Artículo de investigación

Analysis of the karyotype of the Russian apple tree clonal rootstocks bred at the Michurinsk State Agrarian University

Анализ кариотипа российских клоновых подвоев яблони селекции Мичуринского государственного аграрного университета

Análisis del cariotipo de portainjertos clonales de manzano ruso criados en la Universidad Agraria del Estado de Michurinsk

Recibido: 9 de junio del 2019 Aceptado: 10 de julio del 2019

Written by:
M.L. Dubrovsky[244]
ORCID ID: 0000-0003-0883-2867
https://elibrary.ru/author_items.asp?authorid=636133
R.V. Papikhin[245]
ORCID ID: 0000-0001-8015-4242
https://elibrary.ru/author_items.asp?authorid=404422

Abstract

For the first time, karyotypes of six forms of the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University and their main cytogenetic features have been studied. In analyzing the mitotic division of root cells, it has been found that all studied genotypes in the (2n = 2x) diploid set have 34 chromosomes, which make 17 pairs of components. By the morphological structure, all the chromosomes are classified as metacentric and submetacentric; with that, no acrocentric and telocentrics chromosomes have been found. All chromosomes are small — 0.85 to 4.58 μm, their average length by genotypes varies from 2.05 ± 0.13 μm for the 2-15-2 hybrid to 2.71 ± 0.21 μm for the 2-9-83 form. This makes identification of homologous pairs of chromosomes difficult. Depending on the genotype, one to eight pairs of satellite chromosomes were found. Correlations of the relative indicators of chromosomes morphology among the studied clonal rootstocks were the following: by the index of their length Lr +0.85...+0.99; by the index of arm lengths -0.41...+0.35; and by the centromeric index -0.44...+0.31. By the obtained morphometric data of the metaphase plates, individual and generalized idiograms of the chromosomes of the clonal apple tree rootstocks have been built. The identified cytogenetic differences in the karyotypes of the studied forms of rootstocks are

Аннотация

Впервые проведено изучение кариотипов 6 форм клоновых подвоев яблони селекции Мичуринского государственного аграрного университета и выявлены их основные цитогенетические особенности. При анализе митотического деления клеток корней установлено, что все изученные генотипы в диплоидном наборе (2n=2x) имеют 34 хромосомы, составляющие 17 пар. По морфологическому строению все хромосомы являются метацентрическими и субметацентрическими, при этом акроцентрических и телоцентрических хромосом не выявлено. Все хромосомы мелкие – от 0,85 до 4,58 мкм, их средняя длина по генотипам варьирует от 2,05±0,13 мкм у гибрида 2-15-2 до 2,71±0,21 мкм у формы 2-9-83. Это затрудняет идентификацию гомологичных пар хромосом. В зависимости от генотипа отмечено наличие от 1 до 8 пар спутников хромосом. Корреляции относительных показателей морфологии хромосом среди изученных клоновых подвоев составили: по индексу их длины Lr +0,85...+0,99; по индексу длин плеч -0,41...+0,35; по центромерному индексу -0,44...+0,31. По полученным морфометрическим данным метафазных пластинок построены индивидуальные и обобщенная идиограммы хромосом

[244] Federal State Budgetary Educational Institution of Higher Education «Michurinsk State Agrarian University», Internatsionalnaya str., 101, Michurinsk, Tambov region, 393760, Russia
[245] Federal State Budgetary Educational Institution of Higher Education «Michurinsk State Agrarian University», Internatsionalnaya str., 101, Michurinsk, Tambov region, 393760, Russia

not essential, as evidenced by these genotypes' ability to interbreed with each other.

Keywords: Apple tree, chromosome, centromere, idiogram, karyotype, satellite chromosome.

клоновых подвоев яблони. Выявленные цитогенетические различия кариотипов изученных форм подвоев не являются существенными, что подтверждается способностью данных генотипов скрещиваться между собой.

Ключевые слова: Яблоня, хромосома, центромера, спутник хромосомы, кариотип, идиограмма.

Resumen

Por primera vez, se realizó el estudio de los cariotipos de 6 formas de portainjertos de manzana clonales en la selección de la Universidad Estatal Agraria Michurinsky y se identificaron sus principales características citogenéticas. Al analizar la división mitótica de las células de la raíz, se estableció que todos los genotipos estudiados en el conjunto diploide (2n = 2x) tienen 34 cromosomas, que consisten en 17 pares. Según la estructura morfológica, todos los cromosomas son metacéntricos y submetacéntricos, sin cromosomas acrocéntricos y telocéntricos. Todos los cromosomas son pequeños: de 0.85 a 4.58 micras, su longitud promedio para los genotipos varía de 2.05 ± 0.13 micras en un híbrido 2-15-2 a 2.71 ± 0.21 micras en una forma 2-9 -83. Esto dificulta la identificación de pares de cromosomas homólogos. Dependiendo del genotipo, se observa la presencia de 1 a 8 pares de satélites cromosómicos. La correlación de los indicadores relativos de la morfología cromosómica entre los portainjertos clonales estudiados fue la siguiente: por el índice de su longitud Lr +0.85 ... + 0.99; en un índice de longitudes de hombros-0,41 ... + 0,35; en el índice centromérico de -0.44 ... + 0.31. De acuerdo con los datos morfométricos obtenidos de las placas de metafase, se construyeron ideogramas individuales y generalizados de cromosomas de portainjertos de manzana clonales. Las diferencias citogenéticas identificadas de los cariotipos de las formas estudiadas de portainjertos no son significativas, como lo demuestra la capacidad de estos genotipos para cruzarse entre sí.

Palabras clave: Manzano, cromosoma, centrómero, satélite cromosómico, cariotipo, idiograma.

Introduction

Quantitative attributes of chromosomes are important indicators for characterizing the genotype. A set of chromosomes in a somatic cell of any kind is called a karyotype. S. G. Navashin believed that the species specificity of the number of chromosomes and their morphological characteristics were the first, the most equal traits of organisms; therefore, he suggested calling the metaphase plate of the chromosomes an ideogram, i.e., a species formula (Navashin, 1911). The body of any chromosome has a primary constriction, the position of which determines the relative length of the chromosome shoulders. The primary constriction always has a light zone – the centromere with a small body or a granule. The centromere defines the entire dynamics of chromosomes in the mitosis, as it is attached to the thread of the achromatic spindle that ensures the anaphase division of daughter chromosomes.

All existing chromosomes classifications by the morphological principle are built on their shape, size, the position of the centromere, the presence of secondary constrictions and satellites.

By their shape and the arms ratios, the chromosomes are divided into:

- Metacentric, in which arms have the same or nearly the same size;
- Submetacentric (weakly heterobrachial);
- Acrocentric (strongly heterobrachial); and
- Telocentric (rod-shaped).

One of the important indicators in these classifications is the centromeric index (CI) — the ratio of the length of the short arm of the chromosome to its entire length expressed in percent.

Comparative analysis of the chromosomes in genotypes in the same taxonomic unit is an important method of assessing their difference or

ISSN 2322- 6307

similarity, as well as the homology of chromosomes for indirect diagnostics of the potential mutual combining ability of these forms and fertility. This method has been for a long time modified by various methods and technologies that significantly improve its efficiency (Badaeva, Salina, 2013). Compared to many wild and cultivated herbaceous plants, all fruit crops are characterized by the reduced mitotic index, the small size of chromosomes and, as a rule, their significant number in the diploid set, which makes cytogenetic analysis difficult.

Among fruit crops, one of the leading places in the world belongs to the apple tree. According to one of the hypotheses, the haploid number of chromosomes (n = x = 17) in the *Malus* Mill genus formed as a result of polyploidization of the genome of the original ancestor with n = x = 9 with simultaneous deletion of one chromosome (Velasco et al., 2010). In most varieties and natural forms of apple trees of various species, the diploid number of chromosomes in the karyotype (2n = 2x = 34) was noted; triploids and tetraploids were significantly rarer (Lincoln, McCann, 1937; Einset, Imhoff, 1949; Oldén, Koch 1961; Guo-Lu, 1987; Schuster, Büttner, 1995; Guo et al., 2007; Tuz, Lozitsky, 1970; Solovieva, 1973, 1979; Tuz, 1974; Tuz et al., 1979; Sedov et al., 2008).

Karyological analysis of fruit crops is difficult and thus is not as intense as among many other cultivated plants. Independent studies are devoted to studying the karyotype of the cultivars and wild apple trees species (Guo-lu, Xiao-lin, 1993; Schuster et al., 1997; Bouvier et al., 2000; Guo et al., 2007), wild species of stone fruit crops (Wang et al., 2018). Particularities of the karyotype and the level of ploidy of clonal rootstocks of both domestic and foreign-bred apple trees have not been studied previously.

The Michurinsk State Agrarian University is the Russia's largest center for creating, studying, and identification of new promising genotypes of dwarf clonal apple tree rootstocks, the breeding of which was initiated by V. I. Budagovsky (Budagovsky 1959, 1963, 1976). The cloned apple tree rootstocks obtained here are complex interspecies hybrids that combine in their genotype the material with memory of wild species — *M. pumila var. paradisiaca* (L.) Schneid., *M. niedzwetzkyana* Dieck ex Koehne, *M. baccata var. sibirica* Schneid, *M. sieboldii* (Regel) Rehd. etc. As a result, cytogenetic analysis and determination of the ploidy level of clonal apple trees rootstocks are of great scientific and practical value for breeding.

Objects, conditions, and methods

Six genotypes of dwarf and semi-dwarf apple tree clonal rootstocks bred at the Michurinsk State Agrarian University were chosen as biological objects for the study: zoned in Russia – Paradizka Budagovskogo (PB, B9), 54-118, and promising forms that were passing primary grade testing, – 2-9-83, 2-12-10, 2-15-2, 2-15-15. Young growing roots had been fixed in Carnoy's fixative (three parts of 96 % ethanol: one part of glacial acetic acid), after which the material was stored in 70 % ethanol. The metaphase plates of mitotic divisions were analyzed on temporary crushed acetohematoxylin slices by the method of L. A. Topilskaya, S. V. Luchnikova, and N. P. Chuvashina (1976).

Microscopic analysis of the metaphase plates was performed in transmitted light on microscope Leica DM2500. Photographying of the studied slices was performed with digital camera DCM-500 using the ScopePhoto software.

The types of chromosomes according to their proportions were determined according to the generally adopted classification (Levan et al., 1964).

The index of the relative length of the Lr chromosome was calculated by the following formula (Dosba, Cauderon, 1973):

$$Lr = \frac{l+s}{\sum(l+s)} \times 17$$

The index of arm lengths of each chromosome (brachial index, l/s) was determined by dividing the values of the long arm (l) by the length of the short arm (s).
The centromeric index (I_c) was calculated as the ratio of the length of the short arm to the length of the entire chromosome: $\frac{s}{l+s}$ (without expression as a percentage).

The obtained experimental data were statistically processed in the Microsoft Office Excel 2016 software suite.

ISSN 2322- 6307

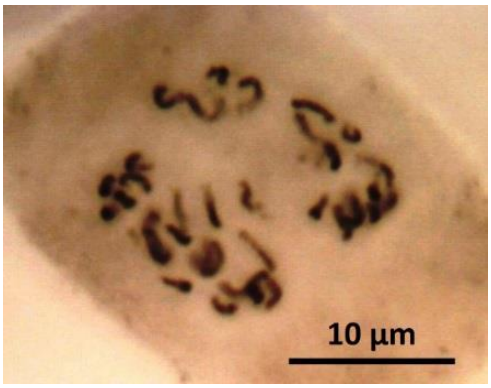
Results

As a result of the research, basic karyograms of the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University were compiled and analyzed.

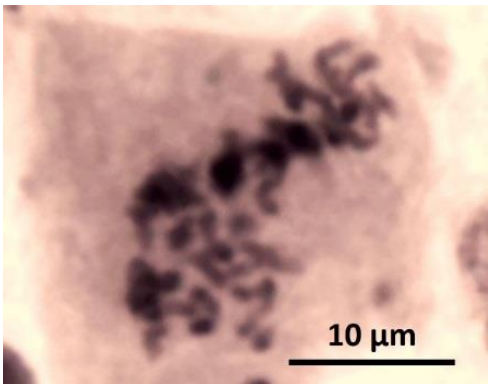
The number of chromosomes in zoned and promising clonal apple tree rootstocks bred at the Michurinsk State Agrarian University (Fig. 1) has been determined for the first time: all studied forms of the *Malus* Mill. genus have 34 chromosomes in their vegetative cells, i.e., are diploids (2n = 2x = 34). This allows for crossing forms with the same number of chromosomes, as well as planning schemes of hybridization of the diploid rootstock with the genotypes that have high ploidy level, which are donors of unreduced gametes.

Based on the fixed images of the microslices with contrast-stained chromosomes of metaphase plates of clonal rootstocks, their base karyograms were built, which had characteristic differences for each rootstock (Fig. 2).

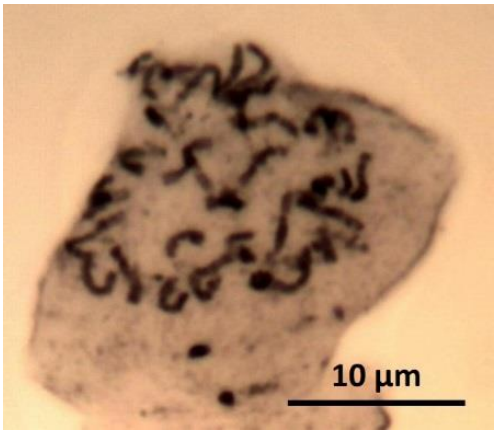
By the morphological structure, all the chromosomes of the rootstock apple tree forms are classified as metacentric and submetacentric; with that, no acrocentric and telocentrics chromosomes were found. All chromosomes were small — 0.85 to 4.58 µm, their average length varied from 2.05 ± 0.13 µm for the 2-15-2 hybrid to 2.71 ± 0.21 µm for the 2-9-83 form. This complicates identification of homologous pairs of chromosomes by visual analysis, and "dithers" the boundary between metacentric and submetacentric chromosomes.



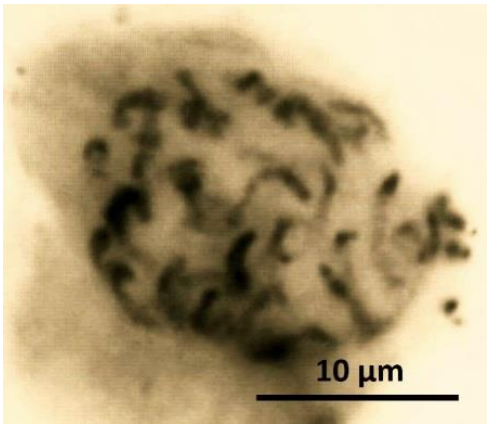
54-118 (2n = 2x = 34)



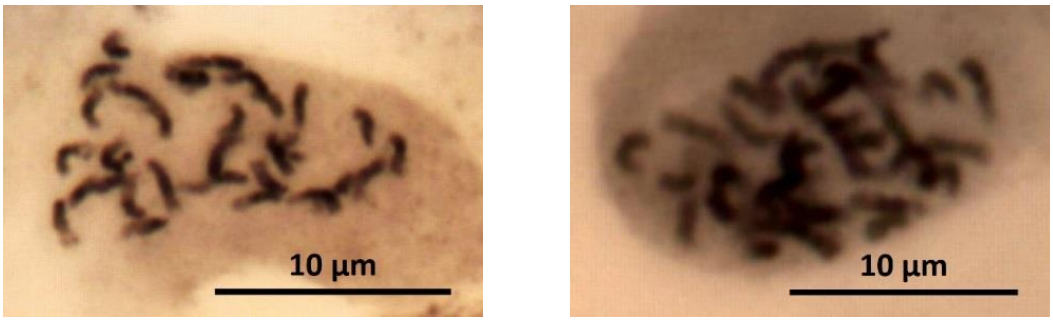
B9 (2n = 2x = 34)



2-9-83 (2n = 2x = 34)



2-12-10 (2n = 2x = 34)



2-15-2 (2n = 2x = 34) **2-15-15** (2n = 2x = 34)

Figure 1. Metaphase plates of mitotic division in the cells in the growth zone of young roots of the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University.

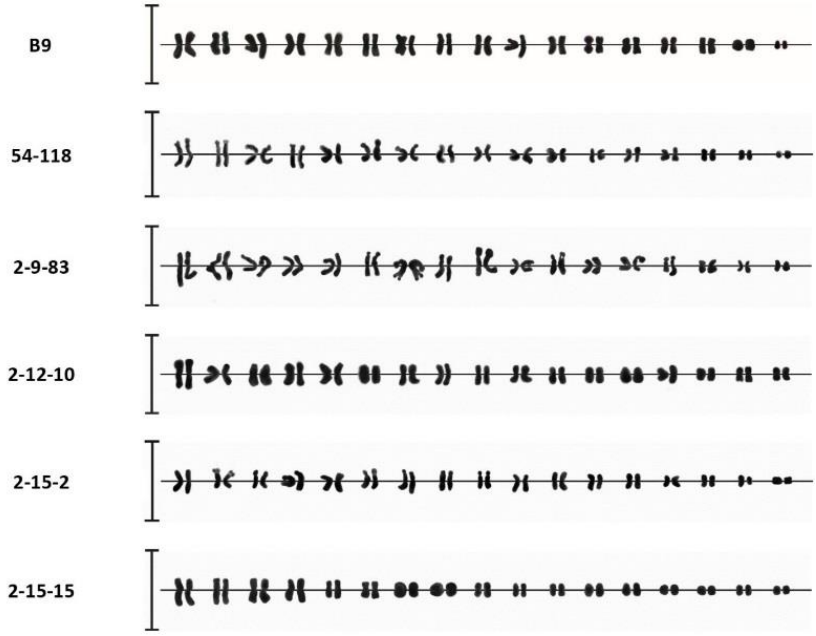


Figure 2. Pairs of homologous chromosomes of the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University. Scale bar — 10 micrometers

Depending on the genotype of the rootstock, the presence of one to eight pairs of satellite chromosomes was noted, which was visually well-expressed (Fig. 3). This is consistent with the available literature data: for instance, using the FISH technology, in the diploid genome of apple tree on the example of cultivar of Pinova, eight nucleolar organizer regions (NOR) were identified (Schuster et al., 1997). However, only 1 – 2 satellites were identified during further studies of chromosomes in various forms of apple trees, and the others were hardly visible due to a very small size (Bouvier et al., 2000).

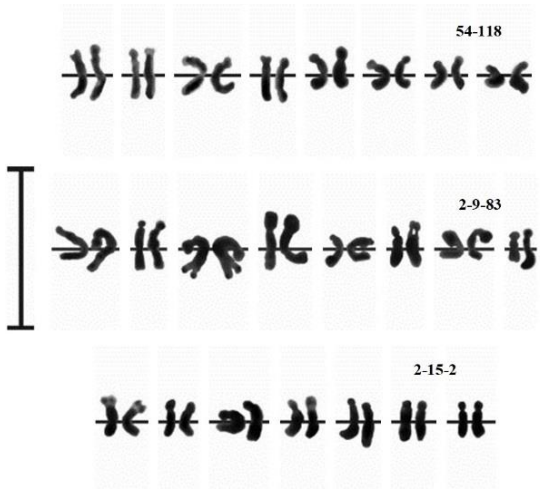


Figure 3. Satellite chromosome in the genotypes of clonal dwarf apple tree rootstocks. Scale bar — 10 micrometers

Morphometric features of chromosomes and the chromosomal formula of the studied clonal apple tree rootstocks are shown in Table 1.

Table 1. Morphological characteristic of the karyotype of the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University

Genotypes	Diploid set of chromosomes (2n = 2x), pcs.	Length of chromosomes, µm			Number of satellite pairs, pcs.	Chromosomal formula
		average, M ± m	minimum	maximum		
B9	34	2.38 ± 0.13	1.08	3.15	2	16m + 1sm
54-118	34	2.07 ± 0.14	0.85	3.17	8	14m + 3sm
2-9-83	34	2.71 ± 0.21	1.39	4.58	8	12m + 5sm
2-12-10	34	2.46 ± 0.13	1.61	3.70	–	16m + 1sm
2-15-2	34	2.05 ± 0.13	0.92	3.07	7	15m + 2sm
2-15-15	34	1.97 ± 0.18	1.08	3.53	1	14m + 3sm

By the obtained morphometric data of the metaphase plates, individual and generalized idiograms of the chromosomes of the clonal apple tree rootstocks were built (Fig. 4, 5).

ISSN 2322- 6307

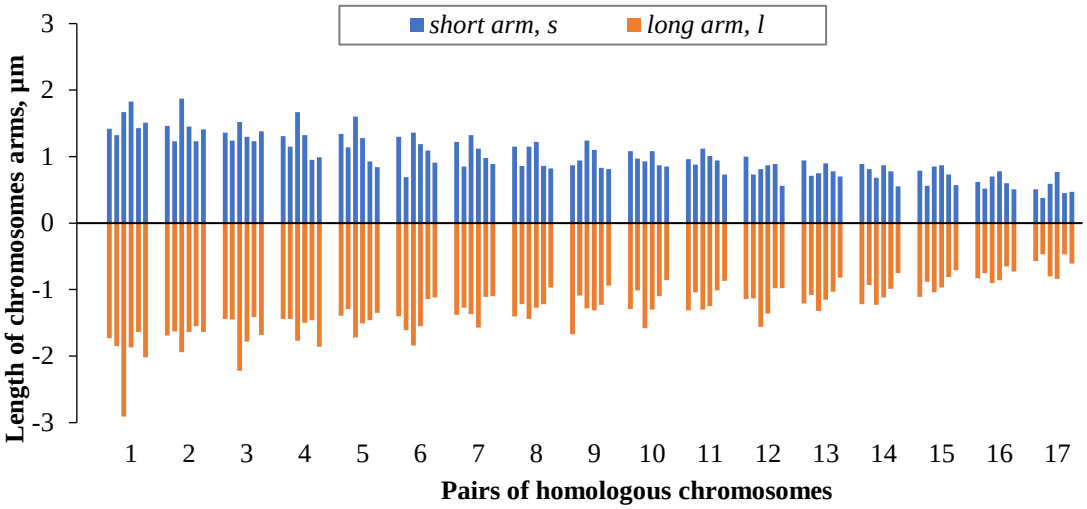


Figure 4. Comparative analysis of the ratio of chromosome arms in the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University (from left to right – shapes B9, 54-118, 2-9-83, 2-12-10, 2-15-2, 2-15-15)

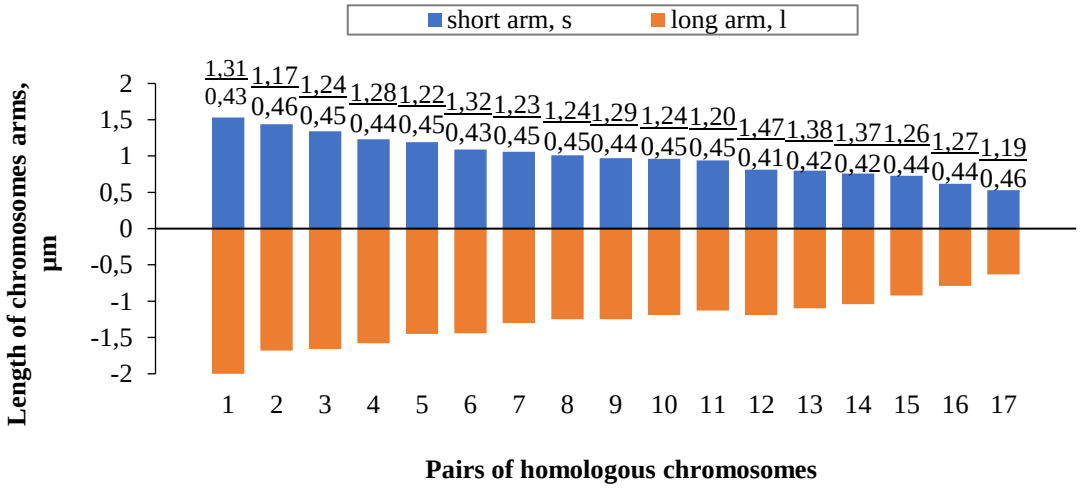


Figure 5. Idiogram of the chromosomes in the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University (based on comparative analysis of six genotypes). The numerator of the fraction is the averaged index of chromosome shoulders, the denominator is the centromeric index

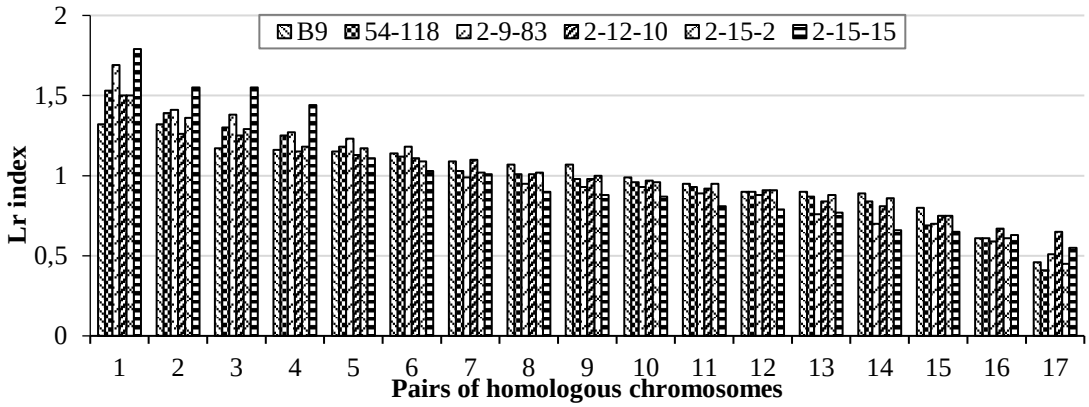
The average values of the absolute size of each pair of chromosomes and their relative shape indices in the studied clonal apple tree rootstocks are shown in Table 2.

Table 2. Characteristic of the chromosomes in the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University (based on comparative analysis of six genotypes)

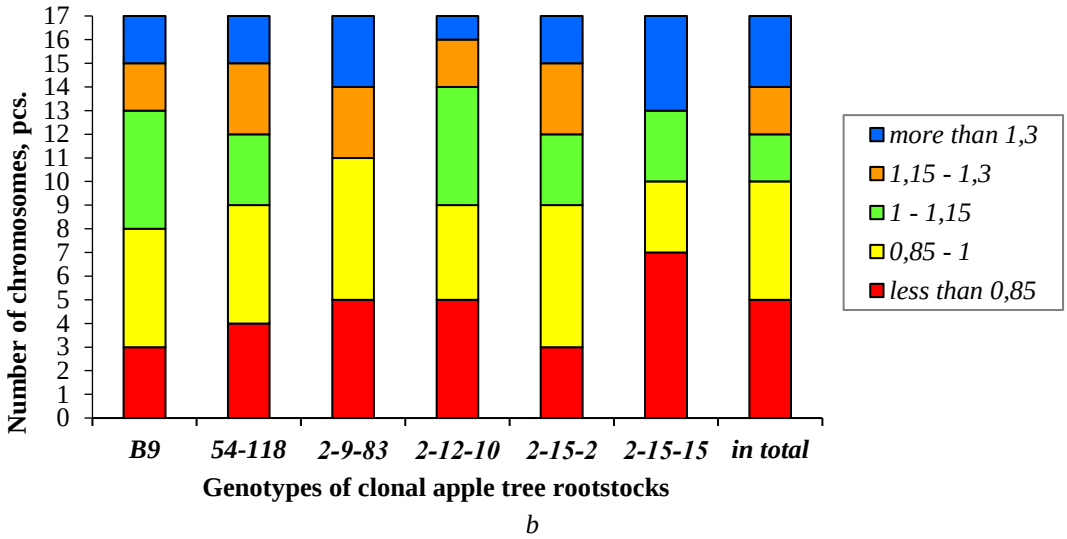
Chromosome pairs	Chromosome linear dimensions, µm			Lr index	Arm length index (brachial index), l/s	Centromeric index, s/l+s	Chromosome type
	Short arm length (s)	Long arm length (l)	Total length (l+s)				
1	1.53	2.00	3.53	1.55	1.31	0.43	m
2	1.44	1.68	3.12	1.37	1.17	0.46	m
3	1.34	1.66	3.00	1.32	1.24	0.45	m
4	1.23	1.58	2.81	1.24	1.28	0.44	m
5	1.19	1.45	2.64	1.16	1.22	0.45	m
6	1.09	1.44	2.53	1.11	1.32	0.43	m
7	1.06	1.30	2.36	1.04	1.23	0.45	m
8	1.01	1.25	2.26	1.00	1.24	0.45	m
9	0.97	1.25	2.22	0.98	1.29	0.44	m
10	0.96	1.19	2.15	0.95	1.24	0.45	m
11	0.94	1.13	2.07	0.91	1.20	0.45	m
12	0.81	1.19	2.00	0.88	1.47	0.41	m
13	0.8	1.10	1.90	0.84	1.38	0.42	m
14	0.76	1.04	1.80	0.79	1.37	0.42	m
15	0.73	0.92	1.65	0.73	1.26	0.44	m
16	0.62	0.79	1.41	0.62	1.27	0.44	m
17	0.53	0.63	1.16	0.51	1.19	0.46	m

The morphometric characteristics of the chromosomes of the studied clonal apple tree rootstocks bred at the Michurinsk State Agrarian University show close values of the average specific indicators (Table 2). By the size groups of chromosomes determined by the relative index of length Lr, certain similarity is also observed in all studied forms (Fig. 6). The comparative analysis of the chromosomes of the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University by the arm length index and centromeric index showed great variability between various genotypes (Fig. 7, 8).

Correlations of the relative indicators of chromosomes morphology among the studied clonal rootstocks were the following: by the index of their length Lr +0.85...+0.99; by the index of arm lengths -0.41...+0.35; and by the centromeric index -0.44...+0.31.



a



b

Figure 6. The characteristic of the karyotype of the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University on the relative index of the lengths of the chromosomes Lr: *a* – comparison by genotypes; *b* – chromosomes grouping by intervals of index values

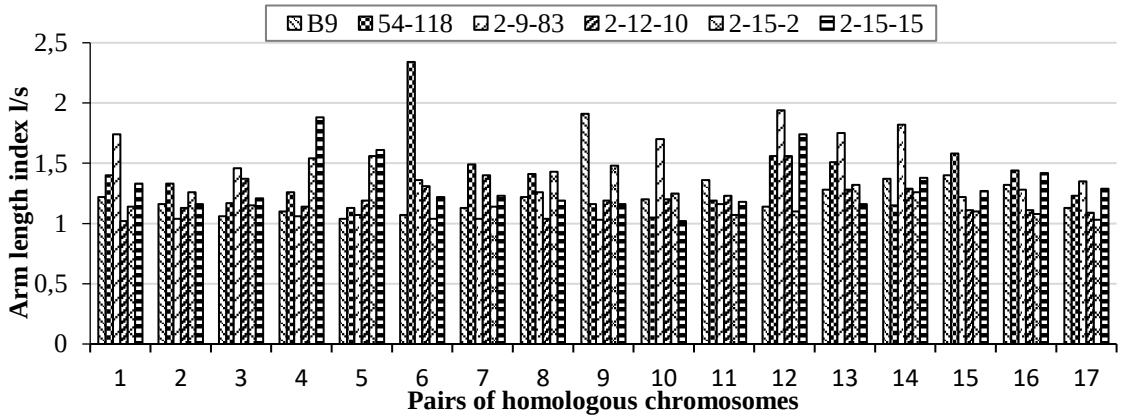


Figure 7. Comparative analysis of the chromosomes of the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University by the arm length index

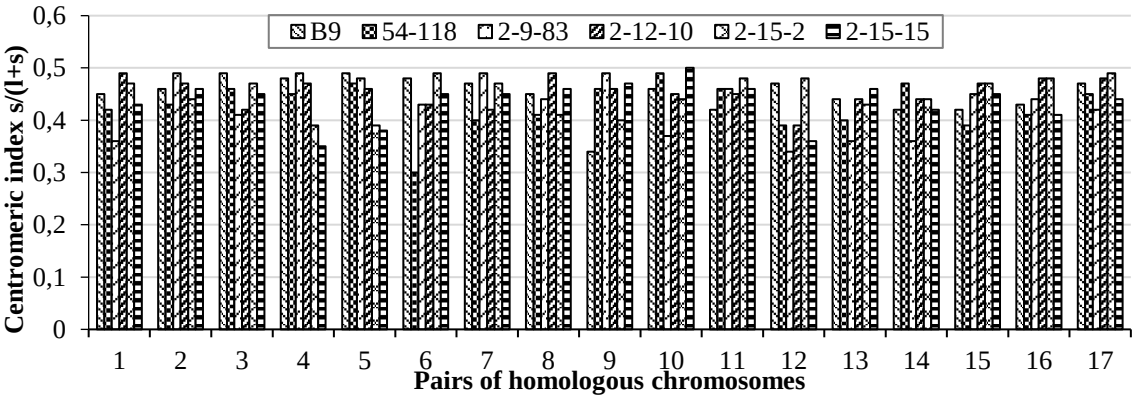


Figure 8. Comparative analysis of the chromosomes of the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University by the centromeric index

Conclusion

Based on the cytogenetic studies, karyotyping of six forms of the clonal apple tree rootstocks bred at the Michurinsk State Agrarian University was performed for the first time, and their features were identified. In analyzing the mitotic division of root cells, it has been found that all studied genotypes in the (2n = 2x) diploid set have 34 chromosomes, which make 17 pairs of components.

By the morphological structure, all the chromosomes are classified as metacentric and submetacentric; with that, no acrocentric and telocentric chromosomes have been found. All chromosomes are small — 0.85 to 4.58 µm, their average length by genotypes varies from 2.05 ± 0.13 µm for the 2-15-2 hybrid to 2.71 ± 0.21 µm for the 2-9-83 form. This makes identification of homologous pairs of chromosomes difficult. Depending on the genotype, one to eight pairs of satellite chromosomes have been found. Correlations of the relative indicators of chromosomes morphology among the studied clonal rootstocks were the following: by the index of their length Lr +0.85...+0.99; by the index of arm lengths -0.41...+0.35; and by the centromeric index -0.44...+0.31. By the obtained morphometric data of the metaphase plates, individual and generalized idiograms of the chromosomes of the clonal apple tree rootstocks have been built.

All the identified cytogenetic differences of the karyotypes of the studied forms of rootstocks are not significant, which is confirmed in practice – all these genotypes can interbreed with each other, forming viable pollen and seeds.

Acknowledgment

The studies were performed at the core facility center "Selection of Crops and Technologies of Producing, Storing and Processing of Functional and Preventive Care Food Products" of the FSBEI HE Michurinsk State Agrarian University in the framework of the State Task of the Ministry of Agriculture of the Russian Federation No. 30 "Breeding Winter-Hardy Dwarf Clonal Rootstocks using Molecular Markers" in 2019.

References

Badaeva E. D., Salina E. A.. (2013). Struktura genoma i khromosomnyi analiz rastenii [Structure of genome and chromosomal analysis of plants]. *Vavilov journal of genetics and plant breeding*. 17(4/2), 1017 – 1043.

Bouvier L., Lespinasse Y., Schuster M. (2000). Karyotype analysis of ahaploid plant of apple (Malus domestica). Proc. EUCARPIA Symp. on Fruit Breed. and Genetics / Eds M. Geibel, M. Fischer & C. Fischer. *Acta Hort*, 538, 321-324.

Budagovsky V. I. (1959). *Karlikovye podvoi dlya yabloni* [Dwarf apple tree rootstocks]. M.: Selkhozgiz, 352.

Budagovsky V. I. (1963). *Promyshlennaya kultura karlikovykh plodovykh derevyev* [Industrial culture of dwarf fruit trees]. M., 382.

Budagovsky V. I. (1976). *Kultura slaboroslykh plodovykh derevyev* [The culture of dwarf fruit trees]. M.: Kolos, 304.

Dosba F., Cauderon Y. (1973). Analyse statistique du caryotype d'Aegilops ventricosa. *Tausch. Ann. Amélior. Plantes*, 23, 133-143.

Einset J., Imhoff B. (1949). Chromosome numbers of apple varieties and sports II. *Proc. Amer. Soc. Hort. Sci*, 53, 197-201.

Guo Q., Yu Y., He Q., Li X., Liang G. (2007). AFLP analysis of four wild Malus Mill. *Acta Horticulturae*, 760, 131-136.

Guo-Lu L. (1987). Observation of Chromosomes of Malus species in China. *Acta Phytotaxonimca Sinica*, 25, 437-441.

Guo-lu L., Xiao-lin L. (1993). Chromosome studies of species of Malus Mill. *Acta Phytotaxonomica Sinica*, 31 (3), 236-251.

Levan A., Fredga K., Sandberg A.A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas*, 52 (2), 201-220.

Lincoln F.B., McCann L.P. (1937). Polyploidy in Native Species of Malus. *Proc. Amer. Soc. Hort. Sci.*,34, 26.

Navashin S. G. (1911). *Ob individualnykh i vidovykh otlichiyakh khromozom* [On individual and species differences of chromosomes]. Minutes of a meeting of the Kiev Society of Naturalists.

Oldén, E. J., Koch, A. (1961). *Stone fruits, root-stocks and strawberries*. Institute of Fruit and Berry Breeding. Report on activities in the year, 16-24

Schuster M., Büttner R. (1995). Chromosome numbers in the Malus wild species collection of the genebank Dresden-Pillnitz. *Genet. Resour. Crop Evol.*, 42(4), 353-361.

Schuster M., Fuchs J., Schubert I. (1997). Cytogenetics in fruit breeding-localization of ribosomal RNA genes on chromosomes of apple (Malus domestica Borkh.). *Theor. Appl. Genet.*, 94, 322-324.

Sedov E. N., Sedysheva G. A., Serova Z. M. (2008). *Breeding apples at the polyploid level.* Orel: VNIISPK, 368.

Solovieva L. V. (1979). Issledovanie ploidnosti kulturnykh sortov Malus domestica (L.) Borkh. i

dikorastushchikh vidov roda Malus Mill. [Studying the ploidy cultivars Malus domestica (L.) Borkh. and wild species of genus Malus Mill]. *Cytology and genetics*, 13(5), 366 – 369.

Solovyieva L. V. (1973). Khromosomnye chisla elitnykh seyantsev i sortov yabloni [Chromosome numbers of elite seedlings and apple varieties]. *Genetics*, 9(11), 66 – 72.

Topilskaya L. A., Luchnikova S. V., Chuvashia N. P. (1976). Metodika prigotovleniya atsetogematoksilinovykh preparatov [Methods of preparing acetohaematoxylin preparations]. *Cytological studies of fruit and berry crops*. Michurinsk, 58 – 60.

Tuz A. C., Lozitsky A. Y. 1970. Poliploidnye sorta yabloni i grushi [Polyploid varieties of apples and pears]. *Genetics*, 6(9), 41 – 50.

Tuz A. C., Varshanina T. P., Romanova N. I. (1979). Poliploidnye sorta yabloni Malus Mill. i grushi Pyrus L. [Polyploid apple cultivars Malus Mill. and pear cultivars Pyrus L.]. *Genetics*, 15(4), 684 – 690.

Tuz. A.C. (1974). Poliploidiya u vidov Malus Mill. i Pyrus L. [Polyploidy in species Malus Mill. and Pyrus L.]. *Reports of the meeting of scientists before the XIX International Horticulture Congress, Poland, Warsaw*. M.: Kolos, 278 – 282.

Velasco R., Zharkikh A., Affourtit J. et al. (2010). The genome of the domesticated apple (Malus × domestica Borkh). *Nature Genetics*, 42(10), 833-841.

Wang Y., Du H.-M., Zhang J., Chen T., Chen Q., Tang H.-R., Wang X.-R. (2018). Ploidy level of Chinese cherry (Cerasus pseudocerasus Lindl.) and comparative study on karyotypes with four Cerasus species. *Scientia Horticulturae*, 232, 46-51.

ISSN 2322- 6307