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Application of Flow Cytometry for Detection of Influence of Abiotic Stress on Plants

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Abstract : The goal of study was the elaboration of easy applicable flow cytometry method for detection of influence of abiotic stress factors on plants, which could be useful for detection of environmental stresses in urban areas. The lime tree Tillia vulgaris H. is a popular tree species used for urban landscaping in Europe and is one of the main species of street greenery in Riga, Latvia. Tree decline and low vitality has observed in the central part of Riga. For this reason lime trees were select as a model object for the investigation. During the period of end of June and beginning of July 12 samples from different urban environment locations as well as plant material from a greenhouse were collected. BD FACSJazz® cell sorter (BD Biosciences, USA) with flow cytometer function was used to test viability of plant cells. The method was based on changes of relative fluorescence intensity of cells in blue laser (488 nm) after influence of stress factors. SpheroTM rainbow calibration particles (3.0–3.4 μm, BD Biosciences, USA) in phosphate buffered saline (PBS) were used for calibration of flow cytometer. BD PharmingenTM PBS (BD Biosciences, USA) was used for flow cytometry assays. The mean fluorescence intensity information from the purified cell suspension samples was recorded. Preliminary, multiple gate sizes and shapes were tested to find one with the lowest CV. It was found that low CV can be obtained if only the densest part of plant cells forward scatter/side scatter profile is analysed because in this case plant cells are most similar in size and shape. The young pollen cells in one nucleus stage were found as the best for detection of influence of abiotic stress. For experiments only fresh plant material was used–the buds of Tillia vulgaris with diameter 2 mm. For the cell suspension (in vitro culture) establishment modified protocol of microspore culture was applied. The cells were suspended in the MS (Murashige and Skoog) medium. For imitation of dust of urban area SiO2 nanoparticles with concentration 0.001 g/ml were dissolved in distilled water. Into 10 ml of cell suspension 1 ml of SiO2 nanoparticles suspension was added, then cells were incubated in speed shaking regime for 1 and 3 hours. As a stress factor the irradiation of cells for 20 min by UV was used (Hamamatsu light source L9566-02A, L10852 lamp, A10014-50-0110), maximum relative intensity (100%) at 365 nm and at ~310 nm (75%). Before UV irradiation the suspension of cells were placed onto a thin layer on a filter paper disk (diameter 45 mm) in a Petri dish with solid MS media. Cells without treatment were used as a control. Experiments were performed at room temperature (23-25 °C). Using flow cytometer BS FACS Software cells plot was created to determine the densest part, which was later gated using oval-shaped gate. Gate included from 95 to 99% of all cells. To determine relative fluorescence of cells logarithmic fluorescence scale in arbitrary fluorescence units were used. 3x103 gated cells were analysed from the each sample. The significant differences were found among relative fluorescence of cells from different trees after treatment with SiO2 nanoparticles and UV irradiation in comparison with the control.

Keywords : flow cytometry, fluorescence, SiO2 nanoparticles, UV irradiation
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