

Cytogenetic polymorphism of seed progeny of walnut (*Juglans regia* L.) during introduction in the Central Chernozem Region

Vladislav N. KALAEV¹, Svetlana S. KARPOVA^{2*},
Firas R.H. AL-HACHAMI¹, Irina V. IGNATOVA¹,
Vasiliy A. SLAVSKIY³

¹Voronezh State University, Faculty of Medicine and Biology, 1 Universitetskaya pl., 394018, Voronezh, Russian Federation; dr_huix@mail.ru; firmasrah@gmail.com; irina777.84@list.ru

²Voronezh State Institute of Physical Culture, 59 Karla Marksa st., 394036, Voronezh, Russian Federation; karpovass@mail.ru (*corresponding author)

³Voronezh State University of Forestry and Technologies named after G.F. Morozov, Faculty of Forestry & Timiryazeva str., 394087, Voronezh, Russian Federation; vasiliy1980@mail.ru

Abstract

We studied the cytogenetic characteristics of the seed progeny of the walnut (*Juglans regia* L.) trees introduced to and grown within the territory of the Central Russian Upland. Three seedling groups with polymorphic cytogenetic characteristics were revealed: mutable (with a high level of pathological mitoses), low mutable (with a low level of cytogenetic disturbances), and intermediate groups. Cytogenetic characteristics (mitotic activity, parts of cells at various stages of mitosis, the level and spectrum of pathological mitoses, sizes of nucleoli and the spectrum of their types, the occurrence of cells with a persistent nucleolus in the stages of meta-, ana-, and telophase) in each of the selected groups were described; homeostatic mechanisms at the cellular level were discussed. The sizes of polymorphic groups were established. The small number of seedlings with a high level of cytogenetic disturbances (7.5%) and the predominance of seedlings with medium (70%) and low (22.5%) values of pathological mitoses indicated a high degree of adaptation of the introduced walnut mother trees to the environmental conditions of the Central Russian Upland. Predictors for assigning any seedling to one of the selected model groups (mutable or low mutable) were established using ROC analysis methods. The obtained data on the qualitative and quantitative polymorphism of cytogenetic characteristics can be used for the development of recommendations for improving the system of seed production and the selection of new forms of walnut in the Central Chernozem Region.

Keywords: mitotic activity; nucleolar characteristics; pathological mitoses; polymorphism; walnut

Introduction

Walnut (*Juglans regia* L.) occupies the leading place among nut-bearing crops in Russia by consumption, however, the products entering the Russian market are primarily of foreign origin. The homeland of walnut is the territory of Central Asia and Asia Minor. This species is thermophilic, it regenerates, grows and fructifies

well under mild warm climates with a gradual change of the seasons of the year, and therefore the territories of China and India, Central Asia, the southern and central parts of Europe, the North Caucasus, Transcaucasia, Uzbekistan, and Moldova compose the modern habitat of wild and cultivated walnuts. The territory of Russia has a sufficient number of areas suitable for the cultivation of this crop, but the nut growing industry is poorly developed. In this regard, breeders are faced with the tasks of a detailed and comprehensive study of walnut, the allocation of the most valuable forms, and the cultivation of resistant plants with high quality fruits (Ibragimov, 2010; Biganova *et al.*, 2015; Korniyenko and Potanin, 2017).

In recent decades, the walnut has been studied in detail from various perspectives. A lot of analyses have found walnut to contain several groups of chemicals (Martinez *et al.*, 2010; Mao, 2014; Abdallaha, 2015; Beyhan *et al.*, 2017; Jahanban-Esfahlan *et al.*, 2019). Numerous researches have focused on studying the cold resistance of walnuts (Aslani Aslamarz *et al.*, 2010; 2011; Charrier *et al.*, 2011; 2013; Hassankhah *et al.*, 2017), the influence of soil and climatic conditions on productivity (Winter *et al.*, 2009; Cosmulescu *et al.*, 2010; Gauthier and Jacobs, 2011; Figueroa, 2017) and describe morphological features which are important for breeding (Arzani *et al.*, 2008; Amiri *et al.*, 2010; Abedi and Parvaneh, 2016), as well as genetic research was not left out (Bernard *et al.*, 2018a; 2018b; Kefayati, 2019).

However, not many cytological or molecular studies have been carried out to date in nut plant species in general and walnut in particular. They are limited to studies of genetic diversity due to variation in the chromosomes and genome size (Harandi and Ghaffari, 2001; Martinez-Gomez *et al.*, 2003; Rasouli *et al.*, 2014; Vahdati, 2014; Sola-Campoy *et al.*, 2015; Khorami, 2018). Detailed study of the cell division in nuts has not been conducted previously, as well as the nucleolar characteristics (their shape, size and morphology) have not been described. The study of cytogenetic characteristics can be powerful tools to evaluate the stability of the genetic apparatus of plants and the mechanisms of maintaining homeostasis at the cellular level, ensuring the normal development of the organism as a whole (Butorina, 1989; Zoldos *et al.*, 1997). Determining the level of cytogenetic disorders in the meristem of seedlings will allow to assess the quality of seeds and to select maternal plants by progeny test in a short time – during one vegetative period. This is important for seed propagation of the best forms of plants, which have a long breeding cycle (Baranova, 2013). Cytogenetic methods are also used for the detection of polymorphism in natural populations, which can be adaptive and reflect the degree of plant adaptability to habitat conditions (Karpova, 2010; Kalaev and Popova, 2014b).

Previously, the variability of cytogenetic characteristics was studied in the seed progeny of deciduous forest-forming species of the Central Chernozem Region, specifically the pedunculate oak (*Quercus robur* L.) and weeping birch (*Betula pendula* Roth) (Butorina *et al.*, 2000; Kalaev and Butorina, 2006; Vostrikova and Butorina, 2006; Kalaev, 2009; Kalaev *et al.*, 2010; Karpova, 2010; Kalaev and Popova, 2014a). It was shown that changes in the cytogenetic parameters and seed progeny of these plant species were influenced by the growth conditions of the mother trees. Under adverse conditions, a change in the level of mitotic activity (amplification or depression) occurred, the number of pathological mitoses increased and their spectrum expanded, the synthetic activity of the nucleoli changed. Cytogenetic polymorphism in the seed progeny of these native woody plant species was revealed. This polymorphism consisted in the existence of several groups of seedlings different in the degree of stability of the genetic material (mutable, low mutable, and one or more intermediate groups). For pedunculate oak, it was found that seedlings from the group with a high level of cytogenetic disturbances and low nucleolar activity had the worst growth rates (Kalaev and Popova, 2014a). Similar studies were conducted for the rhododendron (*Rhododendron ledebourii* Pojark.), introduced into the Central Chernozem Region (Burmenko *et al.*, 2018).

It was shown that Persian walnut successfully grows and stably fructify within the territory of the Central Russian Upland, including Central Chernozem Region (Veresin and Ulyukina, 1970; Vasin, 2004; Nikolaev *et al.*, 2007; Pomogaybin, 2008). According to long-term research, walnut trees have good indicators of cold resistance, fruiting, excellent taste qualities of nuts and other economically valuable characteristics (Slavskiy *et al.*, 2013; 2015; 2017), so they are a valuable material for breeding and seed propagation in this region.

In connection with the above, the goal of our study was to describe the cytogenetic characteristics and identify polymorphism by cytogenetic parameters in the root meristem of walnut seedlings, mother trees of which were grown for a long time under the conditions of the Central Chernozem region.

Materials and Methods

The research was conducted in the Central Chernozem Region, on the border of the transition of the Central Russian upland to the Oka-Don plain (51° 42' 44" N 39° 12' 3" (39° 13' 52") E, elevation above sea level – 156 m). The soil in the place of growth was ordinary chernozem, the depth of groundwater was 8.5 – 9 m. For the experiment, 4 phenotypically normal trees (without damages by pests and fungal diseases) in Voronezh were selected. These trees were grown on private backyards from the seeds of a walnut that grows in the Zvenigorsky district of Kiev region (Ukraine). They grow on a small slope, in the upper part of it and are protected by buildings. The age of the studied walnut trees is 33-35 years, height – 12 m, crown diameter – 4.5 m.

The fruits of the 2017 harvest in the amount of 200 pieces were put for germination in wet sand at a temperature of 16 - 18 °C, without stratification at 9 a.m. The fixation of seedlings and the production of micro-preparations were carried out according to the method of Wittmann (1962). Forty micro-preparations were examined using a Laboval-4 microscope (Carl Zeiss, Jena) (1 seedling – 1 micro-preparation) at magnification 100×1.5×10. The total number of examined cells (at least 700) and cells at various stages of mitosis, the number and types of mitotic abnormalities using the method of Alov (1972), the number of metaphases, anaphase, and telophase cells with persistent nucleolus were taken into account for each preparation. Based on the obtained data, the mitotic index, the part of pathological mitoses, the part of each type of cell division disturbances, and the part of metaphase-anaphase cells with persistent nucleoli (%) were calculated.

The diameter of the nucleoli was measured using a micrometer nozzle (200 cells were analysed for each micro-preparation) and the surface area of the nucleoli (μm^2) was calculated for the investigation of the nucleolar characteristics in the root meristem cells of walnut seedlings. The number of cells with different number of nucleoli was taken into account and their percentage (%) was calculated; the percentage (%) of various types of nucleoli was determined according to the classification proposed by Chelidze and Zatsepina (1988).

The results were processed statistically using the Stadia 7.0 Professional software package (InCo, Russia) (Kulaichev, 2002). Cytogenetic characteristics of Persian walnut seedlings were compared using the following criteria: the frequencies of cells with persistent nucleoli, with two nucleoli in the nucleus, with different type of nucleoli and pathological mitoses, using the nonparametric Van der Varden rank X-test (these varieties do not have a normal distribution); the mitotic index, the part of cells at different stages of mitosis and nucleolar surface areas were compared using the parametric Student's t-test (these varieties have a normal distribution). The parts of cells with different types of mitotic disturbances were compared using Z-approximation for equivalent frequency criterion. For the determination of correlation dependencies, Spearman's coefficient of rank correlation (r_s) was used. Cluster analysis was made using normalized Euclidean distances method with the complete linkage. All studied cytogenetic parameters were added to the data matrix for each of 40 studied seedlings. The correctness of the classification of seedlings and their assignment to a particular group was confirmed by the results of discriminant analysis using the Mahalanobis criterion.

For the determination of the predictors for attributing seedlings to a mutable or low mutable group, the ROC analysis method (Fawcett, 2006; Grigoryev *et al.*, 2016) in the MedCalc 17.5.3. program for statistical analysis of biomedical research was used (MedCalc Software, Osten, Belgium). Prognostic properties of cytogenetic characteristics were evaluated based on the following parameters: AUC (area under curve) – area under the curve (0.9 – 1.0 – excellent diagnostic value of the indicator, 0.8 – 0.9 – very good, 0.7 – 0.8 – good,

0.6 – 0.7 – average, 0.6 or less – unsatisfactory); sensitivity and specificity of the test; critical values (“cut-off point”) of parameters.

Results and Discussion

The mean values of the cytogenetic characteristics of walnut seedlings are presented in Table 1.

Table 1. Cytogenetic characteristics of walnut seedlings from trees growing under the conditions of the Central Chernozem Region

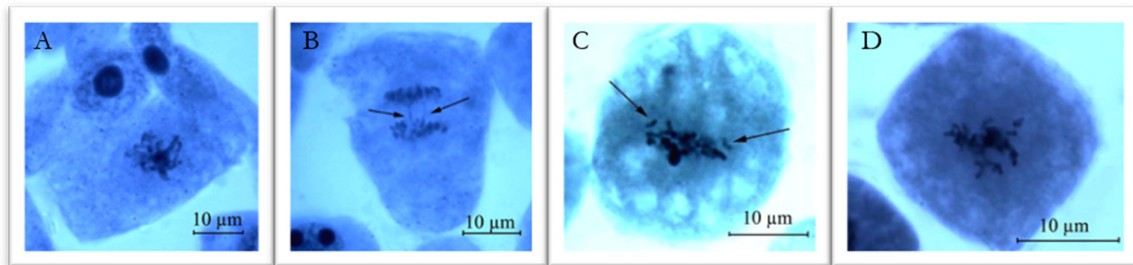
Cytogenetic indices	Mean	Limits of variation (95% confidence interval)	Variation coefficient (%)
Mitotic index (%)	5.8 ± 0.4	5.0 – 6.5	39.7
Mitotic index excluding cells in prophase (%)	3.8 ± 0.3	3.2 – 4.3	44.7
The part of cells (%)			
Prophase	34.5 ± 2.5	29.6 – 39.6	45.7
Metaphase	18.1 ± 1.4	15.4 – 20.9	47.5
Anaphase-telophase	47.4 ± 1.9	44.0 – 51.5	24.7
Pathological mitoses (%)	6.0 ± 0.8	4.4 – 7.6	83.3
Pathological mitoses excluding cells in prophase (%)	9.1 ± 1.1	6.9 – 11.3	78.0
The frequency of persistent nucleoli in mitosis	0.6 ± 0.2	0.2 – 1.0	87.8
The frequency of cells with two or more nucleoli in the nucleus (%)	2.3 ± 0.2	1.9 – 2.7	59.0
The frequency of different type of nucleoli (%)			
“Bark-core”	66.7 ± 3.0	60.7 – 72.3	62.5
“Bark-core” with vacuole	30.2 ± 3.0	24.3 – 36.1	29.0
Vacuolated	0.2 ± 0.1	0 – 0.4	373.7
Compact	3.0 ± 1.2	0.6 – 5.4	254.2
The surface area of the nucleoli in cells with 1 nucleolus in the nucleus (μm ²)	85.52.0	81.7 – 89.4	14.5
The area of the nucleoli of different types (μm ²)			
“Bark-core”	81.1 ± 1.7	77.8 – 84.3	18.8
“Bark-core” with vacuole	101.7 ± 3.0	95.8 – 107.6	13.1
Vacuolated	74.6 ± 2.5	73.1 – 76.2	6.7
Compact	36.6 ± 3.1	32.8 – 40.4	33.6

The distribution of cells according to the stages of mitosis in the root meristem of walnut seedlings reflects the duration of different stages of mitosis under normal physiological conditions: the highest values were observed for the indices “the part of cells in prophase” and “the part of cells in anaphase-telophase”, i.e. during the stages associated with the synthetic processes.

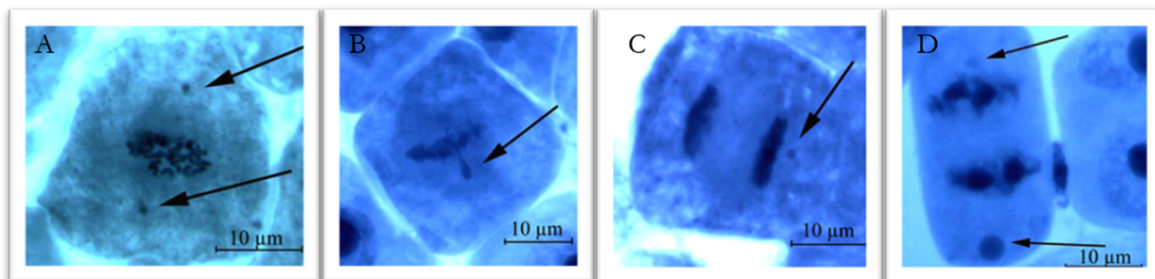
Typical mitotic disorders in walnut (Table 2) were lagging chromosomes in metakinesis and anaphase of mitosis, bridges in the ana- and telophases, as well as agglutination of chromosomes in metaphase. Rare pathologies (1.6%) included multipolar and asymmetric mitoses. No new types of mitotic disturbances were found in walnut that had previously been found in the studied deciduous plants growing under the conditions of the Central Russian Upland. Photos of some pathologies of mitosis are presented in Figure 1.

Table 2. The spectrum of pathological mitoses in the root meristem of walnut seedlings during introduction to the conditions of the Central Chernozem Region

Type of pathology	The frequency of pathology (%)	Limits of variations (95% confidence interval)
Chromosome lagging in metakinesis	37.2 ± 4.1	29.2 – 46
Bridges	29.5 ± 3.9	22.1 – 38.0
Chromosome lagging in anaphase	21.7 ± 3.5	15.3 – 29.7
Agglutination	10.1 ± 2.6	5.9 – 16.6
Rare pathologies	1.6 ± 1.1	0.4 – 5.5

**Figure 1.** Some types of pathological mitoses in the cells of the root meristem of walnut seedlings; (A) Chromosome agglutination in metaphase; (B) Anaphase bridges; (C) Chromosome lagging in metakinesis; (D) Multipolar mitosis

In the meristem of walnut seedlings, the presence of a persistent nucleolus in the metaphase, anaphase, and telophase stages was detected (Figure 2). Persistent nucleoli were observed in the form of rounded formations on one side of the metaphase plate, separately from it or connected to it. In anaphase and telophase, persistent nucleoli were usually pushed to one of the poles of the cell. The appearance of persistent nucleoli in dividing cells was probably due to compensatory protein synthesis necessary for maintaining normal mitosis (Butorina *et al.*, 1997; Burmenko *et al.*, 2018). It should be noted that this functional impairment was rather rare in walnut cells in comparison with other studied deciduous species, which confirms the assumption about the stability of the cell division processes under natural conditions new to the species.

**Figure 2.** Persistent nucleoli in metaphase (A, B), anaphase (C) and telophase (D) of mitosis in the root meristem cells of walnut trees, growing under the conditions of the Central Chernozem Region

In the interphase cells of walnut seedlings, the same types of nucleoli as in native woody species (pedunculate oak, weeping birch) were observed. Photos of nucleoli revealed in cells are shown in Figure 3. The prevalence type of nucleoli in walnut root meristematic cells were the “bark-core” nucleoli: their main structures are RNP-fibrils in central part of nucleolus and RNP-granules forming continual layer in its periphery. Rarely, compact nuclei, which had the high degree of the development of granular component were observed. Both “bark-core” and compact types are high-active nucleoli, which are inherent for active

meristematic tissue. The appearance of vacuole in nucleolus testifies to the reduction of its functional activity, so “bark-core” with vacuole and vacuolated nucleoli are low-active types (Chelidze and Zatsepina, 1988).

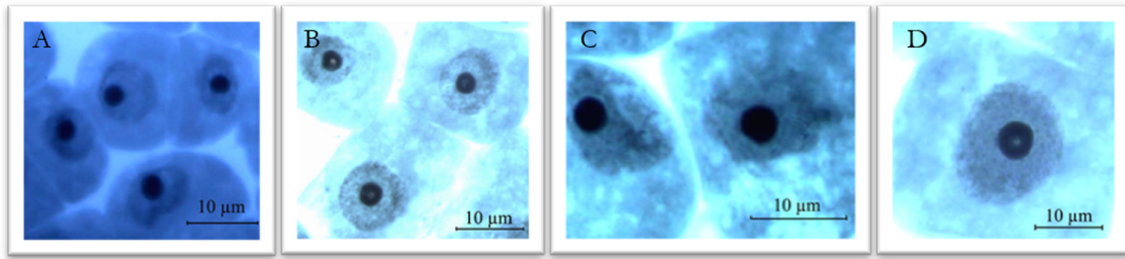


Figure 3. Various types of nucleoli in the root meristem cells of walnut seedlings growing under the conditions of the Central Russian Upland; (A) “Bark-core”; (B) “Bark-core” with vacuole; (C) Compact; (D) Vacuolated

The high variability of the nucleolar characteristics, manifested as change in the size, morphology, and number of nucleoli in the nucleus was revealed in the meristem of walnut seedlings (Table 1), and it is considered by a number of authors as the main trait of the adaptation to adverse environmental factors (Chelidze and Zatsepina, 1988; Kalaev, 2009).

Taking into consideration the fact that many cytogenetic indices of the walnut seedlings had average and high values of the coefficient of variation (Table 1), we performed a cluster data analysis, which allowed identifying three groups of seedlings. The dendrogram of cluster distances according to the studied cytogenetic characteristics is shown in Figure 4. The discriminant analysis confirmed the correctness of the assignment of seedlings to the selected groups ($P < 0.01$). Cytogenetic indices of walnut progeny in the selected seedling groups are presented in Table 3.

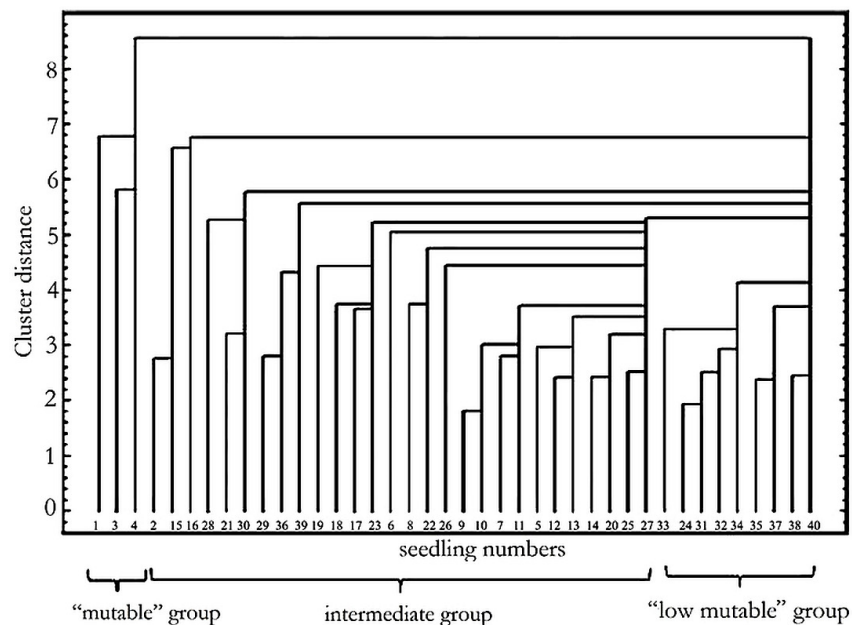


Figure 4. Dendrogram of cluster distances according to cytogenetic characteristics between seedlings of walnut trees growing under the conditions of the Central Chernozem Region

Table 3. Cytogenetic characteristics of different mutability groups of walnut seedlings upon introduction to the conditions of the Central Chernozem Region

Cytogenetic indices	Mutable	Intermediate	Low mutable
The number of seedlings in the group, pcs.	3	28	9
Mitotic index (%)	3.7 ± 0.9 a,b	5.7 ± 0.4	6.7 ± 0.7
Mitotic index excluding cells in prophase (%)	3.0 ± 0.8	4.0 ± 0.3	3.3 ± 0.5
The part of cells (%)			
Prophase	19.1 ± 2.9a, b	31.1 ± 2.6	50.5 ± 3.8b
Metaphase	35.9 ± 6.0a, b	17.8 ± 1.2	13.3 ± 2.1
Anaphase-telophase	45.0 ± 6.8	51.8 ± 1.9	36.2 ± 3.1b
Pathological mitoses (%)	13.7 ± 3.6 a,b	6.7 ± 0.8	1.3 ± 0.4b
Pathological mitoses excluding cells in prophase (%)	17.3 ± 5.0 a,b	10.2 ± 1.2	3.0 ± 1.0.
The frequency of cells with persistent nucleoli (%)	0.9 ± 0.9	0.7 ± 0.3	0.3 ± 0.3
Nucleolus surface area (µm ²)			
Single	77.6 ± 4.9b	88.4 ± 2.4	78.9 ± 2.8b
"Bark-core" with vacuole	97.2 ± 12.2	107.6 ± 3.6	88.9 ± 3.4b
"Bark-core"	77.5 ± 3.0	83.3 ± 2.1	75.2 ± 2.5b
Vacuolated	73.3 ± 3.0	-	-
Compact	47.6 ± 4.0	15.8 ± 3.6	-
The part of nucleoli (%)			
"Bark-core" with vacuole	28.8 ± 4.4	30.1 ± 4.0	30.9 ± 4.7
"Bark-core"	45.9 ± 3.9 a,b	68.1 ± 3.7	69.1 ± 4.7
Vacuolated	2.4 ± 0.7	-	-
Compact	22.9 ± 10.0b	1.8 ± 0.7	-
The part of interphase cells (%) with 2 nucleoli in the nucleus	2.2 ± 0.4a	2.0 ± 0.2	3.1 ± 0.5b

Notes: a – differences with the low mutable group were significant ($p < 0.005$); b – differences with the intermediate group were significant ($p < 0.05$)

The main criterion for distinguishing these groups was the level of pathological mitoses, which often caused the appearance of cells with unbalanced karyotypes, i.e. led to the development of mutations and aneuploidy. Therefore, a group with a high level of pathological mitoses ($13.7 \pm 3.6\%$) can be considered "mutable", and a group with a minimal level of abnormal mitoses ($1.3 \pm 0.4\%$) can be considered "low mutable".

One of the features of the group of seedlings with a high level of cytogenetic instability was the low mitotic activity, and, as can be seen from Table 3, all seedling groups did not differ among themselves by "mitotic index excluding cells in prophase". Thus, a decrease in the number of dividing cells in the mutable group of seedlings occurred due to a decrease in the ratio of prophases. The accelerated passage of the prophase stage in this group of seedlings in comparison with the other two allowed us to suggest that the disturbance of processes of spindle formation and/or chromosome compaction process occurred during this stage. Both of these processes led to the appearance of a high number of pathological mitoses with a predominance of chromosome lagging in the spectrum (86.6%) (Figure 5).

The largest number of cells with persistent nucleoli in mitosis were found in this group of seedlings, which may indicate an increase in the activity of ribosomal genes in the meristem cells under conditions of insufficient functioning of the nucleolar apparatus in interphase. High indices of this criterion were also found in the study of polymorphism based on cytogenetic indices in seedlings of another introduced species - rhododendron belonging to the mutable group. This phenomenon is considered as being a frequent case of increased nucleolar activity and is the mechanism providing the adaptation to adverse environmental conditions (Butorina *et al.*, 1997; Burmenko *et al.*, 2018).

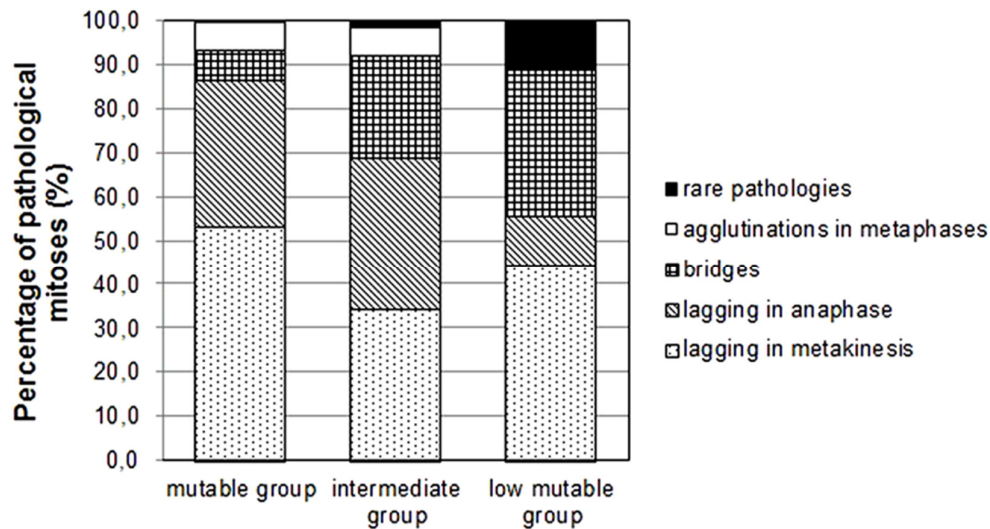


Figure 5. The spectrum of pathological mitoses in groups of walnut seedlings with different mutability

In the mutable group of seedlings, there is a whole spectrum of nucleoli types described for the walnut seedlings. Vacuolated nucleoli with a low level of synthetic activity were only found in this group of seedlings, and those with highly active nucleoli of the bark-core type had the lowest values in comparison with other groups. This probably indicates an insufficient level of nucleolar activity in interphase cells. Therefore, the expansion of the spectrum of nucleolus types in this group due to an increase in compact nucleoli with maximum synthetic activity can be considered as a mechanism compensating the insufficient metabolic activity in the meristem of this group of seedlings (Kalaev *et al.*, 2010; Karpova, 2010).

In the low mutable group of seedlings, an increased number of cells was observed during the prophase stage. This was probably due to the passage of checkpoint-reparation points, leading to a partial reduction of abnormal cell divisions (Kalaev, 2009). In addition, the increase in the number of dividing cells in this group of seedlings can be considered as a compensatory mechanism providing a higher stability of seedlings, since the volume of meristems determines the qualitative and quantitative characteristics of the development of the whole organism (Kosulina, 1993).

The negative correlation between the frequency of pathological mitoses and the frequency of cells with persistent nucleoli on the metaphase-telophase stage of mitosis ($r_s = -0.36$, $P < 0.05$) was revealed in the group of seedlings with a low level of cytogenetic disorders. It indicates the compensatory role of persistent nucleoli in mitosis, which consists in maintaining the synthesis of proteins necessary for the cell under stress conditions (conditions of introduction) and, thus, possibly leading to a decrease of pathological mitoses.

As the analysis of Figure 5 shows, the part of bridges increased in the spectrum of pathological mitoses in the low mutable group of seedlings, compared to other groups, indicating an increase in reparation processes in this group of seedlings (Simakov, 1983).

This group of seedlings is characterized by the absence of compact and vacuolated nucleoli in interphase cells; however, the number of cells with two nucleoli in the nucleus was maximal in comparison with other groups of seedlings. In the study of cytogenetic polymorphism, the highest parts of interphase cells with two or more nucleoli in the nucleus was also observed in a low mutable group of weeping birch seedlings growing in natural and artificial stands of the Central Chernozem Region. The increase in the number of interphase cells with two and multiple nucleoli reflects the mechanisms maintaining stable synthetic activity in the meristem of seedlings of a low mutable group (Kalaev *et al.*, 2010).

It should be also noted that the mutable group accounted for only 7.5% of the total number of analysed seedlings. The low mutable group accounted for 22.5%. The intermediate group included 70% of all studied

seedlings and was characterized by cytogenetic indices transitional between the low mutable and mutable groups of seedlings (Table 2). The obtained data on the size of the groups indicate a good adaptation of the walnut mother trees to the climatic conditions of the Central Chernozem Region during introduction, allowing producing seed progeny with a low level of damage to the genetic material.

We performed a ROC analysis of the cytogenetic parameters of the walnut seed progeny for the identification of critical values (cut-off points of the studied parameters), allowing determining whether any studied seedling belongs to one of the selected model (mutable and low mutable) seedling groups. Cytogenetic criteria with confirmed diagnostic value, showing diagnostic ability in both selected groups of seedlings, were used as predictors of a mutable or low mutable group. These predictors included the part of cells in prophase and metaphase stages, the level of pathological mitoses (both taking into account and without taking into account prophase cells), and the frequency of interphase cells with compact and vacuolated nucleoli. ROC-curves of assignment predictors are presented in Figures 6 and 7. As can be seen from the analysis of the figures, almost all described cytogenetic indices proved to be predictors with good and excellent diagnostic value with high sensitivity and specificity of each criterion.

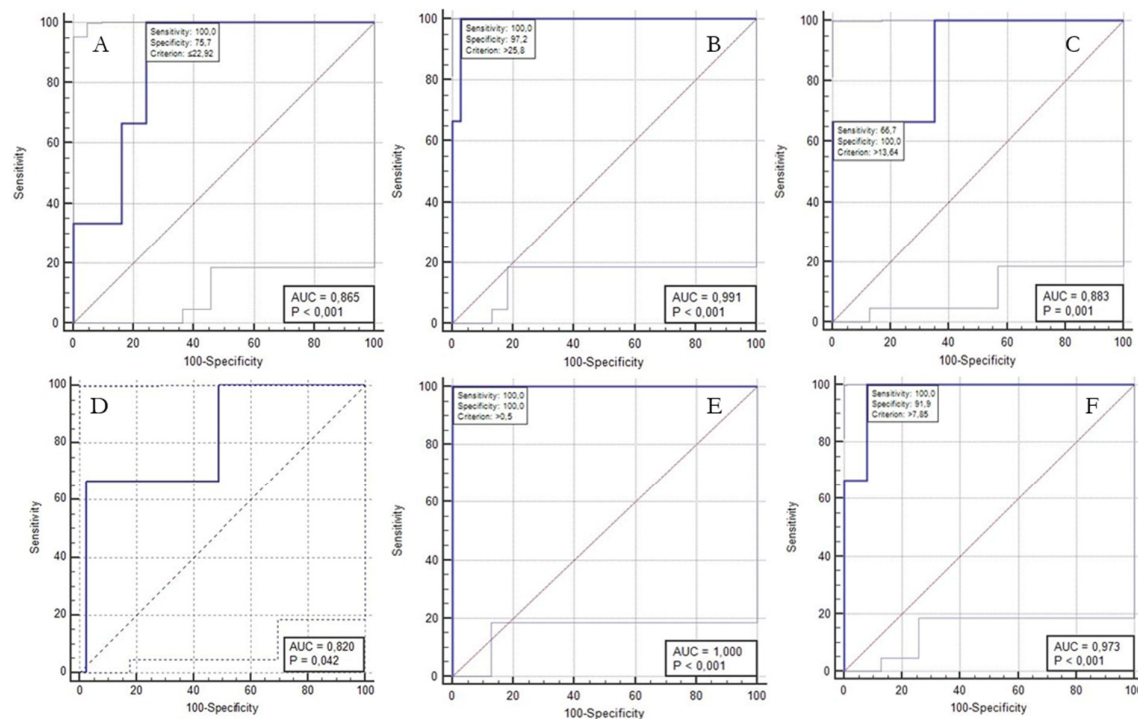


Figure 6. ROC curves of predictors for attributing seedlings to a mutable group; (A) For “the part of cells in prophase%” index; (B) For the “the part of cells in metaphase, %” index; (C) for “pathological mitoses, including prophases, %” index; (D) For “pathological mitoses excluding prophases, %” index; (E) For “the frequency of vacuolated nucleoli” index; (F) for “the frequency of compact nucleoli” index

The parameters of the state of walnut seedlings cytogenetic apparatus, according to which they can be attributed to one of the selected groups – mutable or low mutable with maximum probability, can be determined based on the ROC analysis data. The values of cytogenetic indices (discriminators) for each of the groups are presented in Table 4. Thus, it is possible to attribute seedlings with less than 22.9% of prophase cells in root meristem and increased other indices presented in Table 4 to a mutable group. On the contrary, seedlings without compact and vacuolated nucleoli in the interphase nuclei, with low level of pathological mitoses, an increased part of prophases in mitosis (more than 33.3%) and a low part of metaphases (less than 15.2%) can be classified as a low mutable group. The determination of the values of these predictors will

significantly reduce the labour costs of cytologists in determining the quality of the seed progeny produced by the walnut mother trees. So, mother plants that produce low-mutable offspring can be used for seed propagation and plant plantations.

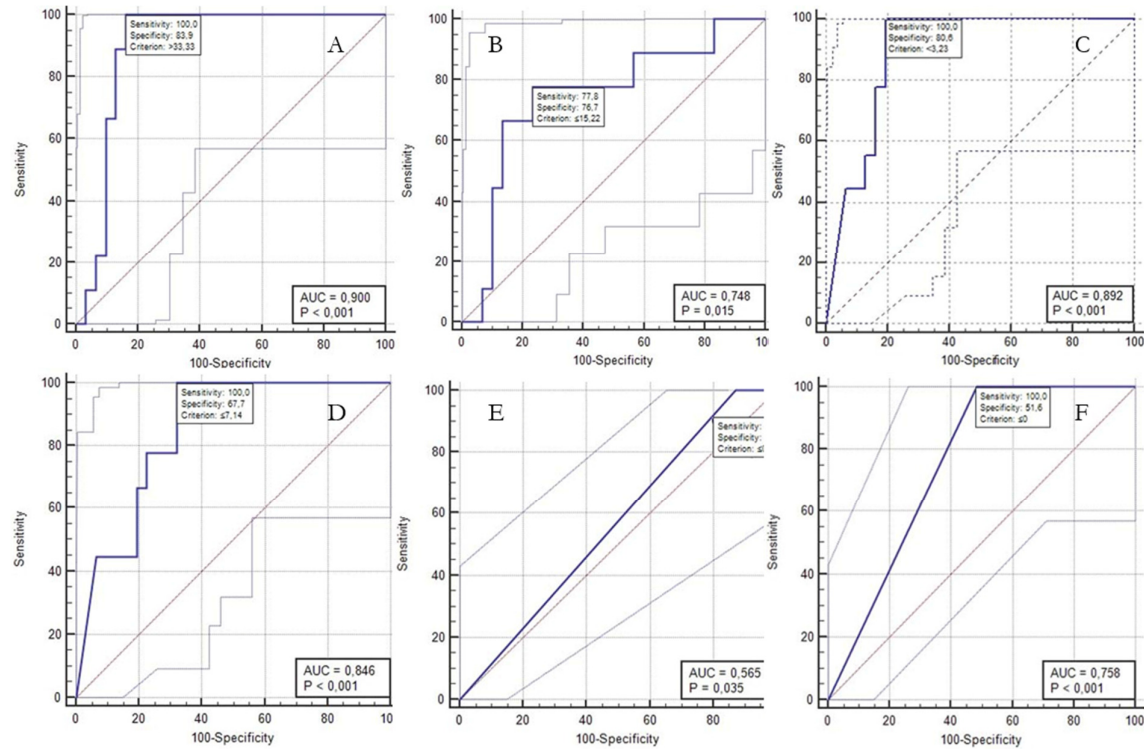


Figure 7. ROC curves of predictors for attributing seedlings to a low mutable group; (A) For “the part of cells in prophase, %” index; (B) For the “the part of cells in metaphase, %” index; (C) for “pathological mitoses, including prophases, %” index; (D) For “pathological mitoses excluding prophases, %” index; (E) For “the frequency of vacuolated nucleoli” index; (F) for “the frequency of compact nucleoli” index.

Table 4. Values of cytogenetic indices – discriminators for classifying seedlings as a mutable or low mutable group (%)

Parameters	Mutable group of seedlings	Low mutable group of seedlings
The part of cells in prophase (%)	≤ 22.9	> 33.3
The part of cells in metaphase-telophase (%)	> 25.8	≤ 15.2
Pathological mitoses including prophases	> 13.6	≤ 3.2
Pathological mitoses excluding prophases	> 17.7	≤ 7.1
The frequency of vacuolated nucleoli	> 0.5	≤ 0
The frequency of compact nucleoli	> 7.9	≤ 0

Conclusions

Based on the cytogenetic indices described above, it can be concluded that mitosis is predominantly stable in the seedlings of walnut trees introduced in the Central Chernozem Region. The identified qualitative and quantitative characteristics of polymorphic groups allow us to speak about a good adaptation of the parent walnut plants to new living conditions. The developed criteria for belonging of seedlings to a mutable or low mutable group of seedlings can be used to identify in a short time the best forms of mother plants that produce low-mutable seeds. The results of research can be used for development of the recommendations for improving the breeding system and creating walnut plantations in the Central Chernozem region of Russia.

Authors' Contributions

Conceptualization: VNK; Data curation: IVI; Formal analysis: SSK; Investigation: FRHA and IVI; Methodology: FRHA and IVI; Project administration: VNK; Resources: VAS; Supervision: VNK; Visualisation: IVI; Writing - original draft: VNK and SSK; Writing - review and editing VNK, SSK, FRHA, IVI and VAS. All authors read and approved the final manuscript.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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