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Original Paper

INFLUENCE OF SiO_2 NANOPARTICLES ON GAMETIC CELLS OF LIME TREES FROM URBAN AREA DETECTED BY FLOW CYTOMETRY

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Silica or silicon dioxide nanoparticles (SiNPs) are one of the most widely spread nanoparticles in the environment, particularly, in urban areas in the form of dust. Influence of SiNPs on plant cells is unclear. This research was conducted to test a hypothesis that plant cell relative fluorescence and SiNP toxicity differ depending on the genetic properties and environmental conditions. Young pollen cells of lime trees in the mid to late one-nucleate developmental stage were found to be more sensitive to detect the influence of SiNPs and UV irradiation. Alteration of cell relative fluorescence depending on tree growth conditions was observed. Cells from trees grown in the urban area of Rīga had much lower reaction to SiNPs in comparison with cells from trees grown in the greenhouse. Lime trees growing for a long time in urban areas have complex adaptive features to a variable environment and can used as source-material to propagate lime trees for growing in such conditions. Flow cytometry can be applied for evaluation of plant reaction to factors that affect plants in the urban environment.

Keywords: *silicon dioxide, UV irradiation, relative fluorescence, urban environment, greenhouse.*

INTRODUCTION

Today approximately half of the world's human population is concentrated in towns and cities. The rapid development of urban environments poses serious environmental problems: climate change, environmental pollution, noise, waste, landscape fragmentation, a reduction in biodiversity, ecosystem degradation, invasive species, etc. (Niemela, 2004; Klaus and Kiehl, 2021). Currently, sustainable urban environment ecological studies are becoming more and more topical and are among the most popular and fastest growing fields of natural science in the world, with the aim of ensuring the quality of living and working environment for people in the city and to provide a basis of knowledge for balanced development. Latvia has nine cities; these urban areas make together only up to 1.1% of the territory of the country, but support 51.1% of the Latvian population. The increase of the population concentration in cities is an important problem, in particular in Rīga, the capital of Latvia: around 70% of all the country's citizens live in urban territories and most of them in Rīga and its surroundings (Krūmiņš, 2023, Official statistics portal, 2024). Rīga is the ninth largest city in northern Europe, with intensive transit flow through harbours, as well as construction of new factories and logistics centres. Although Rīga is rich in gardens and parks, nevertheless there are elevated levels of air pollution by dust (PM10 and PM2.5) and gases (NO_x, O_3), which are the highest among the Baltic Sea cities (European

Environment Agency, 2014). The increased pollution in the city raises importance of conservation of gardens and parks territories and street greenery.

Lime tree (*Tilia* sp.) is a popular tree genus used for urban landscaping world-wide due to its tolerance to various environmental factors, and due to cultural, aesthetical etc. reasons (Sander *et al*., 2003; Sjöman *et al*., 2011; Östberg, 2013; Stravinskiene *et al*., 2015; Tenche-Constantinescu, 2024). During the last years, a high proportion of lime trees in new plantings is also characteristic for Latvian cities, especially Rīga. Unfortunately, within the long-term, decline of street trees and low vitality has been observed in Rīga (Čekstere *et al*., 2005; Cekstere and Osvalde, 2013; Cekstere *et al*., 2020). Tree status can seriously undermine the capacity of the trees to perform a variety of functions such as aesthetical, air pollution reduction, regulation of microclimate, etc. Therefore, *Tilia* sp. was selected as an object for our investigation. While the lime tree populations in urban areas are mainly a mixture of *Tilia* sp. hybrids (Andrew, 1971; Chambers and Godwin, 1971; Pigott, 2012), genetic factors might potentially have influence on individual plant reactions to stress. Trees grown in the urban environment for a long time are affected by various ecological factors, for example air and soil pollution, which influences their growth, development and reproduction (Craul, 1999; Cekstere and Osvalde, 2013).

Silica nanoparticles (SiNPs) and increased intensity UV irradiation are important stress factors for plants in especially in urban areas. SiNPs are one of the most common components of pollution in urban areas (Veliu and Syla, 2008; Campos-Ramos *et al*., 2011; Wang and Pui, 2011). Nanoparticles, or nano-scale particles (NSPs), are defined as particles with dimension less than 100 nm (Ball, 2002). Natural NSPs exist from the beginning of Earth's history as volcanic dust or lunar dust, mineral composites etc. They are constantly present in the environment and play a central role in many natural processes (Kan and Tomson, 1990; Matsunaga *et al*., 2000; Kokina *et al*., 2013). Incidental (waste or anthropogenic) nanoparticles occur as pollution and are a result of different industrial processes (Colvin, 2003; Lin and Xing, 2007; Monica and Cremonini, 2009; Wang *et al*., 2010; Kumar *et al*., 2013; Zajzon *et al*., 2013). Silica or silicon dioxide (SiO_2) nanoparticles are one of the most widely common nanoparticles in the environment, but knowledge of the biological mechanisms of their influence on cells is limited (Kumar *et al*., 2012; Duan, *et al*., 2014). SiNPs have been described as safe, non-toxic and environment friendly for use in the development of nanocomposites, consisting of organic polymers, although there is evidence that amorphous SiNPs can be hazardous (Mizutani *et al*., 2006; Reijnders, 2009). Influence of SiNPs on plant cells, including toxicity of nanoparticles, depends on types, size, and concentration of particles, evaluated plant species and plant physiological state (Ma *et al*., 2010; Wei *et al*., 2010).

UV irradiation is well known as a stress factor (Kostina *et al*., 2001; Hektors *et al*, 2007; Peykarestan and Seify, 2012) that can damage various plant cell processes. These damages can be classified into two categories — damage to DNA that can cause heritable mutations, and effect on physiological processes (Stapleton, 1992). Plants may acclimate to low doses of ultraviolet radiation and show specific changes in morphology and gene expression in response to UV irradiation, even in absence of stress symptoms (Hectors *et al*., 2007). In urban areas plants are exposed to intensive UV irradiation and influence of SiNPs through the year, but there is poor knowledge about plant reaction to both these factors.

The lime trees populations in urban areas are a mixture of *Tilia* sp. hybrids (Andrew, 1971; Chambers and Godwin, 1971), and for that reason genetic factors may potentially have effect on individual plant reaction to stress. Environmental stresses are known initiators of retrotransposon activity, which can have impact on plant adaptation (Civáň *et al*., 2011). In order to study genetic diversity of lime tree *Tilia* sp., the universal retrotransposon based method IRAP (Inter-Retrotransposon Amplified Polymorphism) (Kalendar *et al*., 2010) was chosen in this research. This method allows to reveal high levels of genetic diversity and is cost and labour effective. It was successfully used for investigation of genetic diversity for several species (Vukich *et al*., 2009; Kalendar *et al*., 2010; Smżkal *et al*., 2011; Klavina *et al*., 2014).

Trees such as oaks, ash, birch spruce, pine, and lime have been used as bioindicators using many methods (physiological, biochemical, morphological, and cytogenetic) to determine environmental pollution impact (Nabais *et al*., 1999; Niinemets *et al*., 1999; Hagen-Thorn *et al*., 2004; Yilmaz and Zengin, 2004; Cekstere and Osvalde, 2005; Kosiba, 2008; Cekstere *et al*., 2010). All of these methods are based on determining influence of one or a few environmental factors, or complicated multifactorial influence on one or more traits, which are time and labour consuming. Flow cytometry (FCM) is a widely used method for investigation of several plant cell parameters, including determination of cell oxidative stress on the basis of induced peroxidase intensity (Djaković and Jovanović, 2003; Dožel *et al*., 2007; Dimpka *et al*., 2012). FCM has several advantages: a large number of cells can be evaluated in a very short time, results are with high level of statistical significance and represent a whole population, and on the basis of changes of cell relative fluorescence it is possible collect and analyse more than 20 parameters from each cell. This makes the technique an excellent tool in many research areas (Dožel *et al*., 2007; Bargmann and Birnbaum, 2009; Galbraith, 2010).

The goal of the study was investigate influence of SiO_2 nanoparticles and UV irradiation on cell self-fluorescence of gametic cells of lime trees, using the flow cytometry method for detection of plants environmental stresses.

MATERIALS AND METHODS

Plant material. Inflorescences were collected from 12 trees *of Tilia* sp. (50 inflorescences from each) during the period

from the end of June and beginning of July 2014. All sampled trees were more than 20 years old and represented street greenery of three different urban environment locations of Rīga (capital of Latvia): four of the 12 trees (B2, B4, B5, B10) were growing in Bolderāja, an industrial region of Rīga, six trees (C11, C12, C13, C14, C15, C16) in the city centre and two trees (F6, F9) were located in the territory of Rīga harbour.

Control material was collected from five three-year old trees (20 inflorescences from each) randomly chosen from a nursery's greenhouse plants (K1, K2, K3, K4, K5), where they were cultivated for greening of urban areas. Stipules from inflorescences from all greenhouse trees were analysed as one control group to determine the concentration of several chemical elements and to establish the average relative fluorescence of tree cells (K1–5).

For method elaboration, 200 inflorescences were collected from one more than 20-year-old lime tree growing in a park area of Salaspils city. The collected inflorescences were sorted according to the developmental stage of buds: S1 — very young buds — less than 2 mm in diameter, S3 — young buds — about 2 mm in diameter, S7 — buds about 3 mm in diameter, and S8 — buds more than 3 mm in diameter. For experiments only fresh plant material was used.

Phenotypical evaluation of inflorescence stipules. Stipules of collected inflorescences were visually characterised by damage level using a scale from 0 to 8 points accordingly to two criteria: shape (well-shaped or deformed) and size of necrotic area (in %). Point were scored as follows: 0 points – stipules without damage'; 1–4 points — well shaped stipules with % of necrotic area 1–10% (1 point), 10–20% (2 points), 20–50% (3 points) and > 50% (4 points); 5–8 points — deformed stipules with % of necrotic area 1–10% (5 points), 10–20% (6 points), 20–50% (7 points) and > 50% (8 points).

Chemical analysis of inflorescences stipules. Stipules were dried at +70 (±1) °C for 24 h and ground using a laboratory mill. Samples were dry-ashed in concentrated HNO_3 vapour and re-dissolved in 3% HCl. The level of Ca, Mg, Fe, Cu, Zn, and Mn was measured using an atomic absorption spectrophotometer (AAS) Analyst 700 (PerkinElmer, Singapore) with acetylene-air flame (Page *et al*., 1982; Ferreira *et al*. 2018). Concentration of K and Na was detected with a flame photometer JENWAY PFPJ (Jenway Ltd, Gransmore Green, Felsted Dunmow, Essex, UK). The concentration of P was determined by colorimetric method using ammonium molybdate in acid-reduced medium with a spectrophotometer JENWAY 6300 (Barloworld Scientific Ltd., Gransmore Green Felstad, Dunmow, Essex, UK) (Rinkis *et al*. 1987; Osvalde, 2011).

Genetic diversity analysis. Buds from all selected lime trees were collected to perform DNA isolation. The DNA was isolated using standard NucleoSpin® Plant II protocol (Genomic DNA from plant user manual, Macherey-Nagel).

A total of specific polymerase chain reaction's (PCR) retrotransposon-based primers (Kalendar *et al*., 2010) were tested on a preliminary sample set of *Tilia* sp. trees, which represented several localities of the trees. Of these 16 primers, two primers: 2232 and 2373 were selected for screening all genetic material in the research. The repeatability of the two primers was tested by duplication of ten top polymorphic loci produced samples and processed by the Totallab 1D software.

DNA amplification was performed by a Gene Amp® PCR System 9700 thermocycler (Applied Biosystems) under the following conditions: denaturation for 3 min at 95 °C, 30 cycles (denaturation for 45 sec at 95 °C, primer annealing for 50 min and storing at 4 °C. 4 ng of the isolated DNA was used for each reaction. The total volume of the PCR mixture was 25 µl and consisted of: 2.5 µl 10× Dream Taq Buffer (Fermentas), 0.5 µl of 10 mMdNTPs (Fermentas), 2.5 µl primer, 4 µl stock solution (Invitrogen), and 0.25 µl of 5 u/µl *DreamTaq* polymerase (Fermentas), and 2.5 u/µl of 0.025 *Pfu*DNA polymerase (Fermentas).

The PCR products were processed by 1.7% agarose gel electrophoresis with a 25 × 10 gel track and 1XTAE buffer at 90 V for 4 hours. The gel was dyed for 40 minutes in water solution (50 ml/l) ethidium bromide and rinsed for 10 minutes in deionised water. After visualisation the PCR products were photographed by a digital camera.

The amplified DNA fragments were scored by presence (1) or absence (0) of a band of given length in bp. Data was evaluated using POPGENE software version 1.32 (Yeh *et al*., 1999). Nei's index of diversity, number and percentage of polymorphic bands, as well as genetic distances among lime trees were determined and principal coordinate analysis was performed using NTSYS software.

Immature microspore cell culture preparation. Preparation of immature microspore cell culture was based on the modified method of Kasha *et al*. (2003). The optimal stages of microspores were determined by a light microscope with magnification $\times 10^3$ (Barnabás, 2003). *Tilia* sp. inflorescences with buds (diameter 2 mm) containing microspores at mid to late uninucleate developmental stage were collected and placed in 200 ml jars with deionised water and wrapped with plastic foil. Jars with donor inflorescences were kept at + 4 °C in dim light till cell culture establishment, but no more than for 14 days.

2-gram samples of the buds were collected, placed in a Waring Blender 8011 and ground in 0.3 M solution of D-mannitol with speed in mode No. 2 up to five times, each for 20 seconds, till the suspension was visually homogeneous, then filtered through mesh (50 µm) three times and the obtained liquid was collected in 45 ml-centrifuge plastic tubes. Then the samples were centrifuged (Eppendorf Centrifuge 5810R) at 4 °C, 900 rpm for 15 min. The liquid phase was decanted and cells from sediment were washed with 45 ml 0.3 M D-mannitol solution and centrifuged again at 4 °C, 900 rpm for 15 min. According to previous

observations (Kasha *et al*., 2003), 1 ml of such cell sediment contains about 600,000 cells. The liquid phase of the samples was poured off, then 1 ml of cell sediment was suspended in 4 ml of liquid MS medium (Murashige and Skoog, 1962) and mixed. The cell culture quality was determined by light microscopy in magnification $\times 10^3$.

The influence of SiNPs and UV on lime tree young pollen cells. A suspension of nanoparticles was prepared by the dilution of silicon dioxide (SiO_2) nanoparticles (SiNPs) (Sigma–Aldrich inc., size 10–20 nm, purity 99.5%) in distilled water in proportion 1 mg per 1 ml. After dilution, the suspension flask was placed in a Bandelin® RK-31 ultrasonic bath (frequency 35 kHz, effective US power 40 W) for 30 min sonification.

1 ml of SiNP suspension was added to 10 ml of cell suspension, then cells were incubated in speed shaking regime either for 1 or 3 hours. Control cells were incubated in the same conditions without adding SiNPs. Irradiation of the cells for 20 min by UV was used as a stress factor. Irradiation was provided by a mercury-xenon lamp (Hamamatsu light source L9566-02A, L10852 lamp (A10014-50-0110) that had two maximums of relative intensity: at 365 nm (100% intensity) and at 310 nm (75% intensity). The selected duration of irradiation provided a dose of UV irradiation equal to a daily dose corresponding to mean UV intensity in a sunny summer day in Latvia (35 W/m^2). Before UV irradiation, a negative pressure air-pump was used to place cells from suspension as a thin layer on a filter paper disk (diameter 45 mm), which was then placed on a Petri dish filled with solid MS media. Cells without treatment were used as a control. Experiments were performed at room temperature (23–25 °C). After UV treatment cells were rinsed off the filter paper into Petri dishes with MS media. After UV irradiation, the cells were suspended in 20 ml of MS media. 1 ml of SiNPs suspension was added to 10 ml of the earlier prepared cell suspension and incubated in speed shaking regime for either 1 or 3 hours. Control (non-radiated) cells were incubated in the same conditions both with and without addition of SiNPs. Before flow cytometer analysis the suspensions were filtered through a flow cytometry — pass filter (mesh 40 μm).

A BD FACSJazz® cell sorter (BD Biosciences, USA) with flow cytometer function was used to test the relative fluorescence of plant cells. The device was equipped with a 100 μm nozzle and phosphate-buffered saline (BD Pharmingen™ PBS, BD Biosciences, USA) was used as a sheath fluid. Cell counting events were triggered by a forward-scattered signal. The excitation of cell fluorescence was produced by a 488 nm Coherent Sapphire Solid State (blue) laser. The fluorescence emission was measured at 530 nm (bandwidth 30 nm) and 585 nm (bandwidth 29 nm). Before measurements, the flow cytometer was calibrated using Sphero™ rainbow calibration particles (3.0–3.4 μm, BD Biosciences, USA) in phosphate buffered saline (PBS). The calibration was considered successful if the coefficient of variance (CV) of relative fluorescence of the calibration particles did not exceed 3%. The mean fluorescence intensity both in 530 nm and 585 nm channels was recorded for each cell suspension sample. Cell counts were gated by the intensity in both fluorescence channels to include 95 to 99% of target cells. The intensity of fluorescence was expressed in arbitrary logarithmic units. No less than 3×10^3 cells were gated and analysed from each sample.

Statistical calculations were made using MS Excel software. The significance level of $p = 0.05$ was used.

RESULTS

Analysis of genetic diversity of lime trees. The estimated genetic diversity of lime trees was high, which can be explained by the hybrid origin of the trees; in total 45 polymorphic loci were recognised. According to Nei's genetic distance, there were three groups of genetically close trees (Fig. 1). The biggest group included trees from two industrial areas of Rīga – Bolderāja (B4, B5, B10) and harbour territory (F6, F9), as well as all trees from the nursery garden (K1, K2, K3, K4, K5). Nei's genetic distance among trees of this group varied from 0.28 to 0.46. Some trees from the Rīga central part (C11, C15 and C16) were genetically similar and formed a distant group. They could represent the same hybrid, or even the same clone. Two genetically similar trees (C12 from Rīga centre and B2 from Bolderāja) were genetically distant from other trees grown in centre of Rīga (genetic distance 0.82) and Bolderāja (0.67). Genetic distance among trees from different groups ranged from 0.86 to 0.92.

Phenotypical evaluation of inflorescences. Visual assessment of inflorescence stipules (Fig. 2) demonstrated variation in stipule damage among trees grown in different conditions. Most of visual damage, including deformed stipules and necrotic areas, were found in stipules collected from trees (F6, F9, B2, B4, B5, B10, C11, C15) grown directly

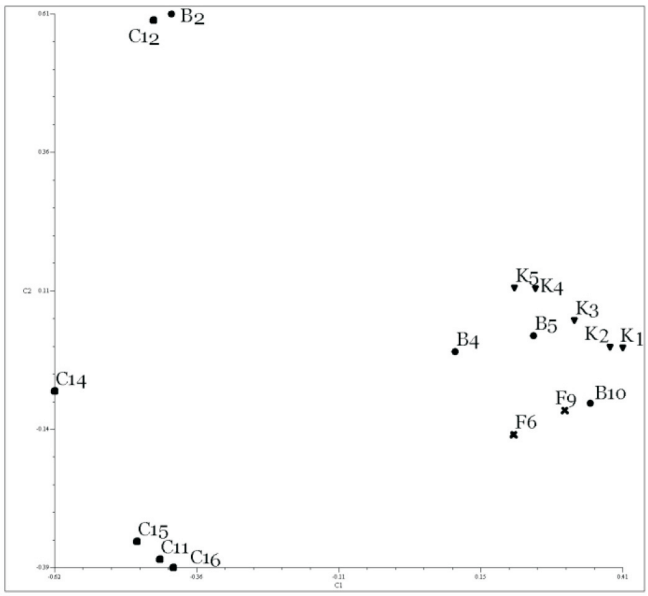

Fig. 1. Principal coordinates analysis of lime trees population based on Nei's genetic distance.

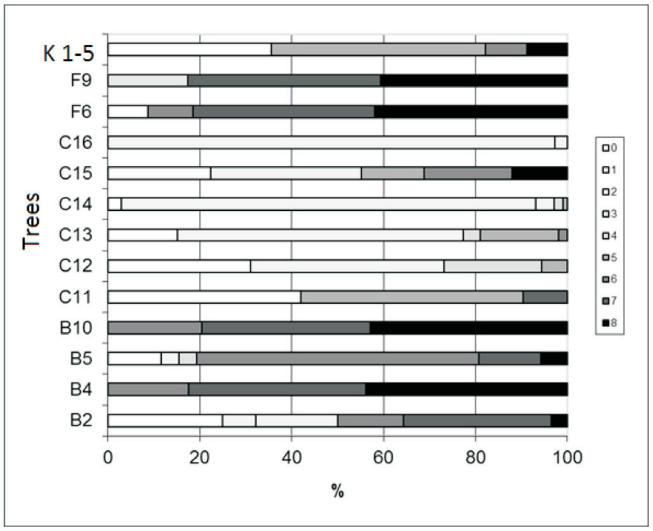

Fig. 2. Percentage of inflorescences stipules characterised by a damage scale (see Materials and Methods) of investigated *Tilia* sp. trees.

near (less than 1.5 m) streets with heavy traffic. The stipules from trees (C12, C13, C14, C15, C16) grown more than 1.5 m from streets had only some necrotic spots. The necrotic areas and deformed shape of some stipules of inflorescences from trees grown in the greenhouse probably were the result of high (more than 80%) humidity.

Chemical analysis of lime tree inflorescence stipules. The estimated nutrient concentrations (Table 1) in *Tilia* sp. inflorescence stipules showed significantly higher concentration of Fe and Cu in trees from the urban area in comparison to samples collected from trees grown in the greenhouse. In stipules grown in different areas of Rīga, Fe concentration was in range 105–1025 mg/kg, the concentration of Cu ranged from 8.0 to 65.0 mg/kg, in comparison to concentration in stipules of greenhouse trees, 57.5 and 3.5, respectively. The stipules of three trees (B4, F6 and F9) had a significantly higher concentration of Ca and Mg. All stipules collected from trees in the central part of Rīga (C11, C12, C13, C14, C15, and C16) had significantly lower concentration of Mn, but four of them (C11, C12, C14 and C15) contained considerably higher concentration of Na. Inflorescence stipules of two trees (B4 and F9) had a significantly higher concentration of several plant nutrients, while one tree (B10) had significantly higher concentration of Zn.

The influence of SiNPs and UV on young pollen cells of lime trees. In the first step of research, configuration of the flow cytometer settings was established. For the emission measurements, the most precise channel was found to be 585/29 nm with logarithmic scale. It was found that low CV (coefficient of variance) of results can be obtained only if the densest part of the forward scatter/side scatter profile of plant cell fluorescence is analysed, which could result in CV less than 10% if oval shape gates were used. The young pollen cells were used in the research, because they all are similar in size and shape, more precise results could be obtained. The most sensitive cells (have highest relative fluorescence) to the influence of SiNPs and UV irradiation (Fig. 3) were from trees growing in the greenhouse and from a tree grown in a green area — S3 (buds with 2 mm in diameter). Light microscopy (magnification $\times 10^3$) demonstrated that more than 80% of the cells were microspores at mid to late one-nucleate developmental stage. The cells of greenhouse samples (K1, K2, K3, K4, K5) demonstrated similar relative fluorescence and for further examination were considered as one sample "K1–5". Samples from other trees had significantly different relative fluorescence, excepting that relative fluorescence of samples K1–5, B10 and F9 was similar (Fig. 4), which can be explained by genetic similarity (see Fig. 1). The cells from trees grown in Rīga in general had higher relative fluorescence than the cells from the greenhouse. In comparison to control cells, greenhouse sample (K1–5) had significant, almost two times higher fluorescence after incubation in medium supplemented with SiNPs. Among the street tree samples, the presence of SiNPs in cultivation medium increased fluorescence only for sample C11 from the Rīga central part. Two

Table 1. Concentration of chemical elements in stipules of *Tilia* sp. inflorescences

	P (%)	Ca (%)	Mg (%)	K (%)	Na (%)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
K1-5	0.14[a]	0.78[a]	0.28[a]	0.85[a]	0.028[a]	57.5[a]	32.5a	47.50[a]	3.50[a]
B2	0.24[b]	1.05[a]	0.40[a]	1.25[a]	0.037[a]	140.0[b]	27.5[a]	45.00[a]	9.75[b]
B4	0.21[a]	1.40[b]	0.55[b]	0.85[a]	0.047[a]	390.0[b]	55.0[b]	52.50[a]	8.75[b]
B5	0.23[b]	1.28[b]	0.35[a]	0.98[a]	0.034[a]	160.0[b]	32.5[a]	40.00[a]	8.00[b]
B10	0.18[a]	1.05[a]	0.33[a]	0.78[a]	0.036[a]	172.5[b]	23.0[a]	75.00[b]	11.00[b]
C11	0.18[a]	0.78[a]	0.33[a]	1.28[b]	0.070[b]	357.5[b]	24.5[a]	42.50[a]	65.00[b]
C12	0.21[a]	0.68[a]	0.23[a]	1.38[b]	0.085[b]	275.0[b]	15.5[c]	45.00[a]	45.00[b]
C13	0.21[a]	0.63[a]	0.21[a]	1.13[a]	0.203[b]	105.0[b]	12.0[c]	37.50[a]	17.00[b]
C14	0.21[a]	0.78[a]	0.35[a]	1.35[b]	0.030[a]	287.5[b]	15.0[c]	30.00[c]	60.00[b]
C15	0.26[b]	0.70[a]	0.15[c]	0.78[a]	0.365[b]	147.5[b]	19.5[c]	40.00[a]	25.00[b]
C16	0.21[a]	0.80[a]	0.33[a]	0.85[a]	0.029[a]	130.0[b]	10.5[c]	32.50[a]	12.50[b]
F6	0.20[a]	1.73[b]	0.43[b]	0.88[a]	0.031[a]	480.0[b]	27.5[a]	45.00[a]	12.00[b]
F9	0.18[a]	2.35[b]	0.70[b]	0.90[a]	0.024[a]	1025.0[b]	55.0[b]	52.50[a]	13.00[b]

Parameters marked with different letters were significantly different (t-test, $p < 0.05$) relates to K1-5 level: a – equal to K1-5 level; b – significantly higher than K1-5 level; c – significantly lower than K1-5 level.

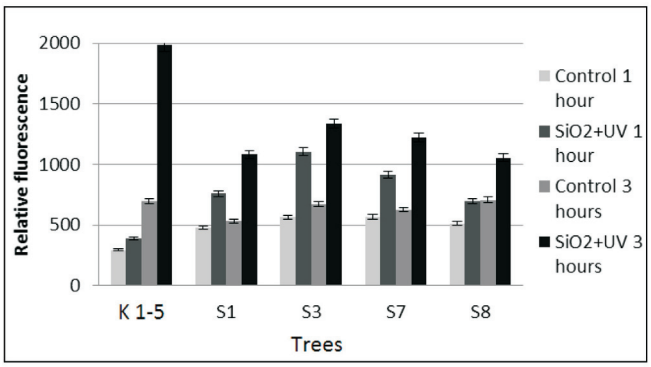

Fig. 3. Influence of SiNPs and UV irradiation on *Tilia* sp. young cells in different developmental stages.

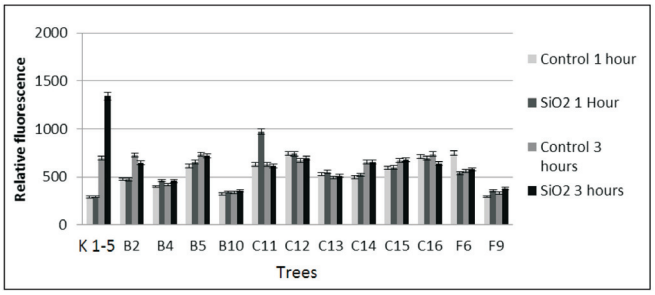

Fig. 4. Relative fluorescence of *Tilia* sp. young cells from trees grown in different city districts after one- or three-hour incubation in medium supplemented with SiNPs.

samples of cells (C16 and B2) had even lower relative fluorescence in comparison with the control.

The UV irradiation treatment increased relative fluorescence of all evaluated trees cells in comparison to the control cells (Fig. 5). The relative fluorescence of control cells (without treatment with UV irradiation) ranged from 290 (K 1–5) to 749 (C16) relative fluorescence units (RFU). Higher fluorescence, ranged from 700 RFU (B4 tree cells) to 1675 RFU (B5 tree cells), observed after three hours cultivation after UV irradiation. Although all sample cells to some extent reacted to UV irradiation, increasing their relative fluorescence, for the samples K1-5, B5, B10, C13, C14, C15, F6, and F9 fluorescence increased almost twice, which could be explained by the individual features of these trees.

The combined effect of UV irradiation and SiNPs had more pronounced influence on sample cell relative fluorescence (Fig. 6) than the separate effect of each factor. Significant changes of relative fluorescence after treatment with SiNPs and UV irradiation were observed in a number of samples. Sample K1-5 cells, cultivated in medium supplemented with SiNPs for three hours, demonstrated the highest increase of relative fluorescence (to 1988 RFU). Increase of cell fluorescence was observed for samples C11, C24, and F9 in both 1- and 3-hour cultivation. Samples B5, C13, and C16 demonstrated significant decrease of relative fluorescence in presence of SiNPs.

Significant difference (Fig. 7) was observed in relative fluorescence of cells after UV irradiation and 1- hour cultivation between greenhouse trees and urban trees in Rīga. Similar results were found for cells treated both with UV irradiation

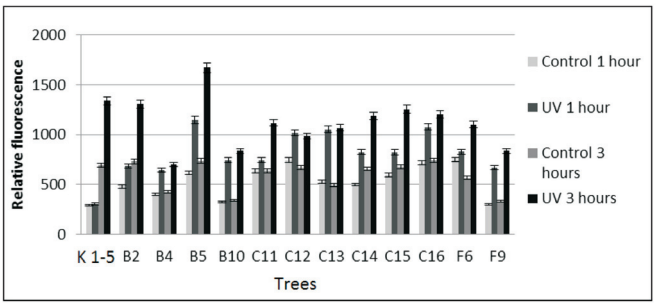

Fig. 5. Relative fluorescence of *Tilia* sp. young cells from trees grown in different city districts after one- or three-hour incubation in medium after influence of UV radiation.

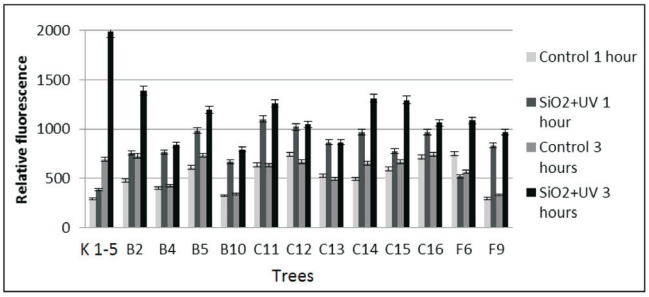

Fig. 6. Relative fluorescence of *Tilia* sp. young cells from trees grown in different city districts after UV irradiation and one- or three-hour incubation in medium supplemented with SiNPs.

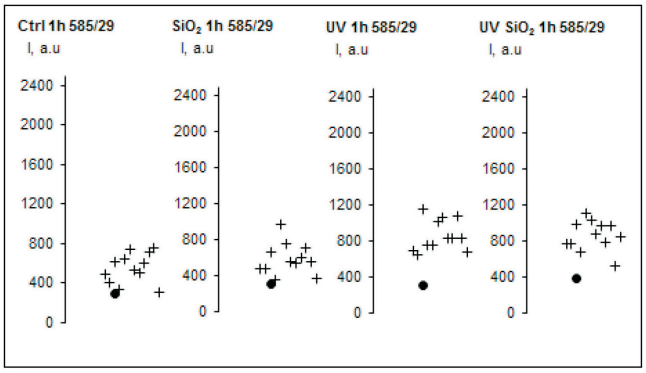

Fig. 7. Scattering diagram of relative cell fluorescence after one hour of cell cultivation. + - city trees data, • - greenhouse trees data.

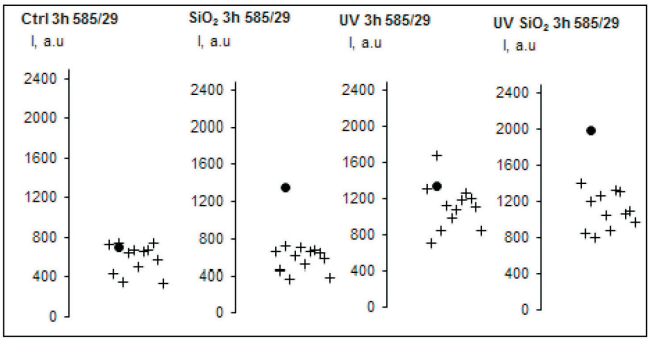

Fig. 8. Scattering diagram of the relative cell fluorescence after three hour of cell cultivation. + - city trees data, • - greenhouse trees data.

and SiNPs. After 1 hour of cultivation cells from trees in Rīga showed higher fluorescence than cells from trees in the greenhouse. Relative fluorescence of cells from the urban area of Rīga form the compact group (Figs. 7, 8), which in-

dicates similar reaction of cells. After three hours of cultivation (Fig. 8) in SiNPs suspension, significant difference of relative fluorescence between cells of greenhouse and Rīga samples was observed. An extreme increase of greenhouse cell fluorescence was observed. After three hours of the UV irradiation, the relative fluorescence increased for all samples, and cultivation of cells in medium with SiNPs after the UV irradiation significantly increased relative fluorescence of cells of the greenhouse samples.

DISCUSSION

The difference in relative fluorescence after laser excitation of cells of lime trees indicates certain properties of individual trees. Depending on the species there are many causes for plant cell fluorescence: plants contain fluorescent pigments, naturally fluorescent products such as proteins, including histones, fluorescent proteins in the chloroplast, and products of cell life processes, such as peroxidase (Neumann and Gabel, 2002; Kimura, 2005; Bargmann, *et al*., 2009; You *et al*., 2015). Fluorescence examination is considered a standard for screening and diagnostics of early stages of infectious processes in plants and changes in reinitiated cell culture; fluorescence can be an indicator of apoptotic cells (Vigneswaran *et al*., 2009; Martinez *et al*., 2010; Carter *et al*., 2013). In this study young pollen cells were used to eliminate the possibility that fluorescence could be produced by chloroplasts, infected or apoptotic cells. Consequently, the estimated relative fluorescence and its alterations are directly linked to physiological state and its changes within the pollen cells. One of the reasons for the difference in lime tree cell fluorescence was based on genetic diversity of trees — genetically congenial tree cells had similar fluorescence. The influence of growth conditions must also be taken into account, included increased pollution in the urban area. Influence of pollution was indicated by results of phenotypical evaluation and chemical analysis of *Tilia* sp. inflorescence stipules. The inflorescence stipules from trees of industrial areas of Rīga were mostly damaged. All inflorescence stipules of urban trees had significantly higher concentration of Fe and Cu, which might affect cellular processes, create disbalance and initiate stress processes. It should be mentioned that the level of Na in stipules from trees growing directly near the streets in the city centre could be characterised as toxic for lime trees (Na concentration in C13 was 0.203%, in C15 — 0.365%). These high Na level occur due to annual use of NaCl as a de-icer for snow melting and to prevent ice formation on streets in Rīga during the winter season (Cekstere *et al*, 2010; Cekstere and Osvalde, 2013). Increase of Cu and Zn concentrations in plants are associated with increasing soil salinity, which affects the Cu-Zn superoxide dismutase protein level and its activity — a part of the salt-induced defence against endogenous oxidative stress (Mittler *et al*., 2004; Miller *et al*., 2010). However, in Rīga, elevated concentrations of Fe and Cu, as well as partly Zn, could be related to accumulation of anthropogenic pollution from intensive traffic, industrial activities etc. directly on stipules. The cells of trees in Rīga could also suffer from stress caused by deficiency or disbalance of other nutrients, e.g., Mg and Mn. The chemical analysis revealed that Mg and Mn concentration in stipules of several urban trees was significantly lower in comparison to the control group. According to previous investigations in Rīga on lime leaves and plants in general, concentration of Mn less than 0.20 mg/kg and Mg less than 0.2% can be characterised as deficiency, causing also visual signs of leaf chlorosis (Bergmann and Neubert, 1976; Čekstere, 2011). Generally, all of the above mentioned factors together can have influence on the cell metabolism and, consequently, on the fluorescence-activated cell relative fluorescence.

It is known that nanotoxicity of nanoparticles is related to their size. The used 10–20 nm diameters of SiNPs have been found to be toxic (Djaković and Jovanović, 2003; Ma *et al*., 2010; Dimkpa *et al*., 2012; Reijnders, 2012). Previous study (Kalteh *et al*. 2014) found that SiNPs can be successfully used as fertiliser, are a good treatment against salinity stress and plant growth stimulator for basil, but in that study the size of used SiNPs was not indicated. The influence of SiNPs on lime tree immature pollen was selective: cells from green house trees were more sensitive to SiNPs (the relative fluorescence of these cells increased twice). In contrast, cells from samples of Rīga urban trees mostly showed no significant changes of relative fluorescence, except for three trees (one tree had relatively little increase and two others had decrease of cell relative fluorescence). The results showed similar (cell relative fluorescence forms a compact group) reaction of cells from the urban area of Rīga samples to addition of SiNPs to cultivation medium. These results can be explained by the adaptation of lime trees grown in Rīga urban area to environmental conditions with high presence of SiNPs (Veliu and Syla, 2008; Campos-Ramos *et al*., 2011; Wang and Pui, 2011). This can be associated with rather high losses of young lime trees that are more sensitive to urban pollutions, which were planted in the Rīga urban area (Cekstere *et al*., 2010) and survival of trees with good adaptability, possible due to epigenetic effects caused by environmental conditions, for example by gene methylation (Kokina *et al*, 2013).

UV irradiation increased the relative fluorescence of all evaluated young pollen cells; different cell reaction was associated with tree individual sensitivity, but for the majority of young pollen cells samples, relative fluorescence increased twice in comparison to the control group. Certainly, the detected increase of cell relative fluorescence after UV irradiation was a result of changes in cell metabolism processes and peroxidase complex activity as responsive reaction to a stress factor (Hektors *et al*, 2007; Karim *et. al.*, 2012; Peykarestan and Seify, 2012).

Young pollen cells from various lime trees cultivated in medium supplemented by SiNPs after UV irradiation differed in relative fluorescence level. Significant association of tree growth conditions and relative fluorescence alteration in both directions was observed. The presence of SiNPs in cultivation medium considerably increased the relative fluores-

cence of young pollen cells from greenhouse trees, but decreased relative fluorescence or had no impact on cells from trees grown in urban Rīga.

Significant differences were found in relative fluorescence of cells from different trees after treatment with SiNPs and UV irradiation in comparison to the control group. Nevertheless, there is no doubt that the largest significant difference in relative fluorescence after UV irradiation without and in presence of SiNPs existed between cells from greenhouse trees and cells samples from in Rīga urban trees. Certainly, the reaction of cells of greenhouse trees to SiNPs had typical characteristics of stress reaction of cells after stress-factor exposure — high increase in activity of the cells in a short period of time. Of particular interest are changes in the relative fluorescence of cells from trees of the Rīga urban area. Changes of relative fluorescence of cells from trees grown in the urban area of Rīga were different, but all of these samples had a much lover reaction rate on SiNPs in comparison to cells from greenhouse trees. This phenomenon potentially could be explained with presence of high amount of different size SiNPs in the urban area of Rīga and consequently in the tree cells, and might be associated with formation of SiNP conglomerates. This can cause the loss of toxic activity of SiNPs, and the formed SiNP conglomerates can act as stress inhibitors (Kalteh *et al*. 2014). Probably, this effect could be associated with changes in the cell membrane peroxidase complex and cell membrane permeability (Karim *et al*., 2012). In our opinion, it is possible that endurance of lime trees grown in urban area to SiNPs is linked to epigenetic changes caused by prolonged influence of urban environment.

The obtained results showed that lime trees grown for a long time in the studied urban area had complex adaptations to a changeable environment and therefore might be good source material for tree nurseries for propagation of lime trees for planting in urban areas.

CONCLUSIONS

Young pollen cells (mid to late one-nucleate developmental stage) were found to be more sensitive (had highest relative fluorescence) to influence of SiNPs and UV irradiation. The cultivation of young pollen cells of lime trees in medium supplemented with SiNPs had influence on relative fluorescence of cells after UV irradiation. Significant association of tree growth conditions with alteration of relative fluorescence was observed: the young pollen cells of greenhouse grown trees were more sensitive than of trees grown in the urban area of Rīga. Changes of relative fluorescence of cells from trees grown in urban Rīga were different, but all showed a much lower reaction rate to SiNPs in comparison to cells from trees grown in the greenhouse. This can be explained by presence of high amount of different size SiNPs in the environment of the urban area of Rīga and, consequently in the tree cells and might be associated with formation of SiNPs conglomerates that caused loss of the toxicity of the used SiNPs. Thus, the formed SiNPs conglomerates might act as stress inhibitors and linked to epigenetic changes in tree cells caused by prolonged influence of the urban environment. The lime trees grown for a long time in urban area had complex adaptability to a changeable environment and might be a good source material for tree nurseries for propagation of lime trees for planting in urban areas. Flow cytometry is suitable for evaluation of plant adaptation to different factors that affect plants in urban environments.

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AR PLŪSMAS CITOMETRIJU NOTEIKTĀ SIO_2 NANODAĻIŅU IETEKME UZ URBĀNĀ VIDĒ AUGOŠO LIEPU GAMETISKĀM ŠŪNĀM

SiO_2 nanodaļiņas ir vienas no izplatītākajām nanodaļiņām urbānā vidē. Parādīts, ka liepu putekšņu šūnām no kokiem, kas auguši urbānā vidē, ir atšķirīga reakcija uz SiO_2 nanodaļiņām, salīdzinot ar šūnām no kokiem, kas auguši siltumnīcā. Secināts, ka plūsmas citometrija var tikt pielietota lai skaidrotu urbānās teritorijas piesārņojuma īpatnības.