Propozycja projektu pracy doktorskiej:
Excess Excitation Energy-Dependent Modulation of Photosynthesis and Wood Formation by Chloroplast Signal Recognition Particle Component (CAO) in Poplar

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The scientific aim of proposed studies is to determine the role of the *CAO* gene in regulation/mechanisms of the tree biomass production, wood composition and plant fitness in the field. The impact of *CAO* mutation will be determined in four poplar transgenic lines. The practical aim is generation of the transgenic trees with improved biomass production and altered wood composition resulted from enhanced resistance to fluctuating stress conditions in field. Increase in stress tolerance by genetically engineering plants to specific expression of *CAO* gene opens opportunities for successful cultivation of trees as a renewable biomass source for bioenergetics purposes.

Task 1. To determine the role of the *CAO* gene in the protection against and acclimation to environmental conditions that promote photooxidative stress

The chloroplast signal recognition particle (cpSRP) is a protein complex consisting of 54- and 43-kD subunits (Klimyuk et al. 1999). The earlier data suggests that *Arabidopsis* cpSRP54 and cpSRP43 have some nonoverlapping roles in integration of the major polypeptides of the light-harvesting chlorophyll *a*/*b* binding protein complex (LHCP) of photosystem II into thylakoid membranes and an accumulation of other photosynthesis protein like the reaction center proteins D1, D2, and psaA/B (Hutin et al. 2002, Yu et al. 2012). The fact that *Arabidopsis* cpSRP54 and cpSRP43 mutants have different phenotypes suggests that the two proteins do not always function as a complex. The function of cpSRP43 is most likely restricted to protein targeting into the thylakoid membrane, whereas cpSRP54 may be involved in an additional process(es), such as chloroplast biogenesis, perhaps through chloroplast–ribosomal association with chloroplast ribosomes. A recessive mutation in *Arabidopsis*, named *chaos* (for chlorophyll *a/b* binding protein harvesting–organelle specific; designated gene symbol *CAO*), was found to be chlorotic, had an elevated Chl *a/b* ratio, was selectively deficient in LHCPs relative to other thylakoid proteins, and was viable in laboratory conditions (Klimyuk et al., 1999). However, *chaos* plants showed significantly higher tolerance to photooxidative stress under both tightly controlled laboratory conditions and highly variable conditions in the field (Klenell et al. 2005). The greater tolerance of *chaos* plants to field conditions was manifested in less photooxidative damage together with faster growth recovery in young seedlings. It was also associated with a lower production of H_2O_2, lower ascorbate levels and less induction of ascorbate peroxidases. Under field conditions, *chaos* exhibited better overall photosynthetic performance and had higher survival rates as well improved plant growth (in contrast to stable laboratory conditions).

Taken together, these data suggest that regulation of the *CAO* gene is part of the plants' system for trimming the photosynthetic machinery in order to avoid and/or protect itself against photooxidative stress and improve plant growth and survival. However, the precise mechanism and the role of the CAO gene remain to be investigated in detail. Since that light absorption and photosynthesis efficiency are the most important factors regulating growth and biomass accumulation of woody plant, thus we plan to test the role of *CAO* mutation in poplar transgenic plant recently generated in our laboratory (*cao1*) and to compare the *CAO*-regulated pathways in poplar and *Arabidopsis*.

<u>The following thesis will be tested</u>:

1. the expression of the *CAO* gene is regulated by a light-dependent chloroplastic redox signalling pathway during acclimation to abiotic stresses, at least high light (in natural field conditions light intensity often exceed even 1500 μmol m^2 s^{-1}) and chilling temperatures

(spring temperature fluctuations). Both environmental factor induce the excess excitation energy (EEE) in chloroplasts that trigger photoinhibition/photoprotection mechanisms.

2. the presence/absence of the *CAO* gene has an impact on photoproduced H_2O_2, and therefore regulation of the *CAO* may be part of the plant's system for acclimation to abiotic stress and cell wall properties, and furthermore a part of mechanisms that determine plant Darwinian fitness in natural field conditions.

Involved methodology: genotyping of transgenic poplar plants, spectrophotometric and histological analysis of accumulation of ROS as well antioxidative systems: SOD, CAT, APX enzymes and GSH/GSSH, SA. Pigments composition analysis by HPLC, overall photosynthetic performance and a rate of heat dissipation as indicated by foliar temperature.

Task 2. To determine the role of the *CAO* in cell division/cell dead mechanisms leading to altered wood accumulation.

Growth may be improved by an overall increase of cell number of e.g. pith and cortex. It was shown in both Poplar and *Arabidopsis* that the effect of the partial suppression of *PtSHRl* and *AtSHR1*, respectively (encoding SHORT-ROOT polypeptide; SHR), leads to the acceleration of the growth rate of shoots and increased biomass production and that higher growth rate is a result of increased mitotic division rate in the VC and the shoot apical meristem. However, there are also mechanisms that did not require cell division. For instance, many plants have avoided shading from the neighbouring plants by increasing height through intensified elongation of internodal cells. Furthermore, cell death is an important process that determine wood formation.

The following thesis will be tested:

3. altered plant growth (stem length and diameter) results from modified balance of cell division and cell death processes that are under control of *CAO* gene.

Involved methodology: plant macroscopic and microscopic morphology, CLSM and FM with different fluorescence dyes for cell division/PCD (DCFH2-DA, DAB, FDA, DAPI, calcofluor white, TUNEL).

Task 3. To determine the physical and mechanical properties of a cell wall that are under control of *CAO*.

Plants devote about 10% of genomes to the construction of their cell walls. Plant cell walls are highly complex structures that are composed of a diverse set of polysaccharides that vary in structure and abundance. However, the main component of the plant cell wall is cellulose, which represents almost 50% of total cell wall material (Liepman et al. 2010). A substantial part of the energy accumulated during photosynthesis is used to produce cellulose and cell walls, therefore every imbalance in effectiveness of photosynthesis should be mimicked in the composition and structure of cell walls. However, up to now there have been no reports concerning detailed biochemical and biophysical studies on cellulose/cell wall formation and fibril organization under stress environmental conditions which lead to EEE.

The following thesis will be tested:

4. Cellulose and the cell wall from *cao1* transgenic lines demonstrate altered composition and resistance to mechanical and chemical factors. These factors are important to enable cost-effective usage of cellulosic biomass (wood) as renewable biomass source for bioenergetics purposes.

Involved methodology:

Cell wall composition as well kinetics of thermochemical decomposition (simultaneous differential scanning calorimetry DSC, thermogravimetry TG combined with quadruple mass spectrometry QMS), mechanical and physical properties of wood, thickness and porosity cell wall/wood (SEM), tensile modulus (TMA), sorption/desorption of water vapour, wetting enthalpy (TG/DSC) as well as wettability and permeability for molecules with different molecular mass (CLSM).