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Cytogenetic Characteristics of Weeping Birch (*Betula pendula* Roth) Seed Progeny in Different Ecological Conditions

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ABSTRACT

The present study of cytogenetic characteristics in seed progeny of woody plants in stress and no-stress conditions is important for better arrangement of green spaces in cities and reforestation on anthropogenically polluted territories because it allows to select maternal trees producing seeds resistant to stress factors. The following cytogenetic characteristics of the root meristematic cells of weeping birch (*Betula pendula* Roth) seedlings were studied: mitotic and nucleolar activities, frequency and spectrum of pathological mitosis, frequency of persistent nucleolus in mitosis. Seeds for this study were collected from maternal trees growing in anthropogenically polluted and ecologically safe areas of the Central Black Earth Region of Russia. Cluster analysis revealed four clusters of seedlings on each territory: "mutable", "low-mutable" and two clusters with intermediate characteristics. The characteristics and differences of these clusters are described in the article.

Keywords: mitotic activity, nucleolar activity, pathological mitosis, persistent nucleolus

INTRODUCTION

Woody plants are objects of intense research due to their great biological, ecological and socio-economic importance. In Russia, morphology, taxonomy, physiology, and biochemistry of the most of wide-spread species of woody plants have been widely studied (see review by Altukhov *et al*. 2004). Genetic diversity and as a result, the differences in morphology, physiology, biochemistry, cytology, and consequently reproduction and optimal adaptation to existing environmental conditions in populations of forestry species also attracts scientific attention (Fadeeva *et al.* 1980; Kaydanov 1996; Butorina *et al.* 2001). The results of deep studies of the genetic structure in populations of woody plants are important for development and implementation of comprehensive programs for conservation, reproduction, and improvement of forestry resources, including selection of proper material for planting in cities and towns. Recently, Russian scientists showed fast-growing interest in genetic and cytogenetic diversity of forest trees, especially of coniferous species (Krutovskii and Bergmann 1995; Butorina *et al*. 2000; Altukhov *et al*. 2004; Doroshev 2004; Cherkashina 2007; Politov 2007; Senkevich 2007). However, the relative lack of information on deciduous woody plants requires further investigation.

Weeping birch (*Betula pendula* Roth) is one of the main forestry species in the Central Black Earth Region of Russia and is widely used for planting in settlements. It is characterized by significant variation of different morphological structures such as leaf shape, bark coloration, crown form, etc. (Popov 2003), but the cytogenetic diversity, and in particular with its respect to different ecological conditions, was not previously studied. The cytogenetic study of seed material, including the analysis of mitotic and nucleolar activity, frequency and spectrum of pathological mitosis, is the basis for monitoring of changes in populations and possible forecast for planting quality and long-term stable reproduction (Muratova 1989; Arhipchuk *et al*. 1992; Zoldos *et al*. 1997; Butorina *et al*. 2001). It provides the premise for 1) the development of recommendations on improvement of artificial populations of birch by selection of maternal trees producing seed progeny resistant to the disadvantageous factors of the environment; 2) understanding cytogenetic processes which occur in populations of woody plants under increasing anthropogenic pollution. The present work aims to assess the cytogenetic diversity of seed progeny of birch trees growing on territories with different levels of anthropogenic pollution.

MATERIALS AND METHODS

Voronezh is the largest industrial city in the Central Black Earth Region of Russia with a population of about 1 million people and various industrial, social, and ecological structures. Two territories, marked for convenience as 'District X' (Yaroslavskaya str., Novosibirskaya str., Savrasova str.) and 'District Y' (Koltsovskaya str., Svobody str., Voroshilova str.), of the city of Voronezh were sampled in 2001 and 2003, respectively. These districts are considered as ecologically problematic zones of the city (Mamchik *et al.* 1997). The main sources of pollution in the 'District X' are the heating-power station and the enterprises of chemical industry producing rubber and tires. The atmosphere of this district is polluted by sulphurous anhydride, nitrogen oxides, ashes, and volatile organic compounds (xylol, toluol, butadiene, acetone, petrol) in concentrations exceeding the maximum allowable in 2.2-4.3 times. More than 30% of soil probes from this territory do not meet the sanitary requirements. The main sources of pollutants (carbon monoxide, nitrogen oxides, and formaldehyde) in 'District Y' are the enterprises of the pharmaceutical and food industry, and auto transport. The concentrations of pollutants exceed the maximum allowable level in 1.9-7.2 times, and more than 50% soil probes do not meet the sanitary requirements (Mamchik *et al.* 1997; Semenova 2009). During the same years, we sampled birch seedlings in the no-stress conditions on the territory of the Biological Educational and Scientific Centre of the Voronezh State University "Venevitinovo" located 30 km westward of Voronezh. This territory is considered as "ecologically safe"; the concentration of the controlled chemical elements in vegetation and the level of radioactive contamination there do not exceed the maximum allowable level (Schetinkina *et al.* 1992).

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For cytogenetic study of the birch seed progeny, seeds were sampled in each district from six mature (approximately 30-40 years old), phenotypically healthy trees (without excessive damages by pests). 300 seeds were collected from each tree and mixed together for each district. In our work, we did not study cytogenetic characteristics in seed progeny of each individual tree, because the previous study (Butorina 1989; Doroshev 2004) did not reveal significant difference between cytogenetic characteristics of individual trees of *Pinus sylvestris* L. and *Querqus robur* L. The seeds were germinated on wet filter paper (Sartogosm 6, 110 mm diameter, 0.33 mm thickness) in the Petri dishes at 20°C. In 6 days, when the length of roots reached 0.5–1 cm, young seedlings were fixed with the 3:1 mixture of ethanol and glacial acetic acid (10 ml of the solution per 50 seedlings) at 9 a.m., when the meristeme of *B. pendula* shows the maximum mitotic activity (Vostrikova 2002). The fixed material was kept refrigerated at the temperature of 4°C. Wittmann's method (Wittmann 1962) was used for permanent micropreparation (one seedling per one slide). 40 microscopic preparations of seedlings from each experimental district were studied under the LABOVAL-4 microscope (Carl Zeiss, Jena) at magnification of 40×1.5×10 and 100×1.5×10. For each specimen, we recorded the total number of cells (at least 500) and an estimated mitotic activity which was calculated as mitotic index (the proportion of dividing cells to the total number of analyzed cells). The number of pathological mitoses and persistent nucleoli, we detected at the stages of metaphase, anaphase, and telophase and calculated their percentage. According to the classification by Alov (1972), all mitotic pathologies were divided in three groups: 1) the pathologies related to chromosomal damage (delay of mitosis in the prophase; disturbances of spiralization or despiralization of chromosomes; early disjunction of chromatids; chromosome fragmentation and pulverization; bridges; lagging chromosomes; formation of micronuclei; irregularities of chromosomal segregation; agglutinations of chromosomes); 2) the pathologies related to injury of mitotic spindle (delay of mitosis in the metaphase; C-mitosis; dispersion of chromosomes in the metaphase; multipolar mitosis; three-group metaphase; asymmetric; monocentric mitosis); 3) the disturbances of cytotomy (cytokinesis) (delay or absent of cytotomy; precocious cytotomy). The frequency of abnormalities was calculated as a percentage to the total number of cells.

To study nucleolar characteristics, a diameter of the nucleolus was measured, using the ocular micrometer (the nucleoli of 200 cells were measured for each sample); and the surface area of the nucleolus was calculated. The number of cells with various types of nucleoli and the number of nucleoli in each cell were also counted; their percentage was calculated. Using the nucleoli classification by Chelidze and Zatsepina (1988), all birch nucleoli can be associated with two types: the highly-active "bark-core" nucleoli and the low-active "bark-core with vacuole" nucleoli, which present in both ecologically safe and disturbed territories (Kalaev and Karpova 2003; Kalaev *et al.* 2006).

The results were statistically analyzed using STADIA 6.0 Professional for Windows software package (InCo Products 1997). The data grouping and processing were done using the method described by Kulaichev (1996). For comparison of birch cytogenetic characteristics the following criteria were used: the Van der Vaerden rank X-test for frequency of persistent nucleoli, pathological mitosis, and for frequency of cells with two, three, and four nucleoli in the nucleus, and the Student's *t*-test for mitotic indexes and nucleolar characteristics (Kulaichev 1996). The proportion of cells with different types of pathological mitosis was compared using their angular transformation and Yeits correction according to Lakin (1990). The correlation was estimated using the Spearman's rank correlation coefficient (Kulaichev 1996). The cluster analysis was performed using normalized Euclidean distances method with the complete linkage. The following cytogenetic parameters for each of 40 seedlings from the studied territories were included in the data matrix: the mitotic index (%), the percentage of cells in the stages of prophase, metaphase, anaphase-telophase of mitosis, the percentage of cells with disturbances in mitosis, the surface area of a solitary nucleolus (μm^2); the percentage of cells with different types of nucleoli; the percentage of cells with persistent nucleoli in the stages of metaphase, anaphase, telophase of mitosis (in % from the total number of the cells on each stage), and the percentage of cells with two, three, and four nucleoli in nucleus. The validity of the partition to classes was defined using the discriminant analysis with the Mahalanobis' distances (Kulaichev 1996).

RESULTS AND DISCUSSION

The dendrograms based on the cytogenetic characteristics of seedlings are presented in **Figs. 1** and **2**, for ecologically safe and disturbed territories respectively. Four clusters of birch seedlings for each of the territories could be detected on each tree. The cytogenetic characteristics of these separated clusters of seedlings are presented in **Tables 1-4**.

Cluster 1 of seedlings is characterized by large number of pathological mitoses and could be considered "mutable" with a high level of cytogenetic instability. The results of cytogenetic study (**Table 1**) show that the frequency of abnormal mitotic cells in this cluster is 5.2-12.2% on the polluted territories and 5.5-6.0% on the ecologically safe territory. Moreover, significant differences with other clusters ($P < 0.05$) also exist. The increase of the pathological mitosis frequency is accompanied by growth of the surface area of the solitary nucleolus in the interphase cells in this cluster of seedlings (**Table 5**). Probably, the increased size of nucleoli is the result of ribosomal gene amplification and/or reinforcement of their transcriptional activity and also dis-

Table 1 Cytogenetic characteristics of birch seed progeny in "mutable" cluster.

Years sampled	**2001**		**2003**	
Cytogenetic characteristics	**'District X'**	**"Venevitinovo"**	**'District Y'**	**"Venevitinovo"**
Total number of seedlings in a cluster	8	16	6	6
Mitotic index, % ($\bar{x} \pm S_{\bar{x}}$)	4.2 ± 0.2	3.7 ± 0.3	5.4 ± 0.9	6.3 ± 0.7
Proportion of cells on mitosis stages, % ($\bar{x} \pm S_{\bar{x}}$)				
prophase	19.3 ± 3.4	25.0 ± 2.0	29.3 ± 5.3	23.8 ± 4.0
metaphase	31.4 ± 2.5	31.4 ± 2.1	24.6 ± 1.7	31.6 ± 2.2
anaphase-telophase	49.2 ± 1.8	43.7 ± 1.8	46.1 ± 5.2	44.6 ± 3.9
Pathological mitosis, % ($\bar{x} \pm S_{\bar{x}}$)	12.2 ± 1.5	6.0 ± 1.3	5.2 ± 1.2	5.5 ± 1.1
Persistent nucleoli' frequency, % ($\bar{x} \pm S_{\bar{x}}$)	18.4 ±3.3	9.1 ± 1.7	17.1 ± 2.7	8.8 ± 2.1
Surface area (μm^2) ($\bar{x} \pm S_{\bar{x}}$) of				
single nucleoli	120.7 ±3.0	103.6 ± 1.5	100.7 ± 3.2	141.2 ± 5.5
"bark-core" nucleoli	108.3 ± 5.6	101.3 ± 1.7	89.2 ± 1.7	113.6 ± 5.9
vacuolate "bark-core" nucleoli	145.7 ± 5.2	126.1 ± 2.4	120.0 ± 2.5	184.5 ± 8.3
Share of different types of nucleoli, % ($\bar{x} \pm S_{\bar{x}}$)				
"bark-core"	67.8 ± 3.9	86.1 ± 2.1	63.9 ± 8.4	60.8 ± 9.0
vacuolate "bark-core"	32.2 ± 3.9	13.9 ± 2.1	36.2 ± 8.4	39.2 ± 9.0
Total number of seedlings / percentage of interphase cells having *n* nucleoli in nucleus				
n = 2	6 / 1.4	15 / 3.8	6 / 1.8	5 / 1.9
n = 3	0	10 / 0.5	3 / 0.3	2 / 0.3
n = 4	0	6 / 0.3	0	0

Table 2 Cytogenetic characteristics of birch seed progeny in "low-mutable" cluster.

Years sampled	**2001**		**2003**	
Cytogenetic characteristics	**'District X'**	**"Venevitinovo"**	**'District Y'**	**"Venevitinovo"**
Total number of seedlings in a cluster	5	7	6	9
Mitotic index, % ($\bar{x} \pm S_{\bar{x}}$)	3.7 ± 0.4	4.0 ± 0.4	6.5 ± 0.9	5.7 ± 0.6
Proportion of cells on mitosis stages, % ($\bar{x} \pm S_{\bar{x}}$)				
prophase	42.4 ± 3.7	21.7 ± 0.9	21.6 ± 4.9	44.7 ± 2.0
metaphase	19.3 ± 2.3	28.1 ± 3.5	27.8 ± 2.0	25.3 ± 1.2
anaphase-telophase	38.4 ± 1.9	50.2 ± 2.7	50.6 ± 4.8	30.9 ± 2.9
Pathological mitosis, % ($\bar{x} \pm S_{\bar{x}}$)	6.0 ± 1.9	2.6 ± 0.7	5.2 ± 2.0	1.9 ± 0.8
Persistent nucleoli' frequency, % ($\bar{x} \pm S_{\bar{x}}$)	7.0 ± 2.4	8.9 ± 2.7	12.5 ± 2.3	12.0 ± 2.8
Surface area (μm^2) ($\bar{x} \pm S_{\bar{x}}$) of				
single nucleoli	97.1 ± 0.8	109.3 ± 3.7	114.2 ± 5.8	103.5 ± 4.1
"bark-core" nucleoli	88.8 ± 2.4	87.8 ± 2.0	111.7 ± 5.1	96.9 ± 2.8
vacuolate "bark-core" nucleoli	118.7 ± 2.0	130.7 ± 4.0	123.9 ± 25.4	121.2 ± 5.2
Share of different types of nucleoli, % ($\bar{x} \pm S_{\bar{x}}$)				
"bark-core"	73.1 ± 6.9	49.5 ± 2.9	93.0 ± 2.4	77.2 ± 5.8
vacuolate "bark-core"	26.9 ± 6.9	50.2 ± 3.2	7.0 ± 2.4	22.8 ± 5.8
Total number of seedlings / percentage of interphase cells having *n* nucleoli in nucleus				
n = 2	5 / 3.5	7 / 4.7	6 / 1.3	9 / 2.2
n = 3	1 / 0.1	1 / 0.3	0	2 / 0.1
n = 4	1 / 0.1	0	0	1 / 0.1

Table 3 Cytogenetic characteristics of birch seed progeny in "first intermediate" cluster.

Years sampled	**2001**		**2003**	
Cytogenetic characteristics	**'District X'**	**"Venevitinovo"**	**'District Y'**	**"Venevitinovo"**
Total number of seedlings in a cluster	12	11	11	10
Mitotic index, % ($\bar{x} \pm S_{\bar{x}}$)	5.2 ± 0.4	3.0 ± 0.3	6.6 ± 0.5	6.7 ± 0.7
Proportion of cells on mitosis stages, % ($\bar{x} \pm S_{\bar{x}}$)				
prophase	17.9 ± 1.6	15.8 ± 1.8	16.9 ± 2.3	21.9 ± 1.9
metaphase	22.2 ± 1.6	27.8 ± 2.6	30.5 ± 2.5	29.4 ± 2.3
anaphase-telophase	59.9 ± 1.5	56.4 ± 1.9	52.6 ± 2.1	48.7 ± 2.7
Pathological mitosis, % ($\bar{x} \pm S_{\bar{x}}$)	6.8 ± 1.1	3.5 ± 1.1	5.3 ± 1.1	4.8 ± 1.0
Persistent nucleoli' frequency, % ($\bar{x} \pm S_{\bar{x}}$)	10.9 ± 1.9	15.8 ± 3.0	12.2 ± 2.8	7.4 ± 1.8
Surface area (μm^2) ($\bar{x} \pm S_{\bar{x}}$) of				
single nucleoli	99.6 ± 1.3	104.5 ± 1.7	111.2 ± 1. 8	112.9 ± 2.5
"bark-core" nucleoli	93.2 ± 1.3	94.7 ± 2.0	99.2 ± 1.9	96.4 ± 3.0
vacuolate "bark-core" nucleoli	120.2 ± 2.4	128.4 ± 2.6	135.2 ± 2.2	129.3 ± 2.7
Share of different types of nucleoli, % ($\bar{x} \pm S_{\bar{x}}$)				
"bark-core"	75.2 ± 2.3	69.2 ± 2.2	66.7 ± 1.1	50.4 ± 2.1
vacuolate "bark-core"	24.8 ± 2.3	30.8 ± 2.2	33.3 ± 1.1	49.6 ± 2.1
Total number of seedlings / percentage of interphase cells having *n* nucleoli in nucleus				
n = 2	10 / 1.8	11 / 2.6	10 / 1.8	9 / 2.5
n = 3	0	0	0	1 / 0.1
n = 4	0	0	0	0

Table 4 Cytogenetic characteristics of birch seed progeny in "second intermediate" cluster.

Years sampled	**2001**		**2003**	
Cytogenetic characteristics	**'District X'**	**"Venevitinovo"**	**'District Y'**	**"Venevitinovo"**
Total number of seedlings in a cluster	15	6	17	15
Mitotic index, % ($\bar{x} \pm S_{\bar{x}}$)	4.6 ± 0.4	3.0 ± 0.3	7.0 ± 0.4	6. 9 ± 0.3
Proportion of cells on mitosis stages, % ($\bar{x} \pm S_{\bar{x}}$)				
prophase	20.7 ± 1.7	23.4 ± 3.1	16.7 ± 1.3	24.8 ± 1.7
metaphase	32.8 ± 2.3	36.3 ± 2.8	31.8 ± 1.5	32.0 ± 1.1
anaphase-telophase	46.5 ± 2.3	40.3 ± 1.0	51.5 ± 1.8	43.3 ± 1.4
Pathological mitosis, % ($\bar{x} \pm S_{\bar{x}}$)	9.8 ± 1.7	3.0 ± 1.4	5.6 ± 0.7	4.1 ± 0.7
Persistent nucleoli' frequency, % ($\bar{x} \pm S_{\bar{x}}$)	14.8 ± 1.7	13.7 ± 4.4	15.9 ± 1.6	9.6 ± 1.6
Surface area (μm^2) ($\bar{x} \pm S_{\bar{x}}$) of				
single nucleoli	94.9 ± 2.1	80.3 ± 2.3	100.4 ± 0.9	121.3 ± 1.6
"bark-core" nucleoli	91.7 ± 2.0	78.7 ± 2.5	94.2 ± 0.9	103.3 ± 1.5
vacuolate "bark-core" nucleoli	113.8 ± 3.1	102.3 ± 5.0	126.6 ± 1.9	148.3 ± 1.9
Share of different types of nucleoli, % ($\bar{x} \pm S_{\bar{x}}$)				
"bark-core"	87.6 ± 1.9	93.3 ± 1.5	80.9 ± 1.7	60.6 ± 1.8
vacuolate "bark-core"	12.4 ± 1.9	6.7 ± 1.5	19.1 ± 1.7	39.4 ± 1.8
Total number of seedlings / percentage of interphase cells having *n* nucleoli in nucleus				
n = 2	13 / 1.2	6 / 5.0	16 / 2.4	15 / 2.8
n = 3	2 / 0.1	2 / 0.3	4 / 0.2	4 / 0.2
n = 4	0	0	1/ 0.0	2 / 0.0

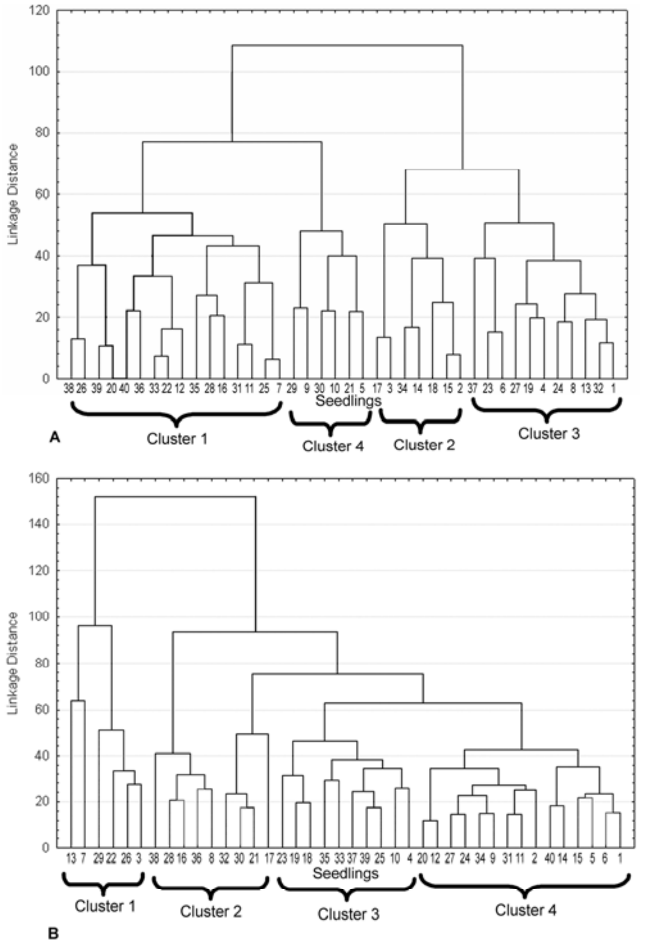

Fig. 1 Dendrograms of normalized Euclidean distances (complete linkage method) between birch seedlings from ecologically safe territory "Venevitinovo", based on their cytogenetic characteristics. (**A**) 2001. (**B**) 2003. Cluster 1 – "mutable", Cluster 2 – "first intermediate", Cluster 3 – "second intermediate", Cluster 4 – "low-mutable". The numbers of seedlings studied are on X-coordinate.

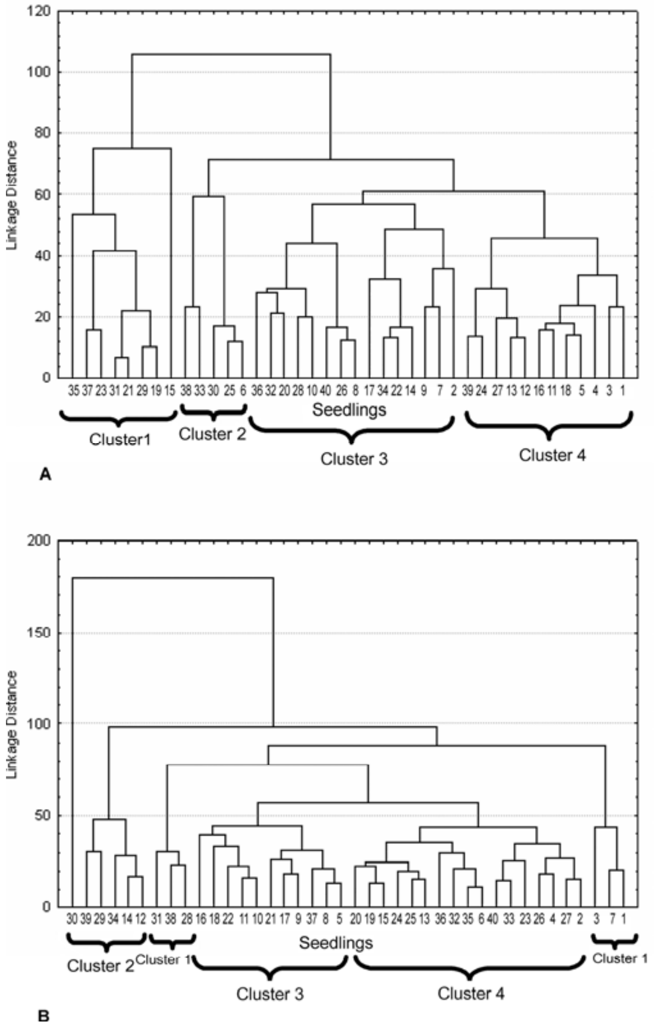

Fig. 2 Dendrograms of normalized Euclidean distances (complete linkage method) between birch seedlings from polluted territories, based on their cytogenetic characteristics. (**A**) 'District X' in 2001. (**B**) 'District Y' in 2003. Cluster 1 – "mutable", Cluster 2 – "first intermediate", Cluster 3 – "second intermediate", Cluster 4 – "low-mutable". The numbers of seedlings studied are on X-coordinate.

order caused by output of the products of ribosomal gene transcription from the nucleus. Both the increased frequency of abnormal mitotic cells and increased size of the nucleolus could be explained as the result of influence of negative environmental factors on plant organism (Chelidze and Zatsepina 1988; Sobol 2001) and are characteristic for the "mutable" cluster of seedlings.

The following trend was observed in "mutable" cluster of seedlings: the frequency of the persistent nucleoli in the metaphase-telophase of mitosis was significantly lower ($P < 0.05$) on ecologically safe territory (8.8-9.1%) compared to the polluted territory (17.1-18.4%, **Table 1**). Earlier, the persistent nucleoli in the stages of metaphase-telophase of mitosis were discovered in the root meristematic cells of pedunculate oak (*Querqus robur* L.) (Butorina and Isakov 1989; Butorina *et al.* 1997, Kalaev and Butorina 2006) and birch (*Betula pendula* Roth) (Vostrikova 2002; Kalaev and Karpova 2003; Kalaev *et al.* 2006) both on ecologically safe and polluted territories. The presence of the persistent nucleoli in the metaphase-telophase testifies for the puffing of chromosomes in the loci of ribosomal genes (Butorina and Isakov 1989). In the present study, the correlation between frequency of the persistent nucleoli and percentage of different types of the nucleoli in the interphase cells was revealed (**Table 5**). The increase of the number of the interphase cells with the highly-active nucleolus of the "bark-core" type correlates with the decrease of the number of the persistent nucleoli in the stages of metaphase-telophase of mitosis. Also, the higher percentage of the interphase cells with the medium-active vacuolate "bark-core" nucleoli type correlates with higher frequency of the persistent nucleoli in mitosis. The appearance of the persistent nucleoli in the

Table 5 Spearman's rank correlation coefficients between some cytogenetic characteristics in the "mutable" (upper line) and "low-mutable" clusters (bottom line).

Cytogenetic characteristics	Pathological mitosis frequency	Share of "bark-core" nucleoli	Share of vacuolate "bark-core" nucleoli
Persistent nucleoli' frequency	–	- 0.41**	0.41**
	- 0.37*	–	–
Percentage of interphase cells having 2, 3, 4 nucleoli in nucleus	–	0.47**	- 0.46**
	–	- 0.31*	0.32*
Surface area of single nucleoli	0.29*	–	–
	–	–	–

*$P < 0.05$, **$P < 0.01$

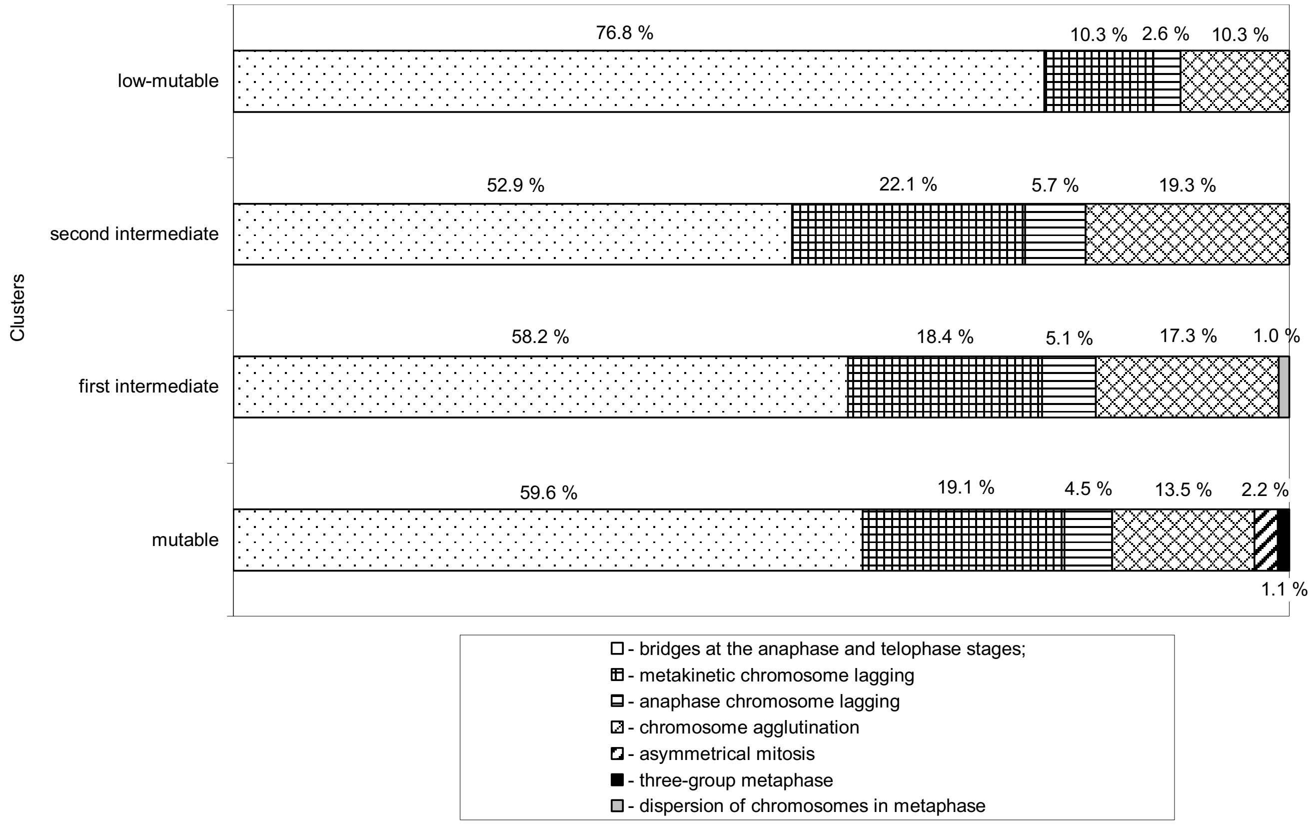

Fig. 3 Spectra of pathological mitosis in the root meristematic cells of birch seedlings in separated clusters.

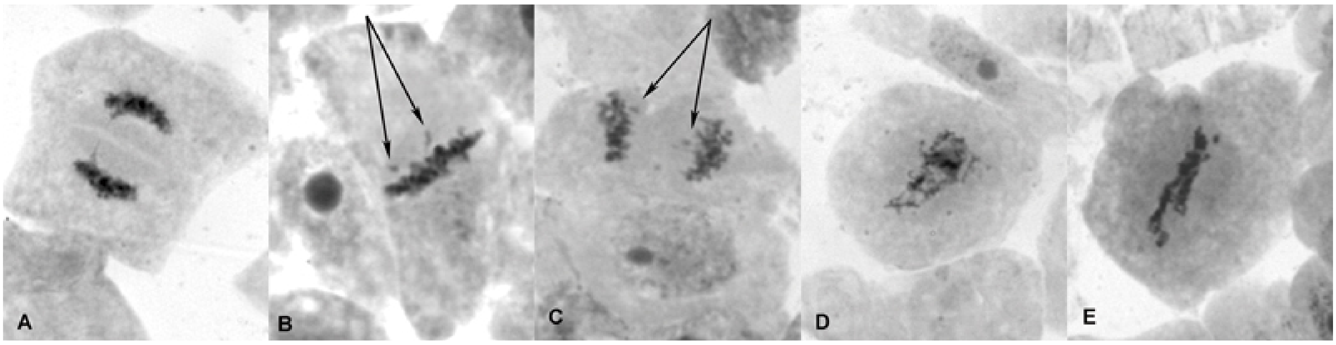

Fig. 4 Birch root meristematic cells with different pathologies in mitosis. (**A**) Bridge in anaphase. (**B**) Metakinetic lagging chromosomes. (**C**) Anaphase lagging chromosomes. (**D**) Agglutination of chromosomes. (**E**) Asymmetrical mitosis.

stages of metaphase, anaphase and telophase of mitosis can be considered as a compensative mechanism of maintaining the normal synthetic function during the cell division under the conditions of insufficient functioning of the nucleolar apparatus during the interphase.

The correlation between the percentage of the cells with two, three, and four nucleoli in the nucleus and the percentage of the cells with the "bark-core" and vacuolate "bark-core" nucleoli (**Table 5**) indicates the possible structural and functional transition in the nucleolar apparatus of the birch meristem in the "mutable" cluster of seedlings. This fact indicates the activation of the latent nucleolar organizer regions of chromosomes by transition to more active nucleolus type ("bark-core"). This is probably a compensative mechanism providing the cell with higher level of protein under negative conditions, and may be considered as a characteristic feature of the "mutable" cluster of seedlings.

Analyzing the spectrum of pathological mitosis (**Fig. 3**), we concluded that the most of abnormal mitotic cells in the "mutable" cluster of seedlings are the pathologies related to the chromosomal damage, namely the bridges, agglutinations, lagging chromosomes in the metaphase and anaphase (96.7%). At the same time, the pathologies related to disturbances of the mitotic apparatus (3.3%) such as asymmetric mitosis and three-group metaphase, are also present in the cells of the "mutable" cluster of seedlings. Moreover, the anomaly of the last type was not observed in the other clusters of seedlings. Some of the mitotic abnormalities mentioned above are illustrated on the **Fig. 4A-E**. The broader spectrum and higher percentage of pathological mitosis are also characteristic features of the "mutable" cluster.

For each of the studied territories, we revealed a cluster of seedlings [cluster 4 (**Table 2**)] with the lowest frequency of pathological mitosis, which was significantly different from all other clusters ($P < 0.05$). The level of pathological mitosis in this cluster is equal 5.2-6.0% on the polluted territories and 1.9-2.6% on the ecologically safe territory. This cluster is marked as a "low-mutable".

Analysis of cytogenetic characteristics in the "low-mutable" cluster of seedlings revealed negative correlation between the frequency of pathological mitosis and the frequency of the persistent nucleoli in the stages of metaphase-telophase (**Table 5**). This proves the compensative role of the persistent nucleoli in mitosis and maintenance of syntheses of protein in the cell on sufficient level under a stress condition (Butorina and Isakov 1989) and possibly, which

Table 6 Main criteria of distinguishing of "mutable" and "low-mutable" clusters of seedlings on studying of diversity of cytogenetic characteristics in birch seed progeny.

Characteristics	"Mutable" cluster	"Low-mutable" cluster
Pathological mitosis level	The highest level (5.2-12.2%)	The lowest level (1.9-6.0%)
Pathological mitosis spectrum	The broadening of pathologies' spectrum due to prevalence of disturbances connecting with the chromosome damages (96.7%), that testifies low intensity of repair activity	The bridges prevalence (76.8%), that testifies high intensity of repair activity
Frequency of persistent nucleoli in mitosis	Decreasing in ecologically safe territory (8.8-9.1%) and increasing in polluted territories (17.1-18.4%)	Rather low values (7.0-12.5%)
Nucleolar characteristics	The increase of percentage of high-active "bark-core" nucleoli (60.8-86.1%) while the frequency of cells with 2 and more nucleoli in nucleus is increasing (1.4-4.6%)	The increase of percentage of medium-active vacuolate "bark-core" nucleoli (7.0-50.2%) while the frequency of cells with 2 and more nucleoli in nucleus is increasing (1.3-5.1%)

leads to reduction of the pathological mitosis frequency.

We observed the following dependency between frequency of cells with two, three, and four nucleoli in the nucleus and the frequency of cells with the nucleoli of different types in the "low-mutable" cluster of seedlings, which is the opposite to the "mutable" cluster: negative correlation with the percentage of the cells with the "bark-core" nucleoli and positive correlation with the percentage of the cells with the vacuolate "bark-core" nucleoli (**Table 5**) was found. Thus, in the "low-mutable" cluster of seedlings, the reduction of the number of cells with several nucleoli in the nucleus was observed when the percentage of the cells with the highly-active "bark-core" nucleoli increases, and, on the contrary, the activation of the latent nucleolar organizer regions of chromosomes occurred when the number of the cells with the medium-active vacuolated "bark-core" nucleoli increases. The described dependency reflects the mechanisms of the maintaining of synthetic activity on the stable level in the interphase cells. This dependency is a characteristic feature of the "low-mutable" cluster of birch seedlings.

The "low-mutable" cluster of seedlings was also characterized by prevalence of bridges in the spectrum of pathological mitosis (**Fig. 3**), which indicates the active functioning of the repair systems (Akopyan 1967; Simakov 1983). The percentage of bridges in 3.3 times exceeds the percentage of other anomalies in this cluster (in the "mutable" cluster in 1.5 times, and in the "intermediate" clusters in 1.1-1.4 times).

The remaining two clusters of seedlings [clusters 2 and 3 (**Fig. 1-2**)] on each of the examined territories were characterized by intermediate cytogenetic conditions between the "mutable" and "low-mutable", clusters (**Tables 3-4**). The main difference between the two intermediate clusters is the presence of seedlings having cells with two, three, and four nucleoli in the nucleus. For each of the studied populations, a cluster of seedlings (we named it the "first intermediate" cluster) was selected, which has the cells of the meristem with only one or two nucleoli in the nucleus. According to Makarova (1989), one pair of chromosomes with the nucleolar organizer regions should present in the cells of *B. pendula*. The other cluster (we named it the "second intermediate cluster") is characterized by presence of seedlings with one, two, three, and even four nucleoli in the nucleus of the root meristematic cells. The increase of the number of nucleoli indicates the activation of the latent nucleolar organizer regions of chromosomes. In the opinion of Lazareva (1999), the number of nucleoli in the interphase nucleus is an indicator of the number of the active nucleolar organizer regions.

In the "second intermediate" cluster, we also revealed the correlation between the frequency of the persistent nucleoli in the stage of metaphase-telophase of mitosis and the surface area of the nucleolus in the interphase cell with solitary nucleolus ($r_S = -0.26$, $P < 0.05$). This confirms the suggestion on the compensative nature of the puffs in mitosis; with decrease of synthetic activity in the interphase cells, the number of the persistent nucleoli in mitosis increases.

On both the ecologically safe and polluted territories, the pair wise comparison of the clusters ("mutable", "low-mutable", and "intermediate") did not reveal significant difference in the parameters of mitotic activity, which allows us to conclude the high resistance of these parameters to an external influence. Besides, there were no differences in the speed of the mitosis stages in the cells. The cell division is a conservative process, playing an important role in ontogenesis of an organism, and the absence of significant difference, besides some cytogenetic parameters, in each of the studied territoris can be considered as an indicator of the homeostasis.

Finally, it is important to note that on the ecologically safe territory, the cluster of "mutable" seedlings dominates (25-40% of the total number of analyzed seedlings). A portion of the "low-mutable" seedlings here is 15-20%. The "low-mutable" cluster of birch seedlings dominates (27-30%) on the polluted territories, the portion of the "mutable" seedlings here is 20-30%. These data indicate the reduction of the gamete and zygote selection on the ecologically safe territory, where the seedlings from the "mutable" cluster can survive; on the other hand, on polluted territories the specimens from the "low-mutable" cluster have advantages in the survival process.

CONCLUSION

In our study, we studied different cytogenetic characteristics in seed progeny of weeping birch trees growing on the ecologically safe and polluted territories. On each of the examined territories, birch seedlings could be divided into four clusters: "mutable", "low-mutable", and two clusters with intermediate characteristics. Generalized characteristics of the "mutable" and "low-mutable" clusters are presented in **Table 6**. Differences of the clusters are shown as the frequency and spectrum of pathological mitosis, percentage of the persistent nucleoli in the stages of metaphase, anaphase, and telophase of mitosis, and as correlations between nucleolar characteristics, which reflect the mechanisms of maintenance of the synthetic activity in the cell on a stable level. The results of this study may be used for development of recommendations on selection of maternal trees for forestry breeding. If the seed progeny of a maternal tree has characteristics of low-mutable cluster (**Table 6**) those seeds can be used to create stable plantings on polluted territories, and the trees producing mutable posterity may be used for selection of new genotypes. Based on the cytogenetic analyses of the seed progeny, an assessment of the environmental pollution could be made.

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