Cytogenetic disturbances in the apical meristem cells of the urban plantations depending on the level of anthropogenic impact

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Abstract. The scope of this research covered cytogenetic indexes of the apical meristem cells of the urban plantations of small-leaved linden (Tilia cordata L.) and silver poplar (Populus alba L.) growing in the areas of different anthropogenic pollution load, such as parks, public gardens, and urban plantation areas along the municipal motorways. The cytogenetic disturbances of the linden and poplar apical meristem cells were studied based on the incidence of micronuclei and nucleus protrusion of different forms. Buds for the analysis were collected from the tree height of 1.5-2 m in the spring season, in March-April. The collected buds were preserved in aceto-alcohol, then the apical meristem cell microslides were prepared and coloured with acetocarmine. The karyological characteristics were analysed and quoted according to the method of L. P. Sycheva. It was demonstrated that the cytogenetic disturbances with the greatest incidence of micronuclei and nucleus protrusion of various types are significantly more frequent in the apical meristem cells of the small-leaved linden and silver poplar buds collected from the plantations along the urban motorways compared to the same indexes of the trees growing in parks and public gardens. Compared to the same cells of silver poplar, the apical meristem cells of the small-leaved linden buds were found to be more sensitive to the technogenic pollution of the environment. The apical meristem cells of the buds of the studied trees and small-leaved linden in particular may serve as the genotoxicity indicators of the urban environment.

1. Introduction
Due to the currently growing negative impact of the technogenic pollution on the people's health [1-3] the environment-related research topics appear to be especially relevant. The pollution problem cannot be solved without monitoring studies intended to assess, first of all, the condition of the natural environment and to identify the dynamics of the environmental changes and the disease incidence among the population. For this reason, the development of efficient approaches to monitoring the anthropogenic impact on the environment plays a special role in this process.

One of such monitoring approaches is a cytogenetic survey, which is widely used for the assessment of genome stability and evaluation of the genotoxic effect of environment damaging factors [4-7].

According to a number of researchers [8-11], the cytogenetic survey is a sensitive method of effective and adequate evaluation of the negative impact made by the harmful factors on the environment and the human health [6,8,12].
The published researches mostly offer information about the cytogenetic disturbances of coniferous plantations caused by the technogenic impact on the environment [8-10,13].

The objective of the research was to study the cytogenetic disturbance in the apical meristem cells of the urban plantation sprouts (linden and poplar) depending on the anthropogenic pollution exposure level.

2. Material and research methods
The research was carried out in the city of Orel. The objects of the research were the small-leaved linden (Tilia cordata L.), silver poplar (Populus alba L.) plantations and their sprout apical meristem samples. The average age of the trees was 12-15 years.

Depending on the anthropogenic pollution exposure, all the studied trees were divided into three groups. The first group included the plantations of the parks, maximally remote from the motorways, i.e. located in the minimum anthropogenic pollution zone. The second group consisted of the trees growing in public gardens located not far away from the motorways, where the anthropogenic pollution level can be assessed as medium. The third group embraced the urban plantations exposed to concentrated exhaust gases and motorway de-icing chemicals used during winter period.

The survey covered three urban parks, three public gardens and nine areas of street plantations along the motorways.

The cytogenetic disturbances of the linden and poplar apical meristem cells were studied based on the incidence of micronuclei and nucleus protrusions of different forms. Buds for the analysis were collected from the tree height of 1.5-2 m in the spring season, in March-April of the years 2009, 2012 and 2015. The collected buds were preserved in aceto-alcohol, then the apical meristem cell microslides were prepared and coloured with acetocarmine. The karyological characteristics were analysed and quoted according to the method of L. P. Sycheva [11].

The mathematical processing was carried out with the variation statistics method with the calculation of the mean value (M), mean error (m) and difference confidence calculation with Student's t-test. The differences were considered relevant at p < 0.05. The statistic analysis of the collected results was carried out with Microsoft Excel and Statistika 6.0 applied software package.

3. Research results
The research results showed (table 1) that the average number of micronuclei per 1000 apical meristem cells of the sprouts of the trees monitored in three parks of the city in the year 2009 constituted 36.8±7.6 or 3.8% for linden and 24.2±6.4 or 2.5% for poplar; in the year 2012, 41.3±6.3 or 4.3% for linden and 28.7±5.7 or 3.1% for poplar; in the year 2015, 49.6±8.4 or 5.1% and 33.2±8.9 or 3.6% respectively. The mean average number of the micronuclei per 1000 cells for the entire survey period in the first group, i.e. the group with the minimum anthropogenic pollution, constituted 41.7±6.5 or 4.3% for linden and 27.7±7.6 or 2.6% for poplar.

The number of micronuclei in the meristem cells of the buds collected from lindens and poplars in public gardens, i.e. places with medium anthropogenic pollution, was obviously bigger compared to the same trees growing in parks. Thus, in the year 2009, the number of micronuclei per 1000 cells was 92.5±10.2 – 9.2% (p=0.998) for linden and 68.4 ±12.3 – 7.1% (p=0.981) for poplar; in the year 2012, 114.7±13.7 – 10.3% (p=0.997) for linden and 86.6±17.4 – 9.7% (p=0.987) for poplar; in the year 2015, 127.8±11.5 – 13.1% (p=0.999) and 98.2±24.6 – 18.6% (p=0.999) for linden and poplar, respectively. The average micronuclei incidence rate for the linden and poplar buds from this group for the survey period constituted 109.4±8.3 – 9.6% (p=0.999) and 85.2±14.8 – 8.4% (p=0.990) respectively.

Testing the urban plantations stretching along the urban motorways and exposed to more intensive anthropogenic pollution, it was concluded that in the apical meristem cells of linden and poplar the micronuclei rate was significantly higher compared to the same index of the trees growing both in parks and public gardens: in 2009, this index constituted 216.3±28.4 or 22.1% (p=0.998) for linden, 168.0±21.2 or 17.2% (p=0.997) for poplar; in 2012, 237.4±23.8 or 24.3% (p=0.998) for linden, 177.8±19.3 or...
18.3% (p=0.988) for poplar; in 2015, 298.5±24.3 for linden and poplar, which makes 29.4% (p=0.999) and 198.2±24.6 or 19.2% (p=0.968) respectively.

The micronuclei rate in the third group of the trees tested during the survey constituted, on the average, 253.7±24.6 or 24.8% (p=0.999) and 180.0 ± 20.9 or 18.4% (p=0.993) respectively.

Table 1. Micronuclei rate in the apical meristem cells of small-leaved linden and silver poplar, M±m.

| Survey years | Number of micronuclei (per 1000 cells) |
|--------------|---------------------------------------|
|              | Linden | Poplar | Linden | Poplar | Linden | Poplar | Linden | Poplar |
|              | Group 1 | Group 2 | Group 3 | Group 1 | Group 2 | Group 3 | Group 1 | Group 2 | Group 3 |
| 2009         | 36.8±7.6 | 92.5±10.2  | 216.3±28.4 | 24.2±6.4 | 68.4±12.3  | 168.0±21.2 | 68.4±12.3  | 168.0±21.2 | 68.4±12.3  |
| 2012         | 41.3±6.3 | 114.7±13.7  | 237.4±23.8 | 28.7±5.7 | 86.6±17.4  | 177.8±19.3 | 86.6±17.4  | 177.8±19.3 | 86.6±17.4  |
| 2015         | 49.6±8.4 | 127.8±11.5  | 298.5±24.3 | 33.2±8.9 | 98.0±16.8  | 198.2±24.6 | 98.0±16.8  | 198.2±24.6 | 98.0±16.8  |

The value differences in the first group are considered relevant at:

\( p < 0.05 \), \( p < 0.01 \), \( p < 0.001 \).

Moreover, the analysis of the survey results demonstrated that the incidence of micronuclei in the apical meristem of the considered trees growing in the covered territories of the city is increasing. Thus, compared to the year 2009, in 2015 the number of micronuclei in the apical meristem of linden and poplar increased: in group 1, by 34.3% and 37.2%; in group 2, by 37.2% and 43.2%; and in group 3, by 38.2% and 18.6% respectively.

As a result of studying cytogenetic disturbances by the index of nucleus protrusions of different kinds (table 2) it was found that in the apical meristem cells of the lindens and poplars growing in the parks, in the covered years, the mean index value constituted 13.4 ± 3.8 (1.1%) and 10.9 ± 2.8 (0.98%) per 1000 cells, while in the second group (public gardens) this index was twice higher compared to that of the first group and constituted 27.9± 4.5 (2.8%; \( p=0.999 \)) and 21.2±3.1 (1.9%; \( p=0.995 \)).

Table 2. Rate of nucleus protrusion of different types in linden and poplar, M±m.

| Survey years | Number of nucleus protrusions of different types (per 1000 cells) |
|--------------|---------------------------------------------------------------|
|              | Linden | Poplar | Linden | Poplar | Linden | Poplar | Linden | Poplar |
|              | Group 1 | Group 2 | Group 3 | Group 1 | Group 2 | Group 3 | Group 1 | Group 2 | Group 3 |
| 2009         | 12.4±2.3 | 24.3±3.4a  | 58.8±7.3b | 8.3±2.2 | 17.3±4.2 | 43.0±5.1b | 12.4±2.3 | 24.3±3.4a  | 58.8±7.3b | 8.3±2.2 | 17.3±4.2 | 43.0±5.1b |
| 2012         | 13.8±5.1 | 28.5±4.2  | 71.0±11.3b | 11.4±2.6 | 19.8±3.5 | 48.4±4.8b | 13.8±5.1 | 28.5±4.2  | 71.0±11.3b | 11.4±2.6 | 19.8±3.5 | 48.4±4.8b |
| 2015         | 16.5±3.7 | 36.7±4.8b  | 96.2±9.7b | 14.2±3.3 | 25.2±5.7 | 59.3±7.9b | 16.5±3.7 | 36.7±4.8b  | 96.2±9.7b | 14.2±3.3 | 25.2±5.7 | 59.3±7.9b |

The value differences in the first group are considered relevant at:

\( p < 0.05 \), \( p < 0.001 \).

The nucleus protrusions were more frequent in the meristem buds of the trees growing along the urban motorways; for linden, the average number of the bud meristem nucleus protrusion was 76.3±8.7 (7.3%; \( p=0.999 \)), and for poplar the index was 51.3±6.0 (5.2%; \( p=0.997 \)). Among the nucleus protrusion types found in the buds of the lindens and poplars growing along the motorways, the most common were type 1 (known as the nuclear bud) found in 32.4% of trees, type 3 (tongue) in 22.9%, atopic shaped nucleus in 14.3%, type 4 (tailed nucleus) in 8.4% and type 2 (broken egg) in 5.1% of trees. Throughout the entire period of the research, the rate of cytogenetic disturbance manifested as nucleus protrusions of different types was noticed to increase among the examined trees; thus, if in the first group both lindens and poplars growing in parks only demonstrated a trend to increasing the protrusion rate, the lindens and poplars in the public gardens and growing along the motorways showed a significant leap in the number of cells with nucleus protrusions of different kinds.
4. Conclusion
The survey demonstrated that the cytogenetic disturbances with the greatest incidence of micronuclei and nucleus protrusion of various forms are significantly more frequent in the apical meristem cells of the small-leaved linden and silver poplar buds collected from the plantations along the urban motorways compared to the same indexes of the trees growing in parks and public gardens. Compared to the same cells of silver poplar, the apical meristem cells of the small-leaved linden buds were found to be more sensitive to the technogenic pollution of the environment. The collected data may prove the high anthropogenic impact making a genotoxic effect on the trees, firstly, on the trees growing along the urban motorways. Therefore, the apical meristem cells of the buds of the studied trees and small-leaved linden in particular may serve as genotoxicity indicators of the urban environment.

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