Change of *Quercus robur* L. acorns seedlings cytogenetic parameters under anthropogenic impact

A Popova* and V Popova

Department of Botany and Plant Physiology, Voronezh State University of Forestry and Technologies named after G.F. Morozov, 8 Timiryazev street, Voronezh, 394087, Russian Federation

*E-mail: lf_popovaaa@vgltu.ru

Abstract. Long-term dynamic of cytogenetic parameters of the seedlings apical root meristem was analyzed for the *Quercus robur* for Central Black-Soil region. Central Black-Soil region located in the European part of Russia (geographical coordinates for Voronezh city: 51.660781; 39.200269) in the temperate climate zone. Annual climate indicators were analyzed to determine the possible influence of precipitation amount and temperature fluctuations during pollination and seed formation in the parent trees. Seed progeny cytogenetic parameters of trees growing in urban and suburban conditions were characterized by an increase in mitotic activity and the proportion of cells with mitotic pathologies, the appearance of micronucleus and residual nucleoli in mitosis. There was possible consequence of the complex technosphere negative factors impact on populations. The mitotic index for urban populations (from 6.8% to 9.5%) was approximately at the same level for all years in long-term dynamics. There was a sharp decrease in mitotic activity (4.6%) for the control area in 2019 compared with the data for 2008 (10.5%). The proportion of cells with mitosis pathologies in the meristems of urban seedlings was increased. There was a significant prophase-cells proportion increase among the seedlings. The reduction of number of pathologies may indicate the check-point repair system operation.

1. Introduction

The forest ecosystems are an important framework of the biosphere in urban and suburban areas, performing important ecological functions [1, 2]. Forest areas are shrinking, and there is a necessity not only to preserve existing forest ecosystems, but also to select planting material with a high growth rate to accelerate reforestation. According to the Food and Agriculture Organization (FAO), the area of forests in 2015 amounted to 3.999 million hectares and decreased by 0.13% in the period from 2014 - 2015. The area also decreased by 0.10 % of other land plots covered with forest vegetation. The natural forest decreased by 0.24 %, while there is a positive dynamics of increasing forest plantations by 1.84 % [3]. Preservation of natural native forest-forming species requires selection for high-quality and active growth of seedlings. The rapid growth markers usually are morphological indicators of seedlings, which include an increase in the mass of plants, the number of cells, linear dimensions (length, height, thickness, diameter, and volume of the plant or its individual parts). Individually, each of these features does not always reliably indicate the presence of growth processes in the plant. This selection can be carried out after a long time after planting for trees species. The levels of organization are interrelated, and the characteristics of cells form the properties and functions of tissues in biological systems. The division of meristems is the basis of growth and development of organisms. The cytogenetic parameters...
allow us to assess the activity of divisions in meristems and the presence of pathologies that can lead to mutations. According to some authors, cytogenetic analysis is the sensitive method for detecting the influence of adverse environmental factors [4, 5]. The determination of cytogenetic parameters was successfully used to count the overall mutation background and the effect of radiation [6]. Previously, the influence of stress factors was shown on the plants cytogenetic parameters: the number of chromosomal aberrations was changed [5, 7], the spectrum of mitosis disorders increased and expanded, and the nucleos characteristics and mitotic activity varied [6]. Generally, cytogenetic parameters are used as biomarkers for assessing the cytogenotoxic potential of substances of chemical [8] and radiation nature [9], as well as an indicator of disorders caused by human diseases and harmful habits, such as smoking [10,11]. In addition, cytogenetic parameters are important in karyotyping as indicators of genetic instability, assessment of hybridization [12]. Cytogenetic monitoring could become an additional indicator of the need to control the urban environment, as a quick marker for the selection of the most vulnerable components of the ecosystem. *Allium cepa* L. is often used as a test plant object [8]. In our study, we try to evaluate the cytogenetic parameters of *Quercus robur* L., a long–lived tree species in the urban environment and to get relevant information for this object. The aim of the study is to evaluate the changes of cytogenetic parameters of the oak seedlings apical root meristem in the period from 2001 to 2019.

2. Methodology

Voronezh is city located in the European part of Russia (geographical coordinates: 51.660781; 39.200269) in the temperate climate zone. According to the local weather station of Voronezh State Agricultural University, the average annual temperature for observation period since beginning of 20th century was +8.0°C, average January temperature -9.5°C, average July – +20°C; the absolute minimum – 36°C (January), absolute maximum +37.5°C (July). The duration of the growing season at the temperatures above +10 °C was 152 days (from April 29 to September 27). The sum of effective temperatures during the growing season was 2800°C. The frost-free period is 150-155 days. The latest frost was observed on June 3, the earliest – in autumn, September 2. The time of onset of stable snow cover was 30-40 cm. Average annual rainfall was 670 mm. South-West, South-East and South winds prevail in winter; in summer – South-West, West and North-West, bringing drought. The relative humidity in summer was 45-60%, in winter – 75-90%. Meteorological conditions in winter, as well as at the beginning and the end of the growing season, determine the results of introduction.

2.1. Territories of study and collection of material

Urban oak populations are located in the South-Western part of the Usman forest (points 1 and 2, figure 1) which as a result of the development of the city was fragmented, isolated from the main forest area, and the population located on the southern edge of the city (point 3, figure 1), these territories are experiencing a high anthropogenic load. The territory of the village of Maklok is defined by us as a control area (point 4, figure 1), is located at a distance from urban areas and closes with the main forest area.

2.2. Collecting and germination of acorns

English oak (*Quercus robur* L.) seed material was collected in the 4 research locations (figure 1, points 1-4). Approximately 150-200 seeds were collected from each area. Acorns was germinated in boxes with moist sand at room temperature. When the roots of plantlets reached a length of 2 to 3 cm, roots apical part was fixed in the 3:1 mixture of 95% ethanol and glacial acetic acid at 11 pm, when the peaks of mitotic activity and pathological mitosis took place [13]. The biological material was kept under 4°C. The roots cytological samples were stained by acetic haematoxylin, squash preparations were made using the method described earlier [6].
Figure 1. Study places: 1 – Zadonskoye highway of the M4 Don, located in the city limits (51.754139, 39.188270); 2 – forest area near the Voronezh state forest engineering University (51.720246, 39.224015); 3 – forest area of Shilovsky forest (51.580645, 39.171152); 4 – forest area of Maklok village (51.809864, 39.414841).

2.3. Preparation stages of pressed preparations
Roots were washed in 18% acetic acid prior incubation for 30 min. After roots were rinsed with 45% acetic acid 5 times, washed in distilled water and filled with acetic haematoxylin for 4 h and washed one more time consistently in acetic acid and in distilled water for 10 min each. Then the 3 mm root tips were placed into the slide to the Goyer liquid, covered by a cover glass and pressed by needle to crushed the material of the root.

Meristem division activity of oak seed progeny was estimated for 280 sample preparations. The cytological analysis was performed using a Laboval-4 microscope (Carl Zeiss, Germany) at magnification of 40×1.5×10 and 100×1.5×10 to study mitosis characteristics and types of pathologies of mitosis 700 cells. Based on the obtained data, we estimated mitotic activity (by calculating the mitotic index – the ratio of quantity of dividing cells to the total quantity of analyzed cells) and mitotic index calculated excluding prophase cells (the ratio of quantity of cells at the mitosis stages of metaphase, anaphase and telophase to the total quantity of analyzed cells – as an express-index of the delays of cells at mitosis stages and functioning of checkpoint-repair system [14]. We calculated the percentage of cells at the stages of prophase, metaphase, anaphase-telophase; the percentage of pathological mitosis out of the total number of cell divisions and the percentage of disturbances at the stages of metaphase, anaphase, telophase to the total number of abberant cells at these stages of mitosis (as the majority of pathologies are recorded at these stages of mitosis); the percentage of every type of mitotic disturbances out of their total number and the percentage of cells with persistent nucleoli at the stages of metaphase – anaphase of mitosis out of the total number of cells at these stages.
Anthropogenic influence in our study was considered as a complex of urban territories factors, including vehicle emissions, changes in microclimate, water regime of soils, recreational load, etc. The climate indicators were taken from the website «Weather and climate» [15] to identify correlations between the studied cytogenetic parameters and air temperature and precipitation. The average and absolute maximum temperatures in vegetation period and annual water precipitation are presented in table 1.

### Table 1. Climate indicators for Voronezh in the studied years.

| Year | Amount of precipitation in the form of rain, mm | Annual precipitation, mm | Average / absolute maximum temperature, °C |
|------|-----------------------------------------------|--------------------------|------------------------------------------|
|      |                                              |                          | April | May | June | July | August |
| 2001 | 141                                           | 669                      | 11.1/25.1 | 14.0/26.7 | 16.9/28.7 | 24.1/36.3 | 20.1/34.4 |
| 2007 | 140                                           | 514                      | 7.1/22.2 | 17.0/35.7 | 19.2/34.6 | 21.0/33.0 | 22.4/36.3 |
| 2008 | 144                                           | 486                      | 11.2/22.2 | 13.7/29.0 | 17.2/28.5 | 21.1/34.0 | 21.0/36.0 |
| 2012 | 144                                           | 829                      | 11.9/29.2 | 18.4/31.8 | 20.1/32.5 | 22.1/32.5 | 20.3/35.4 |
| 2019 | 123                                           | 518                      | 10.2/26.3 | 17.1/31.0 | 19.2/29.7 | 19.4/29.7 | 19.4/33.8 |

3. Results and discussion

The cytogenetic parameters of the apical English oak seedlings root meristem are presented in table 2.

### Table 2. Cytogenetic parameters of English oak seedlings from trees growing in the urban and control areas.

| Cytogenetic parameters | Urban areas | Control area |
|------------------------|-------------|--------------|
| **Year**               | 2001        | 2007         | 2012         | 2019         | 2008 | 2019 |
| Mitotic index, %       | 7.7±0.4     | 7.5±0.3     | 8.4±0.3     | 6.8±0.9     | 7.2±0.9 | 8.4±0.3 | 9.5±1.2 | 8.8±0.9 | 10.5±4.6 |
| Mitotic index excluding prophase cells, % | 5.9±0.3 | 5.1±0.3 | 5.2±0.3 | 3.6±0.3 | 3.9±0.3 | 4.3±0.3 | 5.1±0.3 | 4.9±0.4 | 4.8±1.9 |
| Prophase cells, %      | 36.1±2.0   | 31.8±1.9   | 38.3±2.6   | 40.2±3.3   | 47.4±2.6 | 53.7±2.6 | 45.1±3.4 | 40.8±2.6 | 51.4±53.6 |
| Metaphase cells, %     | 20.9±1.2   | 27.5±1.7   | 20.5±1.2   | 23.1±2.3   | 21.1±1.2 | 15.7±1.2 | 20.1±3.4 | 24.0±1.2 | 16.4±20.3 |
| Anaphase-telophase cells, % | 43.0±1.5 | 40.7±1.3 | 41.2±1.5 | 36.9±2.1 | 30.6±1.1 | 30.6±2.1 | 34.9±2.1 | 35.2±1.1 | 32.9±27.2 |
| Mitotic pathologies, % | 1.5±0.3    | 1.1±0.3    | 1.0±0.3    | 1.1±0.3    | 1.1±0.3 | 1.1±0.3 | 1.1±0.3 | 1.1±0.3 | 1.1±0.3 |
| Mitotic pathologies excluding prophase cells, % | 19.3±1.1 | 16.0±0.8 | 19.1±0.8 | 14.1±0.8 | 24.3±1.1 | 25.0±1.1 | 33.1±1.1 | 26.1±1.1 | 8.9±13.3 |

1 – Zadonsky highway M4 Don located in the city (51.754139, 39.188270); 1.1 - Zadonsky highway M4 Don highway near the interstate; 1.2 - Zadonsky highway M4 Don in the depths of the forest away from highway 1 km; 2 – the forest near Voronezh State University of Forestry and Technologies (51.720246, 39.224015); 3 - forest Shilovsky forest (51.580645, 39.17152); 4 – Maklok forest (51.809864, 39.414841).

The data showed that in 2019, acorn seedlings collected in urban populations of English oak have high level of mitotic index (table 2). In long-term dynamics, the mitotic index for urban populations
(from 6.8% to 9.5%) is approximately at the same level, without sharp fluctuations. There is a sharp decrease in mitotic activity (4.6%), compared with the data for 2008 (10.5%) for the control area in 2019.

The climate indicators vary from year to year, but there were no sharp changes in the analyzed years (table 1). The decrease in the average temperature for the month of April was observed in 2007, maximum temperatures above 23 degrees did not exceed in 2007, 2008 years. The decrease in the average temperature for the month of May occurred in 2001, 2008, maximum temperatures were above 29 degrees in 2007, 2012, 2019 years.

Lowest average temperatures for June were registered in 2001 and 2008, maximum temperatures less than 23 degrees were in 2007, 2012, and 2019 years. The average and maximum temperature for July were highest in 2001. The maximal average temperature for August was found in 2007, maximum temperatures were less than 23 degrees in all studied years.

Cytogenetic indicators of seedlings from urban areas and seedlings from control territories were different. We have shown the increase of mitotic activity, number of cells with impaired mitotic division, and in the long-term dynamics, increased number of mitosis pathologies for urban territories last twenty years (table 2). We also did not observed until 2012 English oak micronuclei. Residual nucleoli during mitosis, another mitotic pathology was also typical for seedlings root meristems from urban areas. Mitotic activity of seedlings from the control area varies in different years and there are not a large number of cells with mitotic pathologies. Long-term cytogenetic analysis of root meristems showed that effect of pathologies increase in urban territory is accumulative. There is a significant proportion of cells at the stage of mitosis prophase, which may indicate the work of the check-point repair system, which reduces the number of pathologies. At the same time, the percentage of professors in the control zone is significantly higher (table 2) than in the urban population.

The dependence of the increase in the mitotic index and the number of pathologies of mitosis on climatic factors was not found.

Cytogenetic parameters are used as biomarkers of exposure to substances of chemical, radiation, biological nature, instability of genetic material. In the case of negative influence of factors, there are violations of the chromosomal aberration, micronuclei, appearance of acentric fragments, a decrease in mitotic activity, and, as a result, a decrease in the growth of tissues and organs [8]. A growth in the number of aberrations indicates a negative effect on the chromosomes and cell division components [7, 11]. The increase in the number of pathological mitoses in the cells of seedlings from urban areas obtained in our study indicates a negative impact of anthropogenic factors on plants. Given that the seeds were sprouted in clean sand moistened with distilled water, the presence of chromosomal aberrations and micronuclei is the result of the accumulation of damaging substances. The proximity of vehicles to collection points indicates the impact of vehicle exhaust, as well as their chemical pollutants.

The decrease in meristems mitotic activity and apexes growth activity is a phenotypic manifestation of various internal processes and is directly related to them. It was shown that, the growth of seedlings was greatly influenced by a variety of both external and internal factors [16]; using microsatellites to assess the genetic diversity and genetic structure of populations, for Q. oleoides showed that signs of gas exchange and growth had strikingly high quantitative variations compared to those associated with leaf morphology, anatomy, and photochemistry. The quantitative genetic variations varied between populations even at geographical scales at a distance of 1 km. Thus, the authors point to a high intraspecific and intra-population heterogeneity of individuals on quantitative grounds. The activity of meristem division is directly reflected in the growth of the plant and affects the competitiveness of seedlings at the early stages of growth and development. It is possible to use the average population values of the proportion of cells with pathological mitoses in the root meristems of seedlings for the selection of English oak.

We suggest that the decrease in mitotic activity may be due to the influence of an anthropogenic factor on the oxidative processes of cells. Thus, it was demonstrated [17] that controlled oxidation was a key feature of the early stages of the cell cycle, but prolonged weak oxidation limits nuclear functions and impairs progression through the cell cycle, leading to a decrease in the number of cells in the root
apical meristem. Reduction of meristem division may be caused by suppression of transcription factors [18]. The effect of high temperatures and water stress on the phenology and growth parameters of the genus *Quercus* was established. In the phenological and growth response of *Q. robur* to spatiotemporal fluctuations, including the reproductive period, it delays budding in control conditions, but outstrip budding in heated seedlings. In seedlings from the mother in controlled warming with the growth of seedlings, it has reduced the diameter of the shoot. Such studies [17, 18] show the complexity of the interaction of processes occurring both at the stage of seed formation and germination, the impact of a complex of environmental and anthropogenic factors affects many systems, but is realized in the division of meristems and subsequent growth of the plant.

The cytogenetic parameters presented us the appearance of a large number of mitotic pathologies in the dividing apical meristem of the root and the intensification of the mitotic cycle in response to increasing the impact of the urban environment on living organisms. In the period from 2001 to 2019, the city increased environmental pressure towards the forest area. Cytogenetic parameters of plant root meristems can be used as biomarkers of environmental pollution, as well as a parameter for selecting the fastest growing plants.

4. Conclusion

The long-term changes of cytogenetic parameters of the seedlings apical root meristems was analyzed for the first time for English oak growing in the Central Black-Soil region in this study. It was found that the cytogenetic parameters of seed progeny of trees growing in urban and suburban conditions are characterized by an increase in mitotic activity and the proportion of cells with mitotic pathologies, the appearance of microkernels and residual nucleoli in mitosis, which may be a consequence of the impact on populations of a complex of city negative factors of the. Voronezh is a large city with a population of 1 million people, developed infrastructure, and a constant increase in the number of vehicles. Considering climate fluctuations, it was found that the parameters vary and do not remain constant, but no sharp fluctuations were detected in the studied years. The climate indicators vary from year to year, but there were no sharp changes in the analyzed years. Variations in climate indicators do not significantly reduce or increase mitotic activity in acorn seedlings.

Acknowledgments

This study was supported by Grant of Russian Fund for basic research (N 19-44-363001-r_mol_a) and by the Russian Federation President grant for support of leading scientific school (NSh – 2535.2020.11, agreement).

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