Determination of Genome Size Differentiation and Ploidy Levels in Some Citrus Rootstock Populations

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Abstract

In plants, knowing the ploidy level of plant material used in breeding studies, and especially for biotechnology applications, carries great importance. The presence of a rapid variety of dynamics in citrus fruits allows their use as rootstock and varieties ensuring adaptability to various climate and soil conditions with different breeding methods. A variety of appropriate rootstocks are used for commercial citrus species. This study investigated the genome sizes and ploidy levels in citrus rootstocks commonly used around the world with flow cytometry in seedling populations. The study used Gou-Tou, C-35, Troyer Citrange, Taiwanica, Citremon, Yuzu, Sunki mandarin, Flying Dragon, Yuma Citrange, Macrophylla and Chinese orange rootstocks. Fresh leaf tissues were mixed with the triploid Tahiti lime leaf tissue, used as standard species, and cell nuclei were isolated. Cells stained with propidium iodide were read with flow cytometry and histograms and cytograms were obtained. According to the obtained results, all seedlings of species had diploid genome volumes. In terms of genome volume, there were differences found between species. Yuzu seedlings were determined to be the species with largest genome volume (0.808 pg/2C), while Flying Dragon trifoliolate had smallest genome volume (0.700 pg/2C).

Introduction

Citrus, originating in southeast Asia, are commonly cultivated in Turkey as in the rest of the world. According to the FAO Statistical Database [9], 4,769,726 tons of citrus fruits from different species were produced in Turkey in 2017 contributing to 3.25% of the total citrus production in the world. Citrus fruits have a very significant share of global fruit production. Citrus species have been cultivated in subtropical and tropical regions for years. Rootstock selection is very important for citrus cultivation. Rootstock has very large effects on tolerance to cold and diseases, adaptation to different climate and soil conditions, fruit yield and quality. As a result, the use of rootstocks for citrus species has become mandatory. An ideal citrus rootstock has high ability to adapt to different soil conditions, is compatible with citrus species and varieties, resistant to biotic and abiotic stress conditions and has high polyembryony rates. Gou-Tou, C-35, Troyer Citrange, Taiwanica, Citremon, Yuzu, Sunki mandarin, Flying Dragon, Yuma Citrange, Macrophylla and Chinese citrus rootstocks are among the rootstocks commonly used in citrus cultivation [6].

As a significant proportion of citrus rootstocks have a tendency toward nucellar embryony, seeds display the same characteristics as main plants and they display performance like clonal proliferation.

Genome volumes are very important for identification of significant citrus genotypes. With flow cytometry analyses, genome volumes and different ploidy structures may be determined in citrus species and varieties. For calculation of the nuclear DNA content of a species, it is necessary to determine ploidy level, chromosome counts and how big chromosomes are. Before beginning breeding studies, differences in ploidy level between individuals to be used as genotype and identification of these differences is very important in terms of breeding studies being successful. Flow cytometry is currently the most sensitive, rapid and reliable method used for determination of nuclear DNA content, and use with this aim has

become more common in recent years [34]. Determination of ploidy level in plants with traditional methods includes counting chromosomes during mitosis division in preparations made from root tip tissues with light microscopy; however, this method is very demanding, slow and performing ploidy analysis of many plant species takes time. Additionally, it is not useful for determination of ploidy levels in plant species with small chromosomes and high ploidy levels, and may cause misclassification of species. As the plant sample for ploidy level determination increases, the light microscopy method may not be sufficient. As studies may not be sufficient for determination of ploidy level using root tip tissues, the flow cytometry method has become a method chosen for ploidy analyses in recent years due to being convenient, rapid, sensitive and reliable [3, 13, 35]. As chromosomes are found in the nuclei of cells in plants, there is a close correlation between the nuclear DNA amount and ploidy level. As a result, due to the convenience, speed and reliability of the flow cytometry method, in recent times ploidy analysis has been performed by determining the nuclear DNA content in plant cells with the flow cytometry method. Flow cytometry analysis has many advantages compared to the chromosome count method in traditional methods such as preparation of samples being easy, it is rapid and there is no need for a root tip cell in mitosis to perform analyses, just a small leaf tissue is sufficient [7]. Flow cytometry was first developed as a method to identify the nuclear DNA content of organisms and it is a very important and easily applied method in these studies [30]. The flow cytometry method is a method based on analysis of light after being absorbed by cells as they pass singly through fluorescence detectors generally. However, in order to ensure light absorption by cells, the cell nuclei from enzymatically degraded leaf samples are freed and cell nuclei stained with fluorescent stains like DAPI or PI are used to determine nuclear DNA amounts. With the flow cytometry method, one person can analyze over 100 plants per day, it performs chromosome counts very rapidly, provides accurate and reliable results, and increases significance and this situation has led to flow cytometry currently being the most chosen method for chromosome counts. However, the disadvantage of the method is that it must be set to the plant species to be analyzed [8]. Applications where flow cytometry is commonly used in plants include determination of ploidy level, nuclear DNA content and estimation of genome volume [25].

In this study, the nuclear DNA amounts in seedling populations of citrus genotypes were examined with the flow cytometry method and ploidy levels and genome volumes were determined. Thus, the ploidy levels between genotypes were investigated for presence of significance from a statistical viewpoint.

Materials And Methods

Plant Materials

In this research seeds and seedlings were used during the experiment.

Two hundred seeds from the following citrus rootstocks were sown and germination rates were precisely determined during four weeks. Seedling populations comprising 100 plants from each rootstock were obtained.

Gou Tou Sour Orange (*Citrus aurantium* L. var. Gou Tou): A rootstock of Chinese origin, apart from being resistant to tristeza disease, there is no information about soil wants or resistance to other diseases. In Florida plants grafted onto Gou Tou rootstock have larger crown structure compared to those grafted on other bitter oranges [31]. Gou Tou sour orange is reported to reduce yield in grapefruits [16]. Gou Tou sour orange is tolerant of *Phytophthora citrophthora* and *Phytophthora* parasitical diseases [17].

C-35 citrange (*Poncirus trifoliata* (L.) Raf. X *Citrus sinensis*. Osb. 'Ruby'): A rootstock obtained by hybridization of Ruby Blood orange and trifoliate orange. It is tolerant of gummosis (*Pytophthora citrophthora* (Smith and Smith) Leon.) and Tristeza diseases and resistant to nematodes. Resistance to cold is equivalent or slightly better than Carrizo citrange. Trees have moderate size and those grafted on Troyer have 25% smaller crown. Good compatibility with sandy, sandy-clayey and clayey soils; however, is more susceptible to limey soils than Carrizo citrange [31].

Troyer citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osb.): A sweet orange and trifoliate orange hybrid. Generally, it has trifoliate properties, with more compliant characteristics in terms of environmental conditions and compatibility with varieties so it is used more often in recent years and in most cases is chosen as alternative to sour orange rootstock. Proliferation with seed and grafting is easy, growth is moderate, yield is high, maturation and fruit setting are early, effects on fruit quality are high and economic lifespan is at moderate levels [23].

Alemow (macrophylla) (*Citrus macrophylla* Wester): Important features are resistance to salinity and boron. Generally, it has good compatibility with all varieties. However, it is used as rootstock for lemon and lime mainly due to susceptibility to Tristeza and Xyloporosis diseases. It appears to be tolerant of Exocortis and Psorosis diseases. Varieties grafted on it grow rapidly and set early fruit. However, quality of fruit is negatively affected. It is susceptible to cold.

Flying Dragon (*Poncirus trifoliata* var. Monstrosa): The trifoliate clone Flying Dragon was found in America in 1915. This rootstock ensures tight crown formation for lime, grapefruit and tangelo, and has the effect of dwarfing mandarin and orange. It is very sensitive to calcium and chlorosis. It develops excessively slowly on mild sandy soils. It is resistant to tristeza (CTV) virus and *Phytophthora* spp. (root neck rot). It is sensitive to Exocortis. The Eureka group showed incompatibility with lemons. Due to showing dwarfing effect on all citrus types and varieties, it is appropriate for dense planting, ensuring convenient harvesting of grafted varieties. It has positive effects on fruit quality, like the trifoliate rootstock. The body having zigzag form and many thorns makes grafting difficult [2]. The Flying Dragon rootstock which is resistant to cold and humid conditions and sensitive to high-lime soils, does not have a tendency to form nucellar plants at high rates [1, 10].

Sunki mandarin (*Citrus sunki* (Hayata) hort ex. Tanaka): It is very widely used as rootstock in China. It is tolerant of Tristeza and Xyloporosis, but sensitive to Exocortis. Studies have reported Sunki is susceptible to *Phytophthora* brown rot. This rootstock is tolerant of salt, has moderate resistance to low temperatures, and can withstand chlorosis in limey soils. It is a polyembryonic rootstock. [16] reported adaptation to limey soils was good, and that it was tolerant to iron chlorosis. Fruit yield, fruit juice

amounts and sugar content in fruit juice was equivalent or superior to fruit obtained from trees grafted on bitter orange [31].

Yuzu (*Citrus junos* Sieb. ex Ten.): It is a common rootstock in the southern regions of China. From China, production spread to Japan and it forms an important commercial rootstock in Japan. Proliferation from seeds is easy, with slow growing features. It is a rootstock with high fruit quality and yield. It is resistant to phytophthora, fungus and nematodes. It is tolerant of tristeza, dwarfing and spalling diseases. It has moderate levels of resistance to limey and salty soils. It has high resistance to low temperatures and polyembryony tendency.

Taiwanica (*Citrus taiwanica* Tan. and Shim.): It is a rootstock that is easily proliferated from seeds, and has moderate levels of tree growth, fruit quality and yield. It is resistant to phytophthora disease, very susceptible to fungal disease and susceptible to nematode damage. It is tolerant of tristeza, dwarfing and spalling diseases. It has moderate levels of resistance to limey soils, with weak resistance of saline soil conditions. It has moderate resistance to low temperatures and is a rootstock with very high tendency for polyembryony.

Yuma Citrange (*P. trifoliata* × *C. sinensis*): A trifoliolate orange hybrid. It matures in the months of October-November. It is a rootstock susceptible to iron deficiency. It has smaller fruits than Citrumelo. It forms trees of moderate size, with trifoliolate leaves, and crown volume in global structure. It has low tendency toward polyembryony, and forms zygotic plants at high rates [12]. It is a very suitable rootstock for grapefruit. In terms of features like susceptibility to disease and nematodes, fruit quality and adaptation to soil types, Yuma citrange is similar to Carrizo and Troyer citranges.

Chinese bitter orange (*C. myrtifolia* Rafinesque): Chinese bitter orange, susceptible to CTV, matures from January-March. Leaves are small and don't have pointed tips. Fruit are small and rounded, with variability in seed numbers from low to high, and it forms small tress. The peel of the fruit has moderate roughness, with color ranging from orange to dark orange. It originated in China.

Citremon (*Poncirus trifoliata* (L.) Raf. X *Citrus lemon* (L.) Burm): Most of these hybrids show abnormal small leaf features. They die in the germination stage or a short while after. Large leaved plants survive, fruit has many seeds and rough structure, trees show rapid development like lemon.

Determination Of Germination Rates

Seeds were provided from open pollinated mature fruits of Gou-Tou sour orange, C-35 citrange, Troyer citrange, Taiwanica, Citremon, Yuzu, Sunki mandarin, Flying Dragon trifoliolate orange, Yuma Citrange, Macrophylla and Chinese orange rootstocks trees in Citrus orchards in Adana – Turkey. 200 seeds from each genotype were sown in growing media containing of vermiculite No:3 in greenhouse. Seedlings were counted 15 days later after seed germination then seedlings with three developed leaves were transferred

into the plastic pots containing peat moss. The seedlings grown well without any blemishes were used for cytometry analysis.

Isolation And Staining Of Nuclei

To release cell nuclei, approximately 50 mg of fresh tissue from each seedling leaf was mixed with Tahiti lime (*Citrus latifolia* Tan.) leaf pieces, which were used as a control, and chopped into small pieces with a sharp razor in a sterile Petri dish containing 300 µl of nuclei buffer (pH7.4) of the following composition: 0.14 M NaCl, 0.003 M KCl, 0.012 M NaH₂PO₄, 0.002 M KH₂PO₄, 0.1% Triton 100, 50 µg of RNase and 100 µl of dithiothreitol. For measurements of absolute DNA values, Tahiti lime leaf tissues were included as an internal standard, as previously described by [19, 20]. Tahiti lime was described as triploid and nuclear DNA content was found to be 1.17 pg/2C by [19, 20]. 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 [33].

For estimation of DNA content of nuclei, the relative fluorescence of nuclei was measured by using a CA-III Flow Cytometer (Partec GmbH, Münster, Germany) with an Argon laser light source operating at a wavelength of 488 nm. Histograms and cytograms were evaluated on DPAC Software (Partec GmbH, Münster, Germany). From 2000 to 5000 nuclei were counted per flow cytometry measurement. The nuclear DNA contents of different seedlings were calculated by comparison of relative positions for G₀₋₁ peaks corresponding to the sample nuclei and the nuclei isolated from Tahiti lime or mungbean, respectively. This permits accurate determination of the unknown DNA content [33].

Data analysis and estimation of nuclear genome size: The nuclear DNA contents of the different rootstock seedlings were calculated by comparison of the relative positions for the G₀₋₁ peaks corresponding to the sample nuclei and the nuclei isolated from Tahiti lime, respectively. This permits accurate determination of the unknown DNA content. Calculation was made according to the formula:

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

where Q = unknown DNA content (pg/2C), R = standard 2C DNA content (1.17 pg), E = sample G₀₋₁ peak mean, and S = standard G₀₋₁ peak mean.

Statistical Analysis

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).

Results

Germination rates the seeds of different citrus rootstocks were given in (Table 1) and compared in (Fig. 2). According to the obtained results germination rates were differed statistically important among the rootstocks. The highest germination rate was found on Troyer citrange (16,3%) at the end of first week. The lowest rates were found in Flying Dragon (5,0%), Taiwanica (5,3%) and Citremon (6,2%). Troyer citrange seeds can be considered that having earliest tendency for germination.

Seed germination continued to increase rapidly in all surveyed rootstocks. The highest germination percentage was determined on C-35 Citrange (40,5%) whereas the lowest in Yuzu (19,0%) 14 days after seed sowing. While the highest germination rate was found in C-35 Citrange (95,2%), Troyer Citrange (94,5%), Macrophylla (92,6%) and Yuma citrange (88,9%) rootstocks whereas the lowest rate was observed in Yuzu rootstock (68,3%) after three weeks of sowing. All viable seeds were germinated at the end of four weeks of sowing. The highest germination percentages were obtained from C-35 citrange (98,0%) and Troyer citrange (96,5%) rootstocks whereas the lowest germination percentage was determined in Yuzu (74,0%).

To summarize, 1921 seedlings were obtained at the end of seed germination studies from 2200 seeds. The number of seedlings per genotype was recorded maximum in C35 citrange (196 seedlings) whereas the minimum in Yuzu (148 seedlings) followed by Flying Dragon trifoliate orange (163 seedlings). The difference for germination rates could be due to genotypic difference. A study conducted by [5] C35 citrange had the lowest germination rate under in vitro germination conditions. Contrarily, C35 citrange had the highest germination rate in our research. The reason for the high difference between two researches could be origin of seed source and seed conservation conditions. Some of the citrus genotypes like C-35 citrange and Troyer citrange produced two or more seedlings from one single seed due to nucellar embryony. [18] stated that Troyer citrange has the highest graft success if two weeks old seedlings used for shoot tip grafting. The seedlings obtained from each rootstock were uniform for further evaluation.

Table 1
Germination rates of citrus rootstock seeds

Rootstock species and hybrids	Germination rates (%)				
	7 days	14 days	21 days	28 days	Total number of seedlings
Yuzu CRC	6,8 ^{cd}	19,0 ^e	68,3 ^d	74,0 ^d	148
Chinese sour orange	8,1 ^{bcd}	22,7 ^{de}	78,0 ^{bcd}	88,0 ^{abc}	176
Yuma Citrange SRA	14,3 ^{abc}	36,5 ^{abc}	88,9 ^a	91,0 ^{abc}	182
C-35 Citrange	15,0 ^{ab}	40,5 ^a	95,2 ^a	98,0 ^a	196
Macrophylla	12,1 ^{abcd}	30,2 ^{abcd}	92,6 ^a	94,5 ^{ab}	189
Troyer Citrange	16,7 ^a	38,0 ^{ab}	94,5 ^a	96,5 ^a	193
Gou-tou	7,5 ^{bcd}	28,8 ^{bcde}	81,0 ^{bc}	82,5 ^{bcd}	165
Citremon	6,2 ^d	30,1 ^{abcd}	80,7 ^{bc}	89,0 ^{abc}	178
Taiwanica	5,3 ^d	24,6 ^{de}	78,5 ^{bcd}	82,0 ^{cd}	164
Sunki Mandarin	9,7 ^{abcd}	25,5 ^{cde}	75,2 ^{cd}	83,5 ^{bcd}	167
Flying Dragon	5,0 ^d	22,1 ^{de}	74,8 ^{cd}	81,5 ^{cd}	163
Significance	**	**	**	**	

Flow cytometry analyses are rapid, preparation of nuclear suspensions is easy and statistical distribution of DNA content of large populations is completed rapidly. Cutting fresh leaf tissues from young seedlings with a sharp razor ensures mixing of large numbers of cell nuclei with the nuclear buffer solution. Leaves from young citrus seedlings and Tahiti lime were mixed and prepared for analysis and flow cytometry formed two large peaks showing G_{0-1} peaks for both species. Calculation of total genome in G_{0-1} peak ratio was successfully completed as published by many researchers. Results obtained from flow cytometry analyses show seedlings of the species used in this study had diploid genome. The G_{0-1} peak ratios were smaller than the Tahiti lime genome used as control species. The mean genome volumes for seedling species analyzed in this study are given in (Table 2) and genome size variations among species are given in (Fig. 1). According to the obtained results, Yuzu (0.808 pg) has the largest genome while

Flying Dragon trifoliolate had the smallest genome (0.700 pg). There were statistical differences determined between the seedling populations of the eleven different rootstocks. The Yuzu rootstock is easily proliferated from seeds, has slow growth characteristic, and is a rootstock with high fruit quality and yield. Yuzu has high resistance to low temperatures and polyembryony tendencies. Though it is resistant to phytophthora, fungus and nematodes and tolerant of tristeza, having dwarfing effect, it has moderate resistance to lime and salty soils so commercial use in the world has not become widespread.

Table 2
Comparison of the relative DNA content of the citrus species seedling populations

Rootstock species and hybrids	Ploidy level	Genome size (pg/2C) means
Yuzu CRC	diploid	0,808±0,04 ^a
Chinese sour orange	diploid	0,780±0,06 ^a
Yuma SRA	diploid	0,759±0,03 ^a
C-35 Citranj	diploid	0,755±0,02 ^a
Macrophylla	diploid	0,754±0,07 ^{ab}
Troyer Citranj	diploid	0,754±0,03 ^{ab}
Gou-tou	diploid	0,743±0,08 ^{ab}
Citremon	diploid	0,734±0,03 ^b
Taiwanica	diploid	0,725±0,09 ^b
Sunki Mandarin	diploid	0,711±0,08 ^b
Flying Dragon	diploid	0,700±0,03 ^b
Significance		**

Diploidy is the most common ploidy level in *Citrus* and its related genera with the basic chromosome number $x = 9$. However, some polyploid genotypes were found in Citrus and related genera. Tetraploid Hong Kong wild kumquat (*Fortunella hindsii* Swing.), Triploid Tahiti lime, tetraploid strains of *Poncirus trifoliata*, allotetraploid *Clausena excavata* Burm. F., tetraploid *Clausena harmandiana* and hexaploid *Glycosmis pentaphylla* are some examples of natural polyploidy found in the germplasm of the Aurantioideae subfamily.

Polyembryony is commonly observed in citrus and related genus of *Clausena*, *Fortunella* and *Poncirus*. With nucellar embryony several embryos occur from one seed and these plants carry all the characteristics of the mother plant. As a result, seedlings obtained from species and varieties with excess

polyembryony tendency have diminished genetic expansion [27, 37]. Seedlings occurring from nucellar embryos may be used as rootstock due to having similar features to the mother. Tetraploid plants can be found in zygotic citrus seedling populations and polyploidy level could be reach up 2.5% in Rutaceae according to the previous articles [4, 29, 33].

A study by [33], determined that trifoliolate seedlings had the smallest genome among citrus species. As a result, both common trifoliolate orange and Flying Dragon trifoliolate orange were revealed to have small genomes. The *Poncirus* genus was determined to have smaller genome volume than the citrus genus. Again, Sunki mandarin was found to have smaller genome volume. The study carried out by [33] determined Cleopatra mandarin has smaller genome than other citrus species.

Mandarins having small genome is considered to be effective on both species having small canopy volume, small leaves and small fruit. As a result, Sunki mandarin and Cleopatra mandarin are two valuable rootstocks especially for mandarin species and varieties. C-35, Carrizo and Troyer citrange species have larger genome compared to trifoliates. The statistical differences between them are significant. The reason for this is associated with citranges having larger genome volume than the other parent of orange. [5] demonstrated C-35 citrange seedlings were diploid with 0.794 pg/2c relative genome sizes. This result confirmed our findings in C-35 seedlings.

Conclusions

Genome size refers to the amount of DNA in an organism's non-replicated haploid set of chromosomes [32]. In diploid ($2n=2x$) organisms, genome size refers to the amount of DNA contained in haploid (n number of chromosomes) chromosomes. Genome size is expressed as the C value, which is the amount of DNA content in the genome in picograms. The 2C value is the amount of DNA in the nucleus of a somatic cell, regardless of its ploidy level [14, 35]. Significant (about 1000-fold) differences are observed between species in terms of genome size (C value). On the other hand, the genome size remains constant among different individuals of a species and therefore becomes species specific. Therefore, the C values of species are extremely important for biology, genetics, taxonomy and evolution studies [21, 22, 24, 26, 28]. Flow cytometry is the newest, fast, sensitive and economical method used to determine genome size today. Since there is a very close relationship between the C values determined by flow cytometry and the chromosome numbers of the species, this parameter is also used to determine the ploidy levels of the species [15, 36]. This study showed that nucellar seedling populations of Gou-Tou sour orange, C-35 citrange, Troyer citrange, Taiwanica, Citremon, Yuzu, Sunki mandarin, Flying Dragon trifoliolate orange, Yuma Citrange, Macrophylla and Chinese orange rootstocks had only diploid genomes. In a study conducted by [11], similar to our study results, they reported that the Gou-Tou sour orange rootstocks they used as study material was diploid. There were no polyploids in the surveyed seedling population due to high tendency to nucellar embryony. Polyploidy may have great potential for citrus rootstock breeding. Autotetraploid and allotetraploid rootstocks may have potential especially for dwarfing of citrus trees.

Declarations

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Author contributions

All authors contributed to the study conception and design. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dr. Murat ŞEKER & Dr. Sefa POLATOZ. PhD. Student Çağlar KAYA contributed to the introduction and discussion part of the study. He also contributed to previous studies on the subject. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflicts of interest: The authors declare that they have no conflict of interest.

No human or animal material was used in this study.

Informed consent: The authors of this study have approved to participate in the study.

References

1. Ashkenazi S, Asor Z, Rosenberg O (1992) High density citrus plantation-the use of flying dragon trifoliolate as an interstock. In V International Symposium on Orchard and Plantation Systems 349 (pp. 203-204)
2. Aubert B, Vullin G (1998) Citrus nurseries and planting techniques. Editions Quae
3. Brummer EC, Cazcarro PM, Luth D (1999) Ploidy determination of alfalfa germplasm accessions using flow cytometry. *Crop Sci* 39(4):1202–1207
4. Cameron JWFH (1968) Genetics, breeding and nucellar embryony. *The citrus industry* 325–370
5. Cimen B (2020) Induction of Polyploidy in C35 Citrange through in Vitro Colchicine Treatments of Seed-Derived Explants. *International Journal of Fruit Science* 20(3):1929–1941
6. Davies FS, Albrigo LG (1994) Citrus. CAB International. Wallingford UK 30-33
7. Dolezel J (1998) Flow cytometry, its application and potential for plant breeding. *Current Topics in Plant Cytogenetics Related to Plant Improvement*. (Ed.): T. Lelely. Universitätsverlag, Vienna 80–90
8. Ellialtıoğlu ŞŞ, Sarı N, Abak K (2000) Haploid Plant Production. *Plant Biotechnology Volume: I*. (Ed: M Babaoğlu, E Gürel, S Özcan), s.138-189. Selcuk University Foundation Publications, Konya
9. FAO (2017) Primary Crops Production Datas. FAO Web Pages (<http://www.fao.org>)
10. Ferguson JJ, Chaparro J (2004) Dwarfing and Freeze Hardiness Potential of Trifoliolate Orange Rootstocks: HS982/HS221, 10/2004. *EDIS* 2004(14).

11. Guo WW, Wu RC, Cheng YJ, Deng XX (2008) Regeneration and molecular characterisation of two interspecific somatic hybrids of Citrus for potential rootstock improvement. The Journal of Horticultural Science and Biotechnology 83(4):407–410
12. Jaskani MJ, Abbas H, Khan MM, Shahzad U, Hussain Z (2006) Morphological description of three potential citrus rootstocks. Pak J Bot 38(2):311
13. Johnson PG, Riordan TP, Arumuganathan K (1998) Ploidy level determinations in buffalograss clones and populations. Crop Sci 38(2):478–482
14. Kaya MM, Şakiroğlu M (2012) Estimating Genome Size and Confirming Ploidy Levels of Wild Tetraploid Alfalfa Accessions (*Medicago sativa* Subsp. *varia*) Using Flow Cytometry. Turkish Journal Of Field Crops 17(2):151–156
15. Kaya M (2010) *Medicago sativa* ssp. *varia* populasyonlarının ploidi seviyesinin flow sitometri yöntemiyle belirlenmesi (Doctoral dissertation, Yüksek Lisans Tezi, Kafkas Üniversitesi Fen Bilimleri Enstitüsü, Kars, 9-11)
16. Louzada ES, Rio D, Setamou HS, Watson M, Swietlik DM (2008) Evaluation of citrus rootstocks for the high pH, calcareous soils of South Texas. Euphytica 164(1):13–18
17. Matheron ME, Matejka JC (1988) Persistence of systemic activity for fungicides applied to citrus trunks to control Phytophthora gummosis. Plant Dis 72(2):170–174
18. Navarro L, Juárez J (2007) Shoot-tip grafting in vitro: impact in the citrus industry and research applications. *Citrus genetics, breeding and biotechnology*, 353–364
19. Ollitrault P, Michaux-Ferrière N (1994) Application of flow cytometry for citrus genetic and breeding. ISC
20. Ollitrault P, Dambier D, Luro F, Duperray C (1994) Nuclear genome size variations in Citrus. Fruits (1978) 49(5–6):390–393
21. Ohri D (1998) Genome size variation and plant systematics. Ann Botany 82:75–83
22. Özkan H, Tuna M, Arumuganathan K (2003) Nonadditive changes in genome size during allopolyploidization in the wheat (*Aegilops-Triticum*) group. J Hered 94(3):260–264
23. Özcan MO, Ulubelde M (1984) Citrus rootstocks. Ege Region Agricultural Research Institute Publications No: 50 Menemen / İzmir.
24. Patrick Ollitrault YF, Dambier D, Luro F, Yamamoto M (2007) 8 Seedlessness and Ploidy Manipulation. *Citrus genetics, breeding and biotechnology*, 197
25. Palomino G, Dolezel J, Mendez I, Rubluo A (2003) Nuclear genome size analysis of Agave tequilana Weber. Caryologia 56(1):37–46
26. Price HJ, Bachmann K (1975) DNA content and evolution in the Microseridinae. Am J Bot 62(3):262–267
27. Polatöz S (1995) Determination of Fruit Yield and Quality Characteristics of Some Local and Foreign Origin Orange Clones in Adana Conditions. Çukurova University Institute of Natural and Applied Sciences Department of Horticulture, Master Thesis. S: 6

28. Rees H, Walters MR (1965) Nuclear DNA and the evolution of wheat. *Heredity* 20(1):73–82
29. Rom RC, Carlson RF (1987) *Rootstocks for fruit crops* (No. 634.0432 R6)
30. Salameh NM (2014) Flow cytometric analysis of nuclear DNA between okra landraces (*Abelmoschus esculentus* L.). *American Journal of Agricultural and Biological Sciences* 9(2):245–250
31. Saunt J (2000) *Citrus varieties of the world* 2. nd ed Sinclair International Norwich
32. Swift H (1950) The constancy of desoxyribose nucleic acid in plant nuclei. *Proc Natl Acad Sci USA* 36(11):643
33. Şeker M, Tuzcu O, Ollitrault P (2003) Comparison of nuclear DNA content of citrus rootstock populations by flow cytometry analysis. *Plant Breeding* 122(2):169–172
34. Teykin EE (2011) Determination of Core DNA Content of *Bromus catharticus* Vahl Accessories by Flow Cytometry. (Master Thesis), Namık Kemal University. Institute of Natural and Applied Sciences Department of Field Crop, Tekirdağ
35. Tuna M (2014) Flow Cytometry and Its Use in Agricultural Research. 2. Flow Cytometry and Use in Agricultural Research Training Program Booklet. Namık Kemal University Faculty of Agriculture Department of Horticulture, Tekirdağ
36. Tuna M, Vogel KP, Arumuganathan K, Gill KS (2001) DNA content and ploidy determination of bromegrass germplasm accessions by flow cytometry
37. Wakana A, Uemoto S (1987) Adventive embryogenesis in citrus I. The occurrence of adventive embryos without pollination or fertilization. *Am J Bot* 74(4):517–530

Figures

Figure 1

Genome size variations among citrus rootstocks (bar indicates the standard error)

Comparison of Germination Rates of Citrus Rootstocks at the end of First, Second, Third and Fourth Weeks

Figure 2

Comparison of germination rates of citrus rootstocks at the end of first, second, third and fourth weeks