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Comparison of Genome Sizes of Persimmon (*Diospyros kaki* L.) and Caucasian Persimmon (*Diospyros lotus* L.) Seedling Populations by Using Flow Cytometry

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Abstract: Flow cytometry is an improved technique for following cell cycle, determination of ploidy levels, calculation of genome sizes and early selection of candidates in plant breeding studies. Persimmon is generally propagated by using *Diospyros lotus* seedlings in Turkey. Moreover some persimmon varieties can be used for propagation as rootstock seed sources. In this study, fresh leaf tissues of *Diospyros kaki* and Diospyros lotus seedlings were used for obtaining of cell nuclei then stained by propidium iodide and were used for estimation of floresence intensities. According to the obtained results caucasian persimmon seedlings were had smaller genome sizes compare to the persimmon genomes and had less variation than persimmons. The relative ploidy level of caucasian persimmon seedlings were diploid where as hexploids in persimmon seedlings. By this experiment it was emphasized that new genotypes which have uniform rootstock charateristics for PCNA (Pollination Constant Non Astringent) and PVNA (Pollination Variant Non Astringent) persimmon varieties are needed for better performance in persimmon growing.

Keywords: Diospyros, rootstock, breeding, early selection, polyploidy, variation

Trabzon Hurması (*Diospyros kaki* L.) ve Artvin Hurması (*Diospyros lotus* L.) Çöğür Populasyonlarının Genom Hacmi Farklılıklarının Hücre Akış Sitometrisi ile Karşılaştırılması

Öz: Hücre Akış Sitometrisi (Flow Cytometry) hücre döngüsünün izlenmesi, ploidi seviyesinin belirlenmesi, genom hacminin ortaya çıkarılması ve ıslah çalışmalarında erken seleksiyon yapılmasına imkân tanıyan gelişmiş bir tekniktir. Trabzon hurması çeşitlerinin çoğaltılmasında genel olarak *Diospyros lotus* (Artvin hurması) türünden elde edilen çöğür anaçları kullanılmaktadır. Ayrıca, Diospyros kaki türüne ait bazı çeşitlerin de tohumları değişik fidanlıklarda kullanılmaktadır. Bu çalışmada, *D. kaki* ve *D. lotus* türlerine ait çöğür populasyonlarında bulunan bitkilerin taze yaprak dokularından elde edilen hücre çekirdekleri propidyum iyodür (DAPI) ile boyanmış ve hücre akış sitometrisi tekniği ile floresant yoğunlukları ölçülmüştür. *D. lotus* türü *D. kaki* bitkilerinden daha küçük genom hacmine sahip olmuş ayrıca tür içi varyasyon *D. lotus* bitkilerinde daha düşük seviyede gerçekleşmiştir. *D. kaki* bitkilerinde yüksek seviyede varyasyon izlenmiştir. Özellikle buruk olmayan çeşitler (PCNA ve PVNA grubu çeşitler) için anaç olarak kullanılması gereken *D. kaki* türünde birörnek çöğür oluşturan genotiplere ihtiyaç bulunduğu vurgulanmıştır.

Anahtar Kelimeler: *Diospyros*, anaç, ıslah, erken seleksiyon, poliploidi, varyasyon

1. Introduction

Persimmon (*Diospyros kaki* L.) fruits, especially with regard to content of vitamin A, C and carbohydrates, as well as plenty of vitamins and minerals, calcium and potassium is important in terms of healthy nutrition.

Persimmon is a kind of fruit which is growing in temperate and subtropical climate zones and it has an increasing importance in fruit growing regions of our country. In our country, in recent years, especially in the Mediterranean region, monocultivar persimmon orchards have been

increasing year by year and attracted great interest from producers.

With the studies, it was seen that the most proper method for propagation of persimmon varieties is possible with grafting. The production of grafted nursery plants is carried out by grafting or budding on the seedlings of different species used as rootstocks.

Although 5 different types of *Diospyros* can be used as rootstock in persimmon sapling cultivation, *D. kaki*, *D. lotus* and *D. virginiana* species are the most commonly used rootstocks because of incompatibilities and different rootstock performances.

Choi et al. (2003), who carried out genome researches in *Diospyros* genus, were reported to be *D. glandulosa* (2n=2x=30), *D. oleifera* (2n=2x=30), *D. lotus* (2n=2x=30)s, *D. virginiana* (2n=6x=90) ve *D. kaki* (2n=6x=90). Persimmon is a polyploid species that hexaploid (6n=90) chromosome structure. On the other hand 'Hiratanenashi' ve 'Tonewase' varieties were found to be 2n = 9x = 135 in a sense nonaploid (Giordani, 2002). However, *D. lotus* species, which widely used in Turkey, have diploid (2x=30) genome. Seedlings of *D. lotus* species in Turkey is widely used for rootstock for Persimmon varieties. In addition to this, grafting incompatibility is frequently observed between *D. kaki* and *D. lotus* species. One of the reasons for the incompatibility problems may be that both species have different levels of ploidy. The definition of ploidy level in plants is important for breeding and physiology studies in different fields.

Cell Flow Cytometry (CFC) is one of the most reliable techniques for investigating ploidy levels in mixoploid cell structures. In this system, cells are stained with fluorescent compounds such as propidium iodide, DAPI, H-33258, passed through individual fluorescence detectors, and the total amount of DNA can be measured rapidly and accurately. Nowadays, CFC is used for many different purposes in medicine, botanic and other biological sciences (Şeker, 2001a; Şeker 2011b; Seker ve ark, 2003; Şeker, 2009).

In this study, fresh leaf tissues of *D. kaki* and *D. lotus* seedlings were used for obtaining of cell nuclei then stained by propidium iodide and were used for estimation of floresence intensities.

2. Materials and Methods

In this research, plants selected in seedling populations of persimmon (*Diospyros kaki*) and caucasian persimmon (*Diospyros lotus*) species. Seedlings were provided from commercial persimmon nurseries in Hatay. 100 seedlings of each species were provided and approximately 100 µg of tissue pieces taken from their fresh leaves were mixed with leaf tissues of the mung bean variety (*Vigna radiata cv* 'Berken'). In a study, it was reported that mung bean, which was used for ploidy level control, has 2x ploidy level and its genome volume is 1,20 pg/2C (Arumuganathan ve Earle, 1991; Seker ve ark., 2003). Leaf tissues were chopped into small pieces with a sharp razor in a sterile Petri dish containing 300 µl of nuclei buffer (pH 7.4) of the following composition: (0.14 M NaCl, 0.003 M KCl, 0.012 M NaH_2PO_4, 0.002 M KH_2PO_4, 0.1% Triton X 100, 50 µg RNAse ve 100 µl dithiothreitol). The suspension was filtered through a 50 µm pore nylon filter into microcentrifuge tubes. After filtration, 100 µl (1 mg/ml) of propidium iodide was added for staining of the DNA. Then the suspensions were incubated for approximately 5 min at room temperature. After incubation, each sample was run on a flow cytometer.

For estimation of the DNA content of the nuclei, the relative fluorescence of the nuclei was measured by using a Bryte HS Flow Cytometer (BIORAD, S.P.D., 20090 Segrate, Milan, Italy) with a xenon light source operating at a wavelength of 450 nm. Histograms and cytograms were evaluated using a Hewlett-Packard computer with WinBryte-BIORAD Software(BIORAD). At least 5000 nuclei were analyzed in each sample (Seker et al, 2003). In this study, only polyploidy levels and genome volume means were investigated.

The nuclear DNA contents of the *Diospyros* species were calculated by comparison of the relative positions of the G0-1 peaks corresponding to the sample nuclei and the nuclei isolated from mung bean, respectively. This permits accurate determination of the unknown DNA content. after cytometric measurement, means of genome volume calculation was made according to the formula:

$Q = R \times (E / S)$

where Q = unknown genome volume of *Diospyros* species (pg/2C), R = standard genome volume of mung bean (1.20 pg/2C), E = sample (*Diospyros* species) G0-1 peak mean, S = standard (mung bean) G0-1 peak mean.

Statistical analyses were carried out with the genome results obtained from each seedling. Significance of genome size variation among the seedlings was determined from analysis of variance by using SAS statistical analysis software. Analysis of variance was computed using the General Linear Model (GLM) procedure of SAS with mean separation using a protected LSD (SAS Institute 1998).

The method used in the research was performed by Dolezel et al. (1994) and Seker et al. (2003).

3. Results and Discussion

Cell Flow Cytometry allowed rapid and precise measurement of stained cell nuclei. The diploid mung bean used was a useful reference species, in particular for distinguishing *D. kaki* plants. The results obtained from the analysis showed that *Diospyros* species had different ploidy levels. Two different histograms were formed when the cell nuclei are isolated by mixing the tissues of *D. kaki* and mung bean and the cytometry analysis is performed by DAPI. The distinction of diploid and hexaploid plants can be clearly determined in these histograms. When the same procedure for *D. lotus* and mung bean was applied, the only peak that shows the diploid genome is revealed. Histograms obtained by cell flow cytometry suggest that two different types of *Diospyros* have different levels of ploidy. The results obtained from Giordani (2002) and Choi et al. (2003) shows compatibility with the indicated results. An example of a cell flow cytometry histogram of the diploid *D. lotus* (2x) and hexaploid *D. kaki* (6x) genome is shown in Figure 1.

Average genome volumes were calculated by quantifying 100 seedlings of both species and the results obtained are given in Table 1. The average genome sizes of *D. lotus* and *D. kaki* seedlings were determined as 0.98 pg 2C-1 and 5.57 pg 2C-1, respectively.

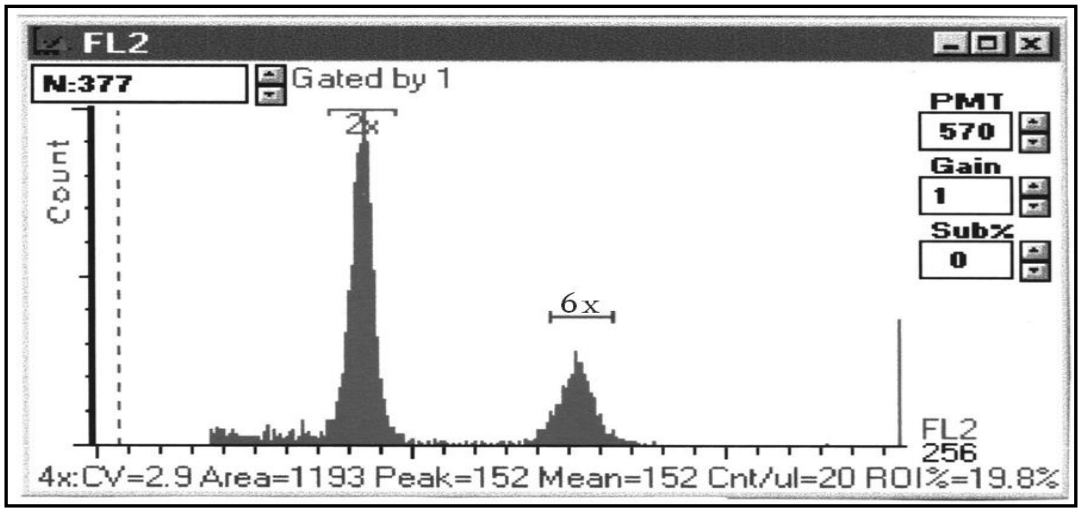

Figure 1. Histograms of propidium iodide stained nuclei of *D. lotus* (2x) and *D. kaki* (6x) obtained in flow cytometry

Şekil 1. *Hücre çekirdekleri propidyum iyodür ile boyanmış D. lotus (2x) ve D. kaki (6x) nin hücre akış sitometrisi histogramları*

Table 1. Ploidy levels and average genome size of *Diospyros* species
Çizelge 1. *Diospyros türlerinin ploidi düzeyleri ve ortalama genom hacimleri*

Species	Ploidy Level	Genome Size (pg/2C)	Standard Variation
Diospyros lotus	**Diploid (2x)**	**0,98 b**	**± 0,15**
Diospyros kaki	**Hekzaploid (6x)**	**5,57 a**	**± 1,14**

D. lotus seedlings showed a lower genome volume variation than *D. kaki* seedlings (Figure 2.). The differences in genome volume of *Diospyros* species were found to be statistically significant.

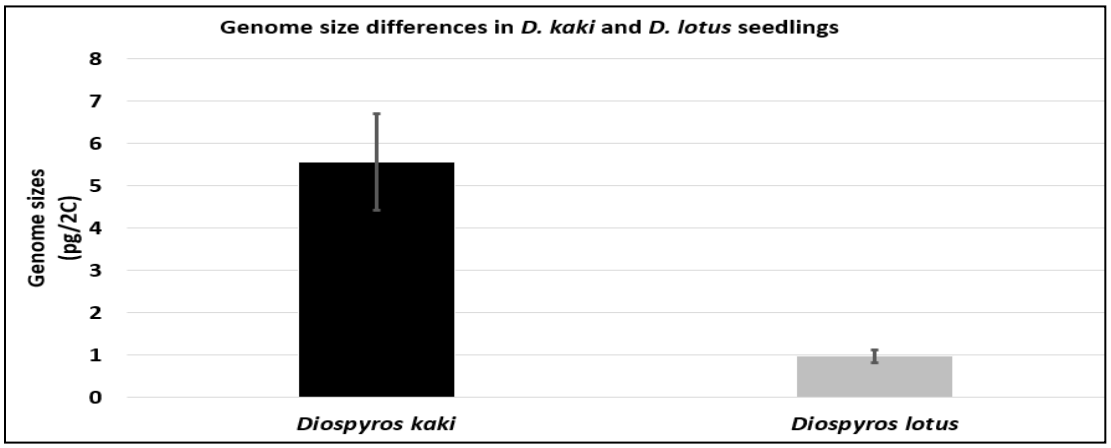

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Figure 2. Genome size differences of D. lotus (2x) and D. kaki (6x) plants

Variations in genome volumes may be related to the technique, staining and measurement procedure used, besides it can be explained by the fact that the variation in genome volume variation is of high ploidy level. Persimmon has the potential to become an important fruit species in the near future. With the increase of cultivation of this species, it will be inevitable to conduct research on dissolving problems such as varieties, rootstocks, cultivation, adaptation to biotic and abiotic conditions will be inevitable. There is a serious potential for persimmon cultivation in our country and it is seen that producers have a great deal of interest in recent years. Therefore, it is necessary to increase and diversify the research on this species. At the beginning of these studies should be on clonal rootstocks for non-stringent, seedless, high fruit flesh firmness and red colored varieties.

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