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Proposition of the research project for a PhD student in „Program doskonalenia dydaktyki SGGW w dziedzinie pozyskiwania surowców roślinnych dla energetyki w kontekście celów Strategii Europa 2020":

Amelioration of biomass production and wood properties by changes of the selected gene expression level in *Populus tremula* L. *x tremuloides*

1. Introduction

Poplars, cottonwoods and aspens (*Populus* sp. L.) are a group of some 30 species of the fastest growing trees in the temperate zone of the northern hemisphere. Recently, some poplar species have been studied extensively, and have become a good experimental model system for tree molecular biology and biotechnology. In general, the development of genetic engineering methods has increased the possibility of producing poplar genotypes and studying, predominantly: 1) integrated tolerance to both biotic and abiotic stresses, 2) lignin metabolism, and 3) growth, development and woody quality.

Among many genes regulating growth and development, mitogen-activated protein kinase (MAPK or MPK) cascades are major components downstream of receptors or sensors that transduce extracellular stimuli into various intracellular responses in plant cells. After phosphorylation of MAP by a kinase (MAPK), the following phosphorylation is mediated by a MAPK kinase (MAPKK), which, in turn, is activated by a MAPKK kinase (MAPKKK). The function of *MPK kinase* (4 *MPK4*) is relatively well-documented in *A. thaliana*, but the role of this gene in other species e.g. in poplar is not recognized at all. The project is planned to obtain several independent transgenic lines of *Populus tremula x tremuloides* with different *MPK4* expression levels. Transgenic lines of poplar are completely new, and it gives unique possibilities to study function of this gene in woody plants in relation to the biomass production and the cell wall composition suitable for bioenergy production.

2. Research project objectives and significance of the project

Based on this, the verification of the hypothesis that *MPK4* in poplar trees plays a crucial role in regulation of photosynthesis and led to a substantial improvement of wood qualities will be

performed. In *P. tremula x tremuloides* understanding of the mechanisms regulating the growth and the quality of wood may have significant practical consequences. These studies are especially important in the case of poplar trees, which are very effective biomass source for biofuels as e.g. ethanol or propanol. Wood biomass can also be used as traditional energetic raw material. In which way we will use that biomass depends on chemical composition of the plant cell wall. Manipulating the content of the cell wall components, such as: cellulose, lignin, and hemicelluloses, we can obtain different wood quality, for cell walls hydrolysis and ethanol production. Therefore our research should have a practical impact on development of alternative and renewable energy sources in Poland. For this reason all efforts leading to identification of potential energy sources have an important sense for our economy.

3. Research methodology

Hybrid poplar (*P. tremula x tremuloides*) will be transformed to obtain plants with reduced and/or overexpressed activity of *MPK4*. The most experiments will be carried out at the experimental field station "Wolica" (52º 8' 30'' N, 21º 4' 12'' E) in Warsaw. To verify the mentioned above hypothesis, assuming changes in the photosynthetic activity and energetic wood properties, a wide range of research methods, such as: gas exchange measurements for net carbon assimilation, chlorophyll *a* fluorescence parameters from photosystem II (PSII), thermoluminescence from PSII, and methods based on the emission of infrared foliar radiation (thermography) will be used. The foliar content of hormones will be analyzed by high performance liquid chromatography (HPLC). Additionally, cellulose, lignin and hemiceluloses content in poplar wood will be determined. Moreover, the level of hydrogen peroxide (H_2O_2), which is involved in lignin metabolism, and the activities of selected antioxidant enzymes will be studied. The wood (secondary xylem) formation will be monitored by use of microscopic observations. Several physical parameters describing wood properties, such as: water binding capacity and wood elasticity will also be determined. Moreover, thermal analysis techniques including: simultaneous differential scanning calorimetry (DSC), thermogravimetry (TG) combined with quadruple mass spectrometry (QMS), which is a useful tool to obtain complete information on physical and chemical properties of the plant cell wall, will be important methods concerning poplar wood properties from a bioenergetic point of view.