LEAF ANATOMY AND PHOTOSYNTHETIC APPARATUS FUNCTIONING OF RED AND GREEN LEAVES FROM SINGLE TREE OF "CRIMSON KING" NORWAY MAPLE

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Introduction: The “Crimson King” Norway maple (Acer platanoides “Crimson King”) is a cultivar with large red leaves. Single tree had one branch with green leaves beside normal red ones. Knowing that red color of these leaves originate from anthocyanin cyanidin-3-glycoside having protecting function on photosynthetic apparatus, the aim of our study was to explore the differences in photosynthetic apparatus functioning and leaf anatomy of red and green leaves sampled from the same tree of “Crimson King” Norway maple.

Materials and Methods: Red and green leaves were sampled in August 2005. Leaves were cut into small pieces, fixed in glutaraldehyde in cacodylate buffer, dehydrated and embedded in methacrylate resin for light microscopy or in Spurr resin for electron microscopy. Measurements of the different tissue areas were made on 3 µm thick transversal sections of leaves stained with Toluidine blue O in benzoate buffer. Ultrastructure was observed using FEI Morgagni 268 D electron microscope. For the determination of photosynthetic pigments content maple leaves were macerated in liquid nitrogen, pigments were extracted with cold acetone and quantified spectrophotometrically. Photosynthetic function of leaves was determined by measuring the maximum efficiency of PS II (Fv/Fm) by saturating pulse method (Mini-PAM, Walz). Photosynthetic oxygen production was measured with gas-phase Clark-type oxygen electrode at 0, 35, 150, 400 and 800 µmol PHOTONS m⁻² s⁻¹. Obtained data were processed using the Student’s t-test.

Results: Red leaves of “Crimson King” Norway maple showed significantly higher levels of chlorophylls a (1,11 mg/g) and b (0,63 mg/g) than the green leaves (Chl a = 0,91 mg/g; Chl b = 0,39 mg/g), while chlorophyll a to chlorophyll b ratio was lower in red ones than in greens (1,77 in reds and 2,31 in greens). Total carotenoids in red leaves (0,35 mg/g) were also higher than in green ones (0,28 mg/g). The value for total chlorophyll to total carotenoids ratio was showed no significant difference between red (4,96) and green (4,72) leaves. The Fv/Fm values (0.80 in red and 0.79 in green leaves) showed no significant difference, although the photosynthetic production of oxygen was significantly higher in green leaves at 150, 400 and 800 µmol PHOTONS m⁻² s⁻¹ (Fig. 1). In opposite, at low light (35 µmol PHOTONS m⁻² s⁻¹) there was no significant difference between red and green leaves in photosynthetic oxygen production (Fig. 1).

Histological measurements revealed that red leaves had significantly higher percent of upper epidermal tissue and spongy parenchyma, in total area of transversal leaf cross-section, than green ones. In contrary, green leaves had significantly higher percent of palisade parenchyma and vascular tissue compared to red ones (Fig. 2). The portions of lower epidermal tissue and intercellular spaces area in total leaf cross-section, exhibited no significant difference between red and green leaves (Fig.2). Also, results pointed out that the cell area of upper epidermis was significantly higher in red (374,4 µm²) than in green leaves (275,9 µm²), although the values of lower epidermis cell area showed no significant difference between green (156,6 µm²) and red leaves (159,4 µm²). The investigations of ultrastructure revealed that chloroplasts in red leaves had more thylakoids per granum than chloroplasts in green leaves (Fig.3). Chloroplasts of both, red and green leaves, had a lot of globules.
Conclusion: Obtained results show that red leaves act as shade ones what is connected with Chl a / Chl b ratio lowering and increased thylakoid number per granum. Differences were indicated in lower O₂ production and higher relative area of spongy parenchyma as well as the increase of cell area in the upper epidermis and the area of upper epidermis it self, in red leaves compared to green ones from the same tree. The measuring of Fv/Fm pointed out fully functional photosystem II in both leaf types.

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Fig. 1. The oxygen production and the maximum efficiency of photosystem II indicated as Fv/Fm in red (white) and green (grey) leaves; * - P (t) < 5%, NS – not significant. Bar indicates standard deviation.

Fig. 2. The mean values of areas of different leaf tissues. The area is expressed as percent of total cross-section; UE-upper epiderma, LE-lower epiderma, PP-palisade parenchyma, SP-spongy parenchyma, I-intercellulars, VT-vascular tissue; * - P (t) < 5%, NS – not significant. Bar indicates standard deviation.

Fig. 3. a – Chloroplasts of green leaves; b – Chloroplasts of red leaf with large number of thylakoids per granum (arrow).