apple species in almost all geographical regions of Anatolia, which has a rich biodiversity. In Anatolia, where apples have been cultured for many years, a large genetic pool has occurred with natural hybridizations of wild and cultivated species. This pool has become even richer with the varieties brought from outside through introduction (Bakır et al., 2022).

In apples, the basic chromosome number is x = 17. Within the genus, ploidy varies between diploid and pentaploid. The majority of apple varieties are diploid (2x = 34); however, triploid (3x = 51) and tetraploid (4x = 68) species and cultivars are also present (Westwood, 1995; Schuster and Büttner, 1995; Höfer and Meister, 2010). Most of the commercially grown apple varieties, including 'Gala', 'Golden Delicious,' and 'Red Delicious' are diploid. 'Jonagold', 'Sunten', 'Jupiter', 'Boskoop', 'Red Jonaprice', and 'Pagacz' varieties are triploid. The use of triploid varieties in apple production dates back to the 17th century, when natural triploids with high yields and large fruits such as

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'Gravenstein' were selected (Hias et al., 2017). Tetraploid apple varieties or species are uncommon compared to diploid and triploid varieties or species.

Since all of the chromosomes in eukaryotic organisms are found in the cell nucleus, there is a tightly positive relation between nuclear DNA content and ploidy (Tamura et al., 1998; Baird et al., 1994). Therefore, the nuclear DNA amounts are considered an expression of the ploidy level (Lu et al., 1998). The use of flow cytometry to determine ploidy level has been reported in many species such as pear (Sehic et al., 2012), persimmon (Tamura et al., 1998; Şeker et al., 2018), medlar (Rothleutner et al., 2016), apple (Tatum et al., 2005; Podwyszyńska et al., 2016), *Citrus* (Çimen et al., 2022), *Brachypodium distachyon* (Savaş Tuna et al., 2019), St. John's wort (Savaş Tuna et al., 2017), alfalfa (Şakiroğlu and Kaya, 2012), *Medicago monantha* (Zarabizadeh et al., 2022), *Dactylis* L. (Tuna et al., 2004), *Bromus* L. (Tuna et al., 2001), and Rosaceae (Dickson et al., 1992).

Plant genetic resources have great importance since they provide the essential raw material for breeding programs to address present and future demands. The development of new apple varieties with a wider genetic base is possible with the effective use of gene resources by breeders. Changing climatic conditions, new disease pressures, and the need to produce fruit with less chemical input to meet consumer demand will result in the need for new apple varieties with higher levels of resistance or tolerance to abiotic and biotic stresses in the future. Overcoming these challenges depends on maintaining sufficient genetic diversity in gene banks and in nature (Bramel and Volk, 2019). Therefore, genetic diversity provides opportunities for breeders to develop new varieties (Sütçü et al., 2022). However, it is necessary to characterize the genetic resources well before using them in any genetic and breeding studies to determine the best strategies and to get maximum benefit from them with minimum time, cost, and labor. As a result, many aspects of plant genetic resources directly related to the origin, genetics, diversity, unique characteristics, current status, conservation, and possible use in plant breeding need to be identified in multifaceted programs. Ploidy information, in particular, is extremely important basic information needed to determine appropriate strategies in plant breeding programs. As a result, ploidy levels of germplasms varying in ploidy such as apple must be determined before using the germplasm in breeding programs. In this study, we used flow cytometry to determine nuclear DNA content and ploidy levels of apple genotypes maintained at the Fruit Research Institute, Eğirdir, Isparta, Türkiye as a living collection and which have critical importance for apple breeding programs.

2. Material and methods

2.1. Plant material

The study was conducted in the field and laboratories of the Fruit Research Institute (37°49′17.97″N, 30°52′22.44″E) and the Plant Genetics and Cytogenetics Laboratory of the Field Crops Department of Tekirdağ Namık Kemal University (TNKU) using the apple germplasm collection grafted on MM111 apple rootstock. Each apple genotype used in the study is represented by three trees. In total, 340 genotypes and 1020 individual apple trees were used in the investigation. The trees were planted in north-south rows at 2.5 m × 4 m spacing in 2011. The research area is located in a region with a transitional climate between the Mediterranean climate and the continental climate, and the soil has a clay-loam structure. The experimental area was irrigated twice a week by drip irrigation and fertilizers were applied based on the results of soil analysis.

2.2. Sample collection

For flow cytometry analysis, approximately 20–30 mg (1 cm^2) of young, fresh, and disease-free leaf tissues obtained from healthy plants were used. Fresh leaf samples were taken at random from three trees for each genotype between May and August. The leaf samples were wrapped in moist filter papers to maintain moisture and transferred to TNKU in locked freezer bags. The leaf samples were kept at +4 °C until they were used.

2.3. Sample preparation for flow cytometry analyses

Partec commercial kit (CyStain PI Absolute P) was used in the preparation of samples for flow cytometry analysis. Common vetch (3.65 pg/2C), which can be easily and freshly obtained in laboratory, was used as an internal standard in the analyses. Approximately, 20 mg of leaf tissue from both sample and standard plants were placed in a petri dish and chopped with a sharp razor blade in 500 µL of nucleus isolation buffer until the tissue was broken into small pieces (30–60 s). The sample prepared in this way was shaken for 15–20 s in the petri dish and transferred into a tube by passing through a filter (30 µm CellTrics). This ensured that the cell nuclei were separated from other cell remnants that make up the leaf tissue. Two milliliters of the previously prepared dye solution (CyStain PI Absolute P) was added to the tube and incubated in the dark for about 60 min. The samples were run in a flow cytometer [CyFlow® Space flow cytometer (Partec GmbH)].

2.4. Calculation of absolute nuclear DNA content and statistical analyses

In the study, 2C nuclear DNA content for each sample was calculated by analyzing approximately 3000 nuclei. The 2C nuclear DNA content was calculated using the fluorescence intensities of the G1 peaks of the sample and the standard (Figure). The formula used in calculation is as below (Podwyszyńska et al., 2016).

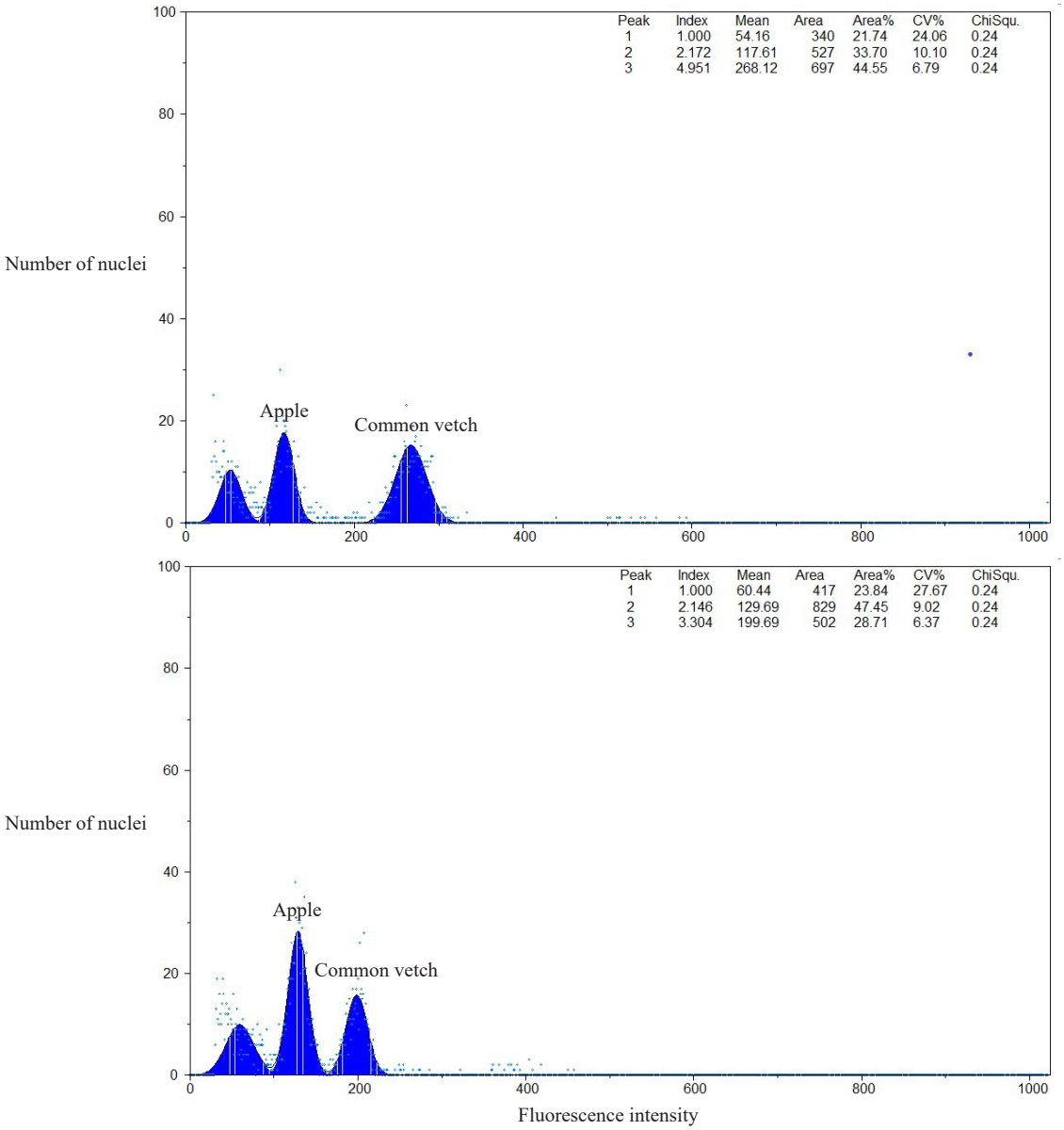

Figure. Flow histograms showing the relative position of G1 peaks of diploid (a) and triploid (b) apple genotypes compared to the G1 peak of common vetch (3.65 pg/2C).

2C Nuclear DNA content (pg) = [Fluorescence intensity of the sample (value of the G1 peak) / Fluorescence intensity of the standard (value of the G1 peak)] × DNA content of the standard (pg).

The standard deviation and confidence interval of each genotype mean were calculated using SPSS software version 26.

3. Results

The 2C nuclear DNA content and ploidy levels of apple genotypes are shown in Table. The 2C nuclear DNA content ranged from 1.46 pg ('Hi, Early Delicious') to 2.45 pg ('Yb-2'). The differences among genotypes were statistically significant. It was possible to separate them into two clearly distinguishable groups based on their 2C nuclear DNA content. The 2C nuclear DNA content within the first group containing 56 varieties varied from 2.04 pg to 2.45 pg. The 2C nuclear DNA content within the second group consisting of 284 apples varied between 1.46 pg and 1.69 pg. While nuclear DNA content values were continuous within the groups, there was a clear gap (0.35 pg) between the two groups (Table).

Apples were classified as diploid or triploid based on the contents of their nuclear DNA. There were 56 triploids

and 284 diploids. A total of 16.47% of the varieties were found to be triploid. Autochthonous apples accounted for 67.85 percent of the triploid group. The average nuclear DNA of these (2.29 pg) was quite similar to the DNA content of foreign cultivars (2.27 pg).

4. Discussion

The nuclear DNA content is species-specific and has a tightly positive correlation with ploidy (Bennett et al., 2000; Höfer and Meister, 2010). However, the nuclear DNA content of angiosperms differs by several orders of magnitude, from 0.0648 pg/1C for *Genlisea margaretae* Hutch to 132.45 pg/1C for *Trillium camschatcense* Ker Gawler (Özkan et al., 2010). In the current study, it was found that the 2C nuclear DNA content of apple genotypes included in the national apple genetic resource collection of Türkiye maintained in the Fruit Research Institute, Eğirdir, Isparta as a living collection varied between 1.46 pg and 2.45 pg. When we consider the characteristics of the nuclear DNA content mentioned above, this large variation was not a surprise since the germplasm collection included apple species and their hybrids varying in ploidy. Similar results were also observed in other plant genera. For example, Tuna et al. (2001) determined that the 2C nuclear DNA content of bromegrass accessions maintained in the USDA collection varied between 6.14 pg and 26.64 pg. The authors also reported that the 2C nuclear DNA contents of the tetraploid, octoaploid, and decaploid accessions maintained in the collection were approximately 2, 4, and 5 times larger, respectively, than the DNA content of the diploid accessions. Özkan et al. (2003) investigated nuclear DNA content change during allopolyploidization in the wheat (*Triticum-Aegilops*) group by carrying out nuclear DNA content analysis on six newly synthesized wheat allopolyploids and their parental plants by flow cytometry. The authors found that 2C nuclear DNA content varied between 10.16 pg and 36.52 pg within the wheat group based on species and ploidy. In this study, the authors compared nuclear DNA contents of the newly synthesized amphiploids with the arithmetic sum of the nuclear DNA contents of their original parents and found that allopolyploidization in the wheat group induced approximately a 2 pg DNA loss. The authors also reported that the loss occurred in the first generation. Vogel et al. (1999) used flow cytometry to determine the base DNA content of the genomes of the perennial *Triticeae* and concluded that the gain or loss of nuclear DNA content occurred during the evolution of perennial *Triticeae* and was probably part of the speciation process. Similar results were obtained in many genera such as *Phleum* (Savas Tuna et al., 2016), *Cristatum* (Savas Tuna et al., 2016), *Koelaria* (Savas Tuna et al., 2016), *Dactylis* (Tuna et al., 2004), *Hypericum* (Savas Tuna et al., 2017), *Vaccinium* (Costich et al., 1993), and *Citrus* (Şeker et al., 2018).

Similar results were reported for the genus *Malus* as well. In one of those studies, Dickson et al. (1992) determined the nuclear DNA content of 18 *Malus* species and they found that 2C nuclear DNA content in the genus *Malus* varied from 1.21 pg (*M. tschonskii* (Maxim.) Schneid. GMAL-1834) to 3.11 pg (*M. coronaria* (L.) Mill. GMAL-3064 (4x)). The study also included Prima, Spartan, and Jonagold apple varieties, also investigated in the current study, and their 2C nuclear DNA content was determined as 1.59 pg, 1.73 pg, and 2.51 pg, respectively. Podwyszyńska et al. (2016) reported the 2C nuclear DNA content of the Jonagold variety as 2.48 pg, which was similar to the result reported by Dickson et al. (1992). The 2C nuclear DNA content of the apple varieties reported in these two earlier studies was a little higher than the values obtained in the current study. In this study, the 2C nuclear DNA content of Prima, Spartan, and Jonagold apple varieties was determined as 1.50 pg, 1.59 pg, and 2.13 pg, respectively (Table). This discrepancy between the results of the current study and earlier studies may be the result of different apple genotypes and standards used (Palomino et al., 2003), cytosolic compounds found in plant tissues (Noirot et al., 2000), and the use of different flow cytometry protocols in the studies. Podwyszyńska et al. (2016) also stated that buffer solutions used in the analysis, dyeing time, and temperature may have contributed to the different results.

The 2C nuclear DNA content of *M. sieversii* (1.58 pg) and *M. x domestica* (1.50 pg) species determined in the current study was similar to the results reported by Höfer and Meister (2010) for the same species, 1.50 pg and 1.51 pg, respectively. Faramarzi et al. (2016) reported the average 2C nuclear DNA content in apples as 1.57 pg in diploids and 2.47 pg in triploids. Similarly, Höfer and Meister (2010) found the average 2C nuclear DNA content to be 1.45 pg in diploids, 2.30 pg in triploids, 3.39 pg in tetraploids, and 3.91 pg in pentaploids. Similar results were also achieved by Dickson et al. (1992), Eaker et al. (2003), and Rothleutner et al. (2016). According to the results of the previous studies, triploid varieties have approximately 1.5 times more DNA compared to diploid varieties.

Therefore, when Table is considered, it is possible to say that apple genotypes with average 2C nuclear DNA content from 1.46 pg to 1.69 pg are diploid while the ones with 2C nuclear DNA content from 2.04 pg to 2.45 pg are triploid. Based on these results, the majority of apples in the Turkish living apple collection are diploid (284) while only 56 of the 340 apples are triploid (Table). In accordance with the literature, the average 2C nuclear DNA content of diploid and triploid genotypes was determined as 1.56 pg and 2.29 pg, respectively, in the current study. Some species

Table. The 2C Nuclear DNA content and ploidy levels of *Malus* genotypes determined by flow cytometry.

Variety	Nuclear DNA content (pg $2C^{-1}$)	Ploidy level	SD**	Confident intervals		Variety	Nuclear DNA content (pg $2C^{-1}$)	Ploidy level	SD*	Confident intervals	
				Min.	Max.					Min.	Max.
*Yb-2	2.45	3x	0.03	2.39	2.52	*32 E 1	1.56	2x	0.05	1.45	1.68
Canada Renette	2.44	3x	0.09	2.22	2.66	*GK 23	1.56	2x	0.03	1.50	1.63
Sinap	2.43	3x	0.04	2.34	2.51	*GK 81	1.56	2x	0.01	1.53	1.59
*Coll 73	2.38	3x	0.06	2.22	2.53	Ranna Çer. Ptitsa	1.56	2x	0.05	1.44	1.69
Double Red Del.	2.37	3x	0.03	2.30	2.43	*Kadir-Hatice	1.56	2x	0.02	1.53	1.60
*Niğde İngiliz	2.36	3x	0.01	2.34	2.38	*GK 67	1.56	2x	0.01	1.54	1.58
Staymared	2.36	3x	0.05	2.24	2.47	Rome Beauty	1.56	2x	0.01	1.54	1.58
*Coll 47	2.36	3x	0.06	2.21	2.50	Idared	1.56	2x	0.01	1.54	1.58
Close	2.35	3x	0.05	2.23	2.48	*Karanfil	1.56	2x	0.03	1.49	1.63
*Göbek (2475)	2.35	3x	0.03	2.27	2.43	*GK 2334	1.56	2x	0.02	1.52	1.60
*546 E	2.35	3x	0.05	2.24	2.47	*Beyaz Elma	1.56	2x	0.03	1.47	1.65
Crown Gold	2.35	3x	0.06	2.20	2.50	*Daldabir	1.56	2x	0.03	1.49	1.63
*Demir (2562)	2.35	3x	0.05	2.22	2.47	*504 J	1.56	2x	0.02	1.51	1.61
*GK 37	2.34	3x	0.01	2.33	2.36	Belle Fleo Jello	1.56	2x	0.02	1.52	1.60
*Piraziz	2.34	3x	0.05	2.22	2.47	*GK 5	1.56	2x	0.07	1.40	1.72
*Adsız	2.34	3x	0.11	2.08	2.60	*GK 2328	1.56	2x	0.01	1.54	1.58
Wilmuta Jonagold	2.34	3x	0.03	2.27	2.41	*GK 35	1.56	2x	0.03	1.49	1.63
*E 71	2.34	3x	0.03	2.26	2.41	Melrose	1.56	2x	0.05	1.44	1.67
*210887 (1-2)	2.34	3x	0.07	2.15	2.52	*Yıldızkıran	1.56	2x	0.07	1.38	1.73
*GK 20	2.34	3x	0.01	2.32	2.35	Ozark Gold	1.56	2x	0.03	1.49	1.62
Jonabress	2.33	3x	0.02	2.29	2.37	Goldstar	1.56	2x	0.01	1.54	1.57
*Karpuz	2.33	3x	0.02	2.27	2.38	Chesapeake	1.56	2x	0.01	1.54	1.57
*GK 14	2.32	3x	0.00	2.29	2.36	Cooper 4	1.56	2x	0.04	1.47	1.64
Mutsu	2.32	3x	0.02	2.27	2.37	*KAGK 114	1.56	2x	0.02	1.53	1.60
*Yaz Elması	2.32	3x	0.05	2.19	2.44	*GK 68	1.56	2x	0.02	1.52	1.59
*392 E	2.32	3x	0.03	2.25	2.38	*42-E-1	1.55	2x	0.03	1.48	1.63
*Sarı Göbek	2.31	3x	0.06	2.18	2.45	Yellow Spur	1.55	2x	0.04	1.45	1.65
*GK 73	2.31	3x	0.07	2.13	2.48	*Tatlı Elma (2511)	1.55	2x	0.04	1.45	1.65
*220887 (3-5)	2.31	3x	0.07	2.12	2.49	Blackjon	1.55	2x	0.02	1.52	1.59
*Sarı Elma	2.30	3x	0.04	2.21	2.39	Victoria	1.55	2x	0.04	1.45	1.65
*Elma (2523)	2.29	3x	0.05	2.18	2.40	*Yaz Tavşanbaşı	1.55	2x	0.03	1.48	1.63
Stark	2.29	3x	0.08	2.10	2.48	Gloster	1.55	2x	0.05	1.43	1.68
*180887 (5-1)	2.29	3x	0.02	2.25	2.32	Rewena	1.55	2x	0.01	1.52	1.58
*GK 76	2.29	3x	0.07	2.12	2.45	*42-A-1	1.55	2x	0.02	1.52	1.59
*2582	2.29	3x	0.07	2.13	2.45	Lena	1.55	2x	0.06	1.40	1.70

Table. . (Continued)

***180887 (1-1)**	2.27	3x	0.03	2.20	2.35	***542E**	1.55	2x	0.05	1.44	1.66
***180887 (5-2)**	2.27	3x	0.02	2.22	2.33	**Democrat**	1.55	2x	0.02	1.51	1.59
***190887 (1-4)**	2.27	3x	0.04	2.16	2.38	***GK 2332**	1.55	2x	0.02	1.51	1.59
***Cigit**	2.27	3x	0.09	2.04	2.50	***Tokat 2**	1.55	2x	0.03	1.48	1.62
***496 E**	2.27	3x	0.05	2.15	2.38	***Karapınar Elması**	1.55	2x	0.04	1.46	1.64
Jonica	2.26	3x	0.06	2.11	2.41	***Aksu 4**	1.55	2x	0.05	1.44	1.66
***GK 55**	2.26	3x	0.10	2.00	2.52	**Enterprise**	1.55	2x	0.03	1.48	1.62
***Coll 72**	2.25	3x	0.03	2.19	2.32	***KAGK 48**	1.55	2x	0.06	1.40	1.70
***GK 33**	2.25	3x	0.03	2.16	2.34	**Starking Delicious**	1.55	2x	0.03	1.48	1.61
Reinette Tardiva	2.23	3x	0.10	1.99	2.47	***32 E 2**	1.55	2x	0.01	1.52	1.58
***Mektep Elması**	2.23	3x	0.11	1.97	2.49	**Galaxy Selecta**	1.55	2x	0.04	1.44	1.65
***Demir (2486)**	2.23	3x	0.12	1.94	2.52	***384 E**	1.55	2x	0.08	1.36	1.73
Black Stay. Imp.	2.22	3x	0.03	2.15	2.30	***Pancarlık**	1.55	2x	0.03	1.48	1.61
***220887 (3-2)**	2.22	3x	0.09	1.99	2.45	***Daldatek**	1.55	2x	0.07	1.39	1.71
Hüryemez	2.21	3x	0.08	2.02	2.39	***GK 2**	1.55	2x	0.03	1.47	1.63
***GK 60**	2.21	3x	0.05	2.08	2.33	***GK 31**	1.55	2x	0.05	1.43	1.66
Black Stayman	2.16	3x	0.10	1.92	2.40	**Zlatna Preyuzho Dna**	1.55	2x	0.06	1.41	1.68
Jonagold	2.13	3x	0.40	1.12	3.13	**Pilot**	1.55	2x	0.04	1.46	1.63
Novaja	2.08	3x	0.45	0.96	3.20	***Demir (2514)**	1.55	2x	0.05	1.43	1.66
Almila	2.07	3x	0.49	0.86	3.29	***KAGK 6**	1.55	2x	0.04	1.45	1.64
***Kalkandelen**	2.04	3x	0.42	0.98	3.09	**Jonathan**	1.54	2x	0.03	1.48	1.61
***Candır**	1.69	2x	0.04	1.59	1.79	**Will Early Red**	1.54	2x	0.03	1.46	1.62
Otawa	1.68	2x	0.02	1.64	1.71	**Cooper 42**	1.54	2x	0.02	1.49	1.60
Braeburn	1.68	2x	0.02	1.62	1.73	**Jonafree**	1.54	2x	0.02	1.51	1.58
***Gelin Elması**	1.67	2x	0.03	1.60	1.73	**Anglyska Zelena**	1.54	2x	0.02	1.51	1.58
***GK 18**	1.65	2x	0.06	1.51	1.79	***Ferik**	1.54	2x	0.01	1.53	1.56
Rubinstein	1.65	2x	0.03	1.57	1.72	***210887(1-4)**	1.54	2x	0.01	1.51	1.57
Pristine	1.65	2x	0.01	1.63	1.66	***Güz Tavşanbaşı**	1.54	2x	0.03	1.47	1.62
***GK 17**	1.64	2x	0.07	1.46	1.81	**Raritan Rose**	1.54	2x	0.03	1.47	1.61
***Harım**	1.64	2x	0.03	1.56	1.72	***Altınok Elması**	1.54	2x	0.03	1.47	1.61
***Tokat 1**	1.63	2x	0.03	1.55	1.71	***Orak 2**	1.54	2x	0.03	1.47	1.61
Wayne Spur Del.	1.63	2x	0.11	1.37	1.90	**Early Red One**	1.54	2x	0.01	1.52	1.56
***May.Tavşanbaşı**	1.63	2x	0.03	1.56	1.70	**Doub. Red Stay. Win.**	1.54	2x	0.09	1.33	1.75
***KAGK 19**	1.63	2x	0.02	1.58	1.68	***372 E**	1.54	2x	0.01	1.52	1.56
***KAGK 45**	1.63	2x	0.05	1.50	1.76	**Elstar**	1.54	2x	0.02	1.50	1.57
***GK 29**	1.63	2x	0.01	1.61	1.64	**Summer Regent**	1.54	2x	0.04	1.44	1.63
S. Early Stripe	1.63	2x	0.02	1.57	1.68	**Cooper 44**	1.54	2x	0.04	1.45	1.62
***Yenice**	1.63	2x	0.05	1.50	1.75	***Tavşanbaşı (2531)**	1.54	2x	0.02	1.48	1.59

Manted	1.62	2x	0.05	1.50	1.75	***Gemlik-3**	1.54	2x	0.05	1.41	1.66
Red Jim	1.62	2x	0.05	1.49	1.75	***GK 48**	1.54	2x	0.01	1.52	1.55
***Kaşel 41**	1.62	2x	0.08	1.41	1.82	**Eden spur**	1.54	2x	0.03	1.46	1.61
***Gelin El. (Kütahya)**	1.62	2x	0.02	1.58	1.65	***KAGK 5**	1.54	2x	0.04	1.43	1.64
***Coll 22**	1.62	2x	0.02	1.56	1.67	***200887 (1-9)**	1.54	2x	0.04	1.44	1.64
Cooper 41	1.61	2x	0.02	1.58	1.65	**Sky Line Supreme**	1.54	2x	0.07	1.36	1.71
Red Elstar	1.61	2x	0.03	1.55	1.68	**Winesap**	1.53	2x	0.01	1.50	1.55
***KAGK 2**	1.61	2x	0.08	1.42	1.80	***Cıncık**	1.53	2x	0.06	1.39	1.68
***KAGK 107**	1.61	2x	0.04	1.51	1.72	**Cripps Pink**	1.53	2x	0.01	1.50	1.56
Elstar Van Viliet	1.61	2x	0.04	1.52	1.70	**Cooper 39**	1.53	2x	0.04	1.43	1.64
Delbarestivale	1.61	2x	0.02	1.57	1.64	***Kırmızı elma**	1.53	2x	0.03	1.45	1.61
Lodi Early Golden	1.61	2x	0.02	1.55	1.66	**Ed Gould Golden Del.**	1.53	2x	0.02	1.48	1.59
***Yayla Pınarı**	1.61	2x	0.02	1.55	1.66	**Ervin Spur**	1.53	2x	0.08	1.33	1.74
***GK 12**	1.61	2x	0.03	1.53	1.68	***GK 47**	1.53	2x	0.03	1.45	1.61
***Gemlik 2**	1.61	2x	0.02	1.55	1.66	***2590**	1.53	2x	0.04	1.44	1.63
Discovery	1.60	2x	0.02	1.57	1.64	**Red Free**	1.53	2x	0.08	1.33	1.74
Golden Weinsberg	1.60	2x	0.03	1.54	1.67	***Tokat 4**	1.53	2x	0.05	1.41	1.66
***Ankara Güzeli**	1.60	2x	0.02	1.55	1.66	***Petek**	1.53	2x	0.02	1.50	1.57
***KAGK 49**	1.60	2x	0.04	1.52	1.69	**Anna**	1.53	2x	0.01	1.50	1.56
***EL-23035**	1.60	2x	0.03	1.52	1.68	***Batum**	1.53	2x	0.03	1.47	1.60
***GK 78**	1.60	2x	0.01	1.58	1.62	***Susuz Elma**	1.53	2x	0.02	1.48	1.58
***KAGK 63**	1.60	2x	0.02	1.56	1.64	***Amasya 351**	1.53	2x	0.02	1.48	1.58
***Amasya 37**	1.60	2x	0.06	1.44	1.76	***Coll 32**	1.53	2x	0.01	1.51	1.55
***Cidagut**	1.60	2x	0.03	1.53	1.67	**Dayton**	1.53	2x	0.02	1.48	1.58
Topaz	1.60	2x	0.01	1.58	1.61	**Cooper 900**	1.53	2x	0.02	1.49	1.57
Red Spur	1.60	2x	0.04	1.50	1.70	***GK 4**	1.53	2x	0.08	1.33	1.73
***GK 15**	1.60	2x	0.04	1.50	1.70	***170887(2-5)**	1.53	2x	0.07	1.37	1.69
***GK 11**	1.60	2x	0.03	1.53	1.66	**York Imperial**	1.53	2x	0.03	1.46	1.60
***Karasakı**	1.60	2x	0.02	1.54	1.65	**Gold Jon**	1.53	2x	0.03	1.44	1.62
***Coll 23**	1.60	2x	0.03	1.53	1.66	***473 E**	1.53	2x	0.03	1.46	1.60
***Yenişehir**	1.60	2x	0.04	1.49	1.70	***Samsun**	1.53	2x	0.06	1.39	1.67
***KAGK 43**	1.60	2x	0.02	1.56	1.63	***62-2**	1.53	2x	0.06	1.38	1.68
Early Blaze	1.59	2x	0.01	1.56	1.62	**Champion**	1.53	2x	0.01	1.50	1.56
***GK 6**	1.59	2x	0.05	1.48	1.71	**Calville Rouge Del.**	1.53	2x	0.06	1.39	1.66
Arlet	1.59	2x	0.01	1.58	1.61	***190887(3-2)**	1.53	2x	0.02	1.49	1.56
Calville R. Datan	1.59	2x	0.04	1.50	1.69	**Red Dykmonszoef**	1.53	2x	0.10	1.28	1.77
***220887**	1.59	2x	0.03	1.53	1.66	**Priam**	1.53	2x	0.05	1.40	1.65
***Tavşanbaşı Küt.**	1.59	2x	0.02	1.56	1.63	***Kış Elması**	1.52	2x	0.01	1.49	1.55
Cooper 40	1.59	2x	0.02	1.55	1.63	***130887 (2-3)**	1.52	2x	0.07	1.35	1.69
Ayvania	1.59	2x	0.02	1.55	1.63	**Rajka**	1.52	2x	0.02	1.47	1.58

***GK 38**	1.59	2x	0.03	1.52	1.66	***Paşa Elması**	1.52	2x	0.01	1.50	1.54
Red Rome 262	1.59	2x	0.01	1.57	1.61	**William's Pride**	1.52	2x	0.03	1.45	1.59
Gallia Beauty	1.59	2x	0.06	1.44	1.74	**Dlatro Prevuzho Dna**	1.52	2x	0.01	1.50	1.54
***Yaz Amasya**	1.59	2x	0.06	1.45	1.73	***210887 (2-1)**	1.52	2x	0.05	1.39	1.65
***J/5/4/59 Bel.**	1.59	2x	0.03	1.51	1.67	**Sky spur**	1.52	2x	0.05	1.39	1.65
***Oltu Elması**	1.59	2x	0.04	1.49	1.69	***Şeker**	1.52	2x	0.02	1.47	1.57
Astramel	1.59	2x	0.03	1.52	1.65	**Santana**	1.52	2x	0.06	1.36	1.68
***385 E**	1.59	2x	0.03	1.51	1.67	***GK 66**	1.52	2x	0.02	1.47	1.57
***Bey Elması**	1.59	2x	0.01	1.57	1.60	**Rose De Benauge**	1.52	2x	0.05	1.41	1.63
Spartan	1.59	2x	0.03	1.51	1.66	***GK 56**	1.52	2x	0.04	1.42	1.62
***KAGK 51**	1.59	2x	0.08	1.39	1.79	***Ak**	1.52	2x	0.02	1.48	1.55
Malus kirghisorum	1.58	2x	0.02	1.53	1.64	***Rize Demir**	1.52	2x	0.03	1.44	1.60
***Tatlı Elma 2492**	1.58	2x	0.08	1.40	1.77	***Petevrek Elması**	1.52	2x	0.01	1.49	1.55
***220887 (1-2)**	1.58	2x	0.01	1.57	1.60	***GK 9**	1.52	2x	0.03	1.45	1.58
All Red Jonathan	1.58	2x	0.04	1.48	1.68	***Kaba elma**	1.52	2x	0.04	1.41	1.62
***180887**	1.58	2x	0.04	1.50	1.67	**Korella**	1.51	2x	0.04	1.42	1.61
***Gümüşhane**	1.58	2x	0.01	1.55	1.61	***Yb 3**	1.51	2x	0.02	1.46	1.57
***M. Yıldızkıran**	1.58	2x	0.03	1.51	1.66	***GK 63**	1.51	2x	0.04	1.43	1.60
***GK 65**	1.58	2x	0.06	1.44	1.72	***200887 (1-2)**	1.51	2x	0.02	1.47	1.55
***KAGK 11**	1.58	2x	0.04	1.49	1.68	***GK 82**	1.51	2x	0.04	1.41	1.61
***KAGK 22**	1.58	2x	0.02	1.53	1.64	***250887 (1-10)**	1.51	2x	0.02	1.46	1.56
***KAGK 25**	1.58	2x	0.02	1.55	1.62	***GK 2411**	1.51	2x	0.07	1.35	1.67
***371 E**	1.58	2x	0.03	1.51	1.65	***24 M 31**	1.51	2x	0.02	1.45	1.56
Malus sieversii	1.58	2x	0.02	1.52	1.63	***63-6-2**	1.50	2x	0.02	1.47	1.54
Mor Spur	1.58	2x	0.04	1.47	1.68	***Malus domestica***	1.50	2x	0.04	1.39	1.61
***130887 (3-4)**	1.58	2x	0.01	1.56	1.59	***Gelendost**	1.50	2x	0.04	1.42	1.59
***Mahsusa Elması**	1.58	2x	0.02	1.54	1.61	***Amasya 532**	1.50	2x	0.02	1.45	1.56
***KAGK 7**	1.58	2x	0.04	1.47	1.68	***GK 21**	1.50	2x	0.01	1.49	1.52
***KAGK 8**	1.58	2x	0.01	1.55	1.61	**Pozmer 20**	1.50	2x	0.03	1.43	1.58
***Sandık**	1.58	2x	0.06	1.42	1.74	***Sivanor Elması**	1.50	2x	0.03	1.41	1.59
***Söğüt Elması**	1.58	2x	0.07	1.40	1.75	***Yaz Elması (2488)**	1.50	2x	0.07	1.33	1.66
Delprim	1.57	2x	0.05	1.46	1.69	**Prima**	1.50	2x	0.03	1.42	1.57
***Süs Elması**	1.57	2x	0.02	1.54	1.61	**Astragan Rouge**	1.50	2x	0.11	1.22	1.78
***GK 57**	1.57	2x	0.01	1.56	1.59	**Red Ingred Marie**	1.49	2x	0.05	1.37	1.62
***Gürcü**	1.57	2x	0.03	1.51	1.64	***Tatlı Tavşanbaşı**	1.49	2x	0.03	1.42	1.57
***GK 50**	1.57	2x	0.05	1.46	1.69	***GK 34**	1.49	2x	0.04	1.38	1.60
Top Red	1.57	2x	0.03	1.51	1.64	**Dallies**	1.49	2x	0.02	1.44	1.54

***GK 19**	1.57	2x	0.05	1.45	1.70	**Reine de Renettes**	1.49	2x	0.09	1.27	1.71
***KAGK 29**	1.57	2x	0.05	1.45	1.70	***180887 (2-1)**	1.49	2x	0.02	1.45	1.52
Winter Banana	1.57	2x	0.04	1.46	1.68	***GK 2329**	1.49	2x	0.04	1.39	1.59
Oregon Spur	1.57	2x	0.02	1.53	1.61	***Kaşel 37**	1.49	2x	0.04	1.39	1.59
***529 J**	1.57	2x	0.00	1.57	1.57	**Priscilla**	1.48	2x	0.05	1.37	1.60
***Yb-1**	1.57	2x	0.01	1.55	1.59	**Red Delcorf**	1.48	2x	0.06	1.32	1.64
***GK 2331**	1.57	2x	0.06	1.42	1.72	***GK 13**	1.48	2x	0.04	1.38	1.58
Cooper 7-SB-2	1.57	2x	0.13	1.26	1.88	***180887 (4-4)**	1.48	2x	0.02	1.43	1.54
***KAGK 17**	1.57	2x	0.04	1.47	1.67	**Wealthy**	1.48	2x	0.01	1.46	1.50
***KAGK 34**	1.57	2x	0.05	1.44	1.70	***E 45**	1.48	2x	0.08	1.29	1.67
Gold Rudi	1.57	2x	0.07	1.40	1.73	**Mollies Delicious**	1.48	2x	0.04	1.39	1.57
***GK 72**	1.57	2x	0.03	1.50	1.63	***Hanım Teni**	1.48	2x	0.03	1.41	1.54
***62-1**	1.57	2x	0.01	1.55	1.58	***383 E**	1.48	2x	0.04	1.38	1.58
McIntosh	1.57	2x	0.03	1.50	1.63	***GK 32**	1.48	2x	0.08	1.28	1.68
Fiesta	1.57	2x	0.02	1.51	1.62	**King Lucious**	1.47	2x	0.04	1.38	1.57
***KAGK 104**	1.57	2x	0.04	1.46	1.67	***Amasya (Uludağ)**	1.47	2x	0.03	1.40	1.55
***GK P36**	1.57	2x	0.01	1.54	1.60	***GK 51**	1.47	2x	0.12	1.18	1.77
***E 70**	1.56	2x	0.02	1.51	1.62	***Malus niedzwetzkyana***	1.47	2x	0.05	1.35	1.58
Hena	1.56	2x	0.04	1.46	1.67	***240887 (1-2)**	1.47	2x	0.05	1.35	1.58
***Uzun Yomra**	1.56	2x	0.02	1.51	1.62	**Hi Early Delicious**	1.46	2x	0.05	1.35	1.57

* Autochthonous apples
** SD; standard deviation

known as diploid such as *M. sieversii*, *M. niedzwetzkyana*, and *M. domestica* (Schuster and Büttner, 1995; Höfer and Meister, 2010), and some cultivars known as diploid such as Discovery, Early Golden, Elstar, Gloster, Jonathan, Prima, and Rajka (Larsen et al., 2018) were included in the diploid group (Table). The varieties known as triploid such as Jonagold and Mutsu (Larsen et al., 2018; Ordidge et al., 2018) were included in the triploid group (Table). These results prove the accuracy of the ploidy estimation of the nuclear DNA content determined by flow cytometry.

5. Conclusion

Fertility, crossing, and inheritance of traits are affected by the ploidy level. In the present study, the ploidy level of the Turkish living apple germplasm collection was assessed. Based on the results, apple genotypes included in the Turkish collection were separated into 2 clear groups: diploids and triploids. The results show that 83.53% of apple genotypes are diploid while 16.47% are triploid. The results of the study will be useful to determine the best strategies in breeding programs as ploidy is one of the most important characteristics in selecting parents for crosses. For this reason, the results of the study will make significant contributions to increasing the efficiency of hybridization studies in apple breeding programs that last for many years and help researchers to save resources, time, and labor.

Acknowledgment

The authors would like to thank The Scientific and Technological Research Council of Türkiye (TÜBİTAK) [project 219O095] for their financial support.

Conflict of interest

No potential conflict of interest was reported by the author(s).

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