CARBOXYLASE AND OXYGENASE ACTIVITY OF RUBISCO IN HEAVY METALS STRESS CONDITIONS - A PRELIMINARY STUDY

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Rubisco (ribulose-bisphosphate carboxylase/oxygenase) is a key-enzyme of the Calvin cycle, which can catalyze carboxylation or oxygenation RuBP, depending on availability of CO_2 and O_2. CO_2 is both substrate and activator of the enzyme. Furthermore, Rubisco activity is regulated by two accompanying enzymes: Rubisco activase (RCA) and carbonic anhydrase (CA). RCA is the enzyme which acts as a chaperone during assembling of Rubisco subunits in the active complex and controls a correct structure of the active center, whilst CA catalyzes conversion of HCO_3^- ion to CO_2.

Our research was carried out on *Arabidopsis thaliana w.t.* Columbia, *w.t. RLD* and *RCA-antisense* mutant RLD 100. Method of *Arabidopsis* cultivation was as follows: all plants were grown in a climate chamber with 10h daylight photoperiode of PPFD of 150 µmol $m^{-2}s^{-1}$ and 23/19ºC day/night temperature on a wet soil for 6 weeks. Then, after root washing, plants where transferred to full Hoagland nutrient solution, 1 plant/pot, and cultivated in the same light/temperature conditions. Liquid nutrient medium was continuously aerated. After 4 days the nutrient medium was changed to fresh one, with heavy metals added in concentrations of: 0 (control), 5 or 25 µM Cd and 5 or 25 µM of additional Cu. After 10 days of growth in stress conditions leaves were harvested for measurements. Parameters of chlorophyll *a* fluorescence induction were measured in modulated actinic light, in normal air and air with addition of CO_2. Enhanced CO_2 level was received by using buffer $NaHCO_3/Na_2CO_3 \cdot 10H_2O$ (pH 9,3). Measurements were run on PAM 100 Chlorophyll Fluorometer (intensity of actinic light was 240 µmol $m^{-2}s^{-1}$ and saturation pulse 8 800 µmol $m^{-2}s^{-1}$), operated by software Win Control V1.48. Before measurements leaves were dark-adapted for 30 minutes.

For all tested *Arabidopsis* ecotypes a negative influence of heavy metals on carboxylase activity of Rubisco was observed as decrease in photochemical quenching rate (q_p) and increase in non-photochemical quenching (q_n). Additional CO_2 improved photosynthesis in all Cd-treated plants. For Cu-treated RCA-antisense mutant and 50 µM Cu-treated *Columbia* w.t. and RLD w.t. higher CO_2 level did not show any beneficial influence on plants photosynthesis. The results suggest that among Cu-treated plants adaptation of carboxylation to stress conditions is dependent on RCA on higher extent than among Cd-treated plants.

Supported by Polish Committee for Scientific Research, grant 3P04C.050.22.