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Influence of SiO_2 nanoparticles on relative fluorescence of plant cells

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Abstract. **Nanoparticles (nano-scale particles (NSPs)) are defined as particles with dimensions less than 100 nm. SiO_2 nanoparticles are one of the most widely common nanoparticles in the environment, particularly in urban areas. The sources of SiO_2 nanoparticles are very different, including natural nanoparticles, anthropogenic and engineered nanoparticles. The SiO_2 nanoparticles could be considered a source of different pollution effects on leaving organisms. Nevertheless, knowledge of the mechanisms, through which the SiO_2 nanoparticles affect cells, is incomplete. The aim of the research was to elaborate a method to determine changes in relative fluorescence of both somatic and immature gametic plant cells in presence of SiO_2 nanoparticles. Relative cell fluorescence was measured with BD FACSJazz® cell sorter using 488 nm exciting laser light. Mean cell fluorescence was determined for samples of purified cell suspension. Gates of different size and shape were preliminary tested to find those with the lowest CV. Cell plots were created by BS FACS Software 1.0.0.650. The densest part of the plot was gated using oval-shaped gate. The gate included 95-99% of all cells. A logarithmic scale in arbitrary fluorescence units was applied to determine cell relative fluorescence. More than 10 000 cells were gated and analysed from each sample. Somatic cell culture from callus culture initiated from leaves of flax (*Linum usitatissimum)* was obtained. The relative fluorescence of the somatic cells had large distribution, since the cells differ by many parameters (size, shape, metabolism etc.). Immature pollen cells (one-nucleus stage) as best for SiO_2 nanoparticles influence investigation were found. The influence of SiO_2 nanoparticles on several plant species (*Cyclamen persicum, Tilia cordata, Hordeum vulgare* and *Triticum aestivum*) immature pollen cells were investigated. A significant increase in relative cell fluorescence was observed for all mentioned plant species cells after incubation in SiO_2 nanoparticles suspension. It was found that cell relative fluorescence was dependent on cultivation duration in SiO_2 nanoparticles suspension.**

Keywords: **plant cell fluorescence; flow cytometry; SiO_2 nanoparticles; urban ecology.**

I INTRODUCTION

Nanoparticles (nano-scale particles, NSPs) are defined as particles with dimensions less than 100 nm. Silica or silicon dioxide (SiO_2) nanoparticles, including natural, anthropogenic and engineered nanoparticles, are one of the most widely common nanoparticles in the environment, particularly in urban areas [1]-[7]. They could be considered a source of different pollution effects on leaving organisms, nevertheless, knowledge of the mechanisms through which nanoparticles affect cells is incomplete**.** The major importance has the understanding of biological mechanisms through which nanoparticles affect cells [8]. SiO_2 nanoparticles were described as non-toxic, environment-friendly and safe for use in nanocomposites consisting of organic polymers. However, there is still evidence that amorphous SiO_2 nanoparticles could be hazardous [9], [10]. Silica also has an important role in plant tolerance to environmental stresses und plant photosynthesis [11], [12].

Flow cytometry methods (FCM) in the last 20 years is widely used method for investigation of different plant cell parameters, including cell oxidative stress [13]-[15]. FCM has several advantages − on the base of changes of cell relative fluorescence it is possible to analyse more than 20 parameters of each cell, large number of cells can be evaluated in a very short time, the results are statistically significant and represent the all studded population. All this make the method an excellent investigation tool in many areas [16]-[18]. The aim of the research was to elaborate a method to determine relative fluorescence of both somatic and immature gametic plant cells in presence of SiO_2 nanoparticles.

II MATERIALS AND METHODS

A. Plant Material

The plant cells from different genus five genetically distant species – lime trees (*Tillia cordata),* cyclamen *(Cyclamen persicum),* wheat *(Triticum aestivum),* barley *(Hordeum vulgare)* and flax *(Linum*

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usitatissimum) were used in the research. The lime trees (three years old), cyclamen, wheat and barley were grown in greenhouse. Flax calli culture was obtained using an earlier elaborated method [19], [20].

B. Cell Culture Preparation

The cell cultures of immature microspores of lime trees, cyclamen, wheat and barley, as well as flax

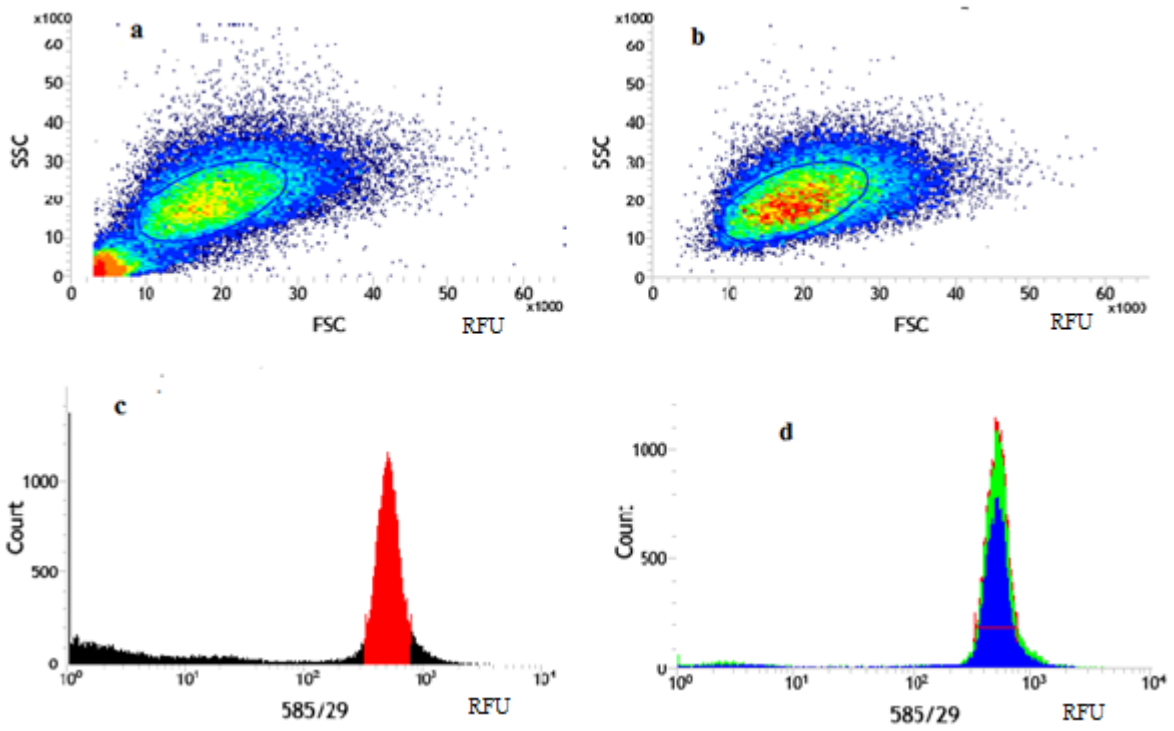

Fig. 1. Flow cytometer analysis of cyclamen *(Cyclamen persicum)* cells: a – oval shape gating of control cells sample; b – oval shape gating for cells cultivated in SiO_2 suspension (1 mg/ml) for 1 hour; c – the relative fluorescence units (RFU) in logarithmic scale for control cells; d – the relative fluorescence units (RFU) in logarithmic scale for cells cultivated in SiO_2 suspension (1 mg/ml) for 1 hour.

somatic cell culture preparation was done using modified methods of cells culture establishment [21]. The optimal stage of microspores for each plant species were determine by light microscope (magnification x10^3) [22]. The buds, spikes and calli were collected and put in the Waring Blender 8011 and grind in 0.3 M solution of D-mannitol. The samples were grinded in the mode Nr. 2 up to five times each during 20 seconds till visually homogeneous suspension. The samples were filtered through mesh (50 µm) three times then the acquired liquid was collected into several 45 ml centrifuge plastic tubes. The samples were centrifuged (Eppendorf Centrifuge 5810R) for 15 min at 4 °C, 900 rpm. After centrifugation the liquid phase was decant, but the sediment (cells) was diluted with 45 ml 0.3 M D-mannitol solution and centrifuged one more time for 15 min at 4 °C, 900 rpm. 1 ml of cell sediment contained about 600 000 cells [21]. The liquid phase was poured off and 1 ml of cell sediment was suspended in 4 ml liquid MS medium [23] and mixed. The cell culture quality was determined by light microscope (magnification x10^3).

C. Evaluation of SiO_2 Nanoparticles Influence

A suspension of SiO_2 nanoparticles was prepared by silicon dioxide (SiO_2) nanoparticles (Sigma – Aldrich inc., size 10-20 nm, purity 99.5%) dilution in distilled water in proportion 1 mg per 1 ml. After dilution the suspension flask was placed into Bandelin® RK-31 ultrasonic bath (frequency 35 kHz, effective US power 40 W) for 30 min for sonification to separate possible nanoparticle conglomerates. Immature pollen cells of cyclamens were used as a model object for elaboration of flow cytometry method. The cells were incubated in MS medium without and in presence of SiO_2 nanoparticles for 1, 2, 3 and 4 hours at 17 °C temperature in speed shaking regime. The concentrations of SiO_2 NSP in media were 0.5, 1.0 or 1.5 mg/ml (0.5 ml, 1 ml or 1.5 ml of SiO_2 NSP suspension was added to 10 ml of cell suspension). After incubation the suspension was filtered through a flow cytometry-pass filter (mesh 40 µm).

For the establishing of relative fluorescence of plant cells of different species immature pollen cells of lime trees (*Tillia cordata),* cyclamen *(Cyclamen persicum),* wheat *(Triticum aestivum) and* barley *(Hordeum vulgare), as well as* somatic cells of flax *(Linum usitatissimum)* calli were used. After cell incubation at temperature 22 °C without and in presence of 1 mg/ml SiO_2 nanoparticles in speed shaking regime for 1 and 3 hours the suspension was filtered through a flow cytometry-pass filter (mesh 40 µm).

D. The Device and the Software for the Research

To test relative fluorescence of plant cells BD FACSJazz® cell sorter (BD Biosciences, USA) with flow cytometer function with a 100 µm nozzle was used; phosphate-buffered saline (BD Pharmingen™ PBS, BD Biosciences, USA) was applied as a sheath fluid.

Sphero™ rainbow calibration particles (3.0–3.4 µm, BD Biosciences, USA) in phosphate buffered saline (PBS) were used for flow cytometer calibration. The calibration was considered successful if the coefficient of variance (CV) of relative fluorescence of the rainbow calibration particles was not exceed 3%. The cytometer settings were: trigger level 1418, trigger detector FSC, scope channel 1 and scope channel 2 585/29, PMT; power voltages PMT1 – 25.73 (FSC), PMT2 – 25.01 (SSC), PMT3 – 43.16 (log 530/40), PMT1 – 41.99 (log 585/29) were used. The method was based on changes of relative self-fluorescence intensity of cells after excitation with 488 nm Coherent Sapphire Solid State (blue) laser. The light emission was measured at 585/29 nm. The information of mean fluorescence intensity from the purified cell suspension samples was recorded. Preliminarily, multiple gate sizes and shapes were tested to find the one with the lowest CV. Using flow cytometer BS FACS Software 1.0.0.650 cells plot was created to determine the densest part that was later gated using oval-shaped gate (Fig. 1). Gate included from 95 to 99% of all target cells. A logarithmic fluorescence scale in arbitrary fluorescence units was used to determine relative fluorescence units (RFU) of cells. 3 x 10^3 gated cells were analysed from each sample.

For statistical analysis of results the *p*-value obtained through TDIST function (MS Excel) was used; *p*-value is a tool to test the null hypotheses in certain level of significance. The significance threshold chosen for the research was p=0.05 (5%).

III RESULTS AND DISCUSSION

Influence of nanoparticles on plant cells depends on particles properties and concentration, evaluated plant cells type und physiological state [24], [25]. Great importance has also the cells wall structure, including the cells wall pores diameter, as well permeability of cell wall pores complex [29]. The SiO_2 nanoparticles with diameters of 10-20 nm used in this investigation were regarded as biologically active [26], [27].

The relative fluorescence of cyclamen immature pollen cells depended on cultivation temperature and time (Fig. 2 and Fig. 3). After cell incubation in SiO_2 nanoparticles suspension with concentrations 0.5, 1.0 and 1.5 mg/ml in 17 °C temperature (Fig. 2) a significant difference from control cells (cultivated without SiO_2 nanoparticles) was found only for the cells cultivated during 1 h in media with 1.5 mg/ml SiO_2 nanoparticles.

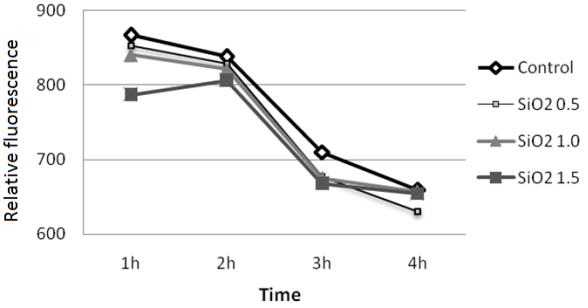

Fig. 2. The relative fluorescence of cyclamen immature pollen cells after incubation in suspension of SiO_2 nanoparticles with 0.5, 1.0 and 1.5 mg/ml concentrations after 1, 2, 3 and 4 hours of incubation (the cultivation temperature was 17 °C).

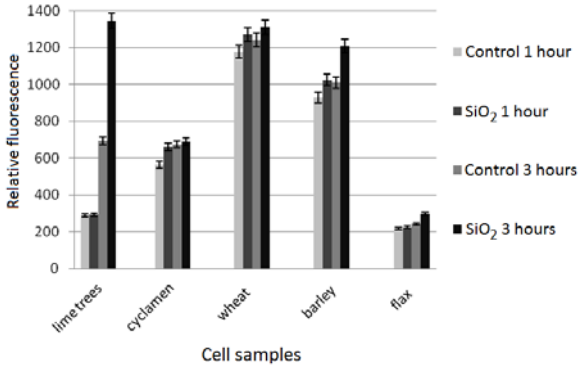

Fig. 3. The relative fluorescence of lime trees (*Tillia cordata),* cyclamen *(Cyclamen persicum),* wheat *(Triticum aestivum),* barley *(Hordeum vulgare)* immature pollen cells and flax *(Linum usitatissimum)* callus somatic cells after incubation at room temperature (+22 °C) without and in presence of 1 mg/ml SiO_2 nanoparticles.

After 3 h of cultivation extreme reduction of relative fluorescence were observed for all cultivated cell samples. It might be because of decrease of activity of cell metabolic processes in 17 °C temperature. After cell cultivation in 22 °C temperature in presence of SiO_2 nanoparticles extension of cell relative fluorescence was observed (Fig. 3). This could be due to the fact that increase of cultivation temperature for 5 degrees increases the cell metabolic activity. The enhancing of cell relative fluorescence, depending of the species, was related with the changes of different cell parameters such as fluorescence of fluorescent pigments, proteins, including histones, fluorescent proteins in the chloroplast, and cell life process products such as peroxidase [28].

The influence of SiO_2 nanoparticles on plant cells is still in discussion: in some investigations [13], [15], [24], [27] SiO_2 nanoparticles were found to be toxic, but in the other studies [12] it was found that SiO_2 nanoparticles has been successfully used as fertiliser. However, it should be noted that authors do not indicated the size of used SiO_2 nanoparticles, but it is also known that in cell reaction on presence of nanoparticles is significant the type, size and

concentration of SiO_2 nanoparticles [11], [24], [25]. All investigated cells after cultivation in 22 °C temperature in presence of SiO_2 nanoparticles showed increase of relative fluorescence for 1 and for 3 hours (Fig. 3). The changes of cell relative fluorescence depending of plant species were observed: the highest changes (from 695 relative fluorescence units (RFU) of control cells to 1345 RFU of cells cultivated in SiO_2 nanoparticles suspension) were detected for lime trees immature pollen cells after cultivation for 3 h in presence of SiO_2 nanoparticles, the lowest relative fluorescence changes were detected for somatic cells obtained from callus culture initiated from leaves of flax (*Linum usitatissimum)*. It ranged from 216 RFU (1 h cultivation, control cell sample) to 298 RFU (3 h cultivation in suspension of SiO_2 nanoparticles). The somatic flax cells fluorescence had large distribution, since the cells differed by many parameters (size, shape, metabolism etc.) and were problematic for gating. In turn, the immature cells (one-nucleus stage) had very small difference in shape and size and were more useful (the cell pool was clear for gating, Fig. 1) to establish changes in cell relative fluorescence after influence of SiO_2 nanoparticles.

IV CONCLUSIONS

The significant increase in relative cell fluorescence was observed after incubation in SiO_2 nanoparticles suspension for several plant species – immature pollen cells of lime trees (*Tillia cordata),* cyclamen *(Cyclamen persicum),* wheat *(Triticum aestivum),* barley *(Hordeum vulgare)* and somatic cells of flax *(Linum usitatissimum)*. Relative cell fluorescence was dependent on cultivation duration in SiO_2 nanoparticles suspension. The influence of temperature on cell fluorescence was observed after cell incubation in SiO_2 nanoparticles suspension. The immature (one nucleus stage) pollen cells of all evaluated species were found to be appropriate for investigation of influence of SiO_2 nanoparticles on plant cells.

V ACKNOWLEDGEMENTS

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