Transgenic amelioration of the cell wall composition in *Populus* sp. for biofuels production.

Only a portion of the absorbed light energy is used by plants for photosynthetic metabolism [1-6]. The amount of absorbed light energy that is excessive and cannot be used for CO_2 fixation is termed excess excitation energy (EEE). Light acclimation processes in plants act to dissipate the EEE and optimise photosynthesis under variable light conditions. The EEE is directly dissipated as light emission by fluorescence or as heat by non-photochemical quenching (NPQ) [7-9]. According to the classical view, the failure to dissipate and quench EEE can be highly damaging to plants, and is often manifested in chlorosis, bleaching or bronzing of leaves due to heat production and the imbalanced reactive oxygen species (ROS) metabolism. Furthermore, it has recently been revealed that EEE-induced activation of the cell death (CD) is regulated by the same genetic system as the hypersensitive disease defence response [10-15] and systemic acquired resistance (SAR) [16-18].

The *LESION SIMULATING DISEASE 1 (LSD1)* mutant *lsd1* belongs to one of the best characterized *Arabidopsis thaliana* mutants in the context of deregulated cell death [10-13, 19-23]. *lsd1* was initially characterized for its ROS- and salicylic acid (SA)-dependent uncontrolled spread of cell death that develops under non-permissive conditions such as long (>16h) or continuous photoperiods, supply of superoxide ion ($O_2^{\cdot-}$), or infection with avirulent pathogens. The runaway CD (RCD) phenotype is indicative for the failure to stop both the initiation and propagation of CD. LSD1 was proposed as a negative regulator of RCD, acting as a ROS rheostat [19, 20] and preventing the pro-death pathway below certain ROS levels [21-23]. However, our previous results report that *LSD1* is also required for acclimation to root hypoxia stress and conditions that promote EEE. The *lsd1* mutant shows reduced stomatal conductance and catalase activity in short-day permissive conditions and increased ethylene (ET) and hydrogen peroxide (H_2O_2) accumulation followed by RCD in non-permissive conditions. The *lsd1/cao* double mutant that has reduced PSII antenna due to the *cao* (chloroplastic signal recognition particle cpSRP43) mutation, displays reduced RCD and higher non-photochemical quenching (NPQ). We linked RCD in the *lsd1* mutant to several parameters: the EEE, the redox changes in PSII and emerging and dynamic changes in NPQ, the stomatal conductance and ultimately the photorespiratory burst of H_2O_2 and ET [10-12]. Importantly, *lsd1* traits depend on *ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1)* and its interacting partner *PHYTOALEXIN DEFICIENT 4* (*PAD4*). The *Arabidopsis* EDS1 and PAD4 proteins constitute a regulatory hub for resistance (R) gene-mediated and basal resistance and are required for accumulation of SA [24, 25].

Recently, however, we demonstrated that *PAD4* is also involved in the regulation of light and root hypoxia acclimatory responses and, in general, in the regulation of plant fitness – all of which depend on SA, ET and ROS homeostasis and signalling. The role of *PAD* in woody plants is not known, therefore, we characterise its function in hybrid aspen and its role in the regulation of ROS metabolism and wood development. Three independent transgenic poplar lines: *pad4/1*, *pad4/10* and *pad4/11* with different suppression levels of poplar *PAD* expression were obtained. All of these lines displayed deregulated ROS metabolism which was manifested by significantly increased foliar H_2O_2 level, higher activities of manganese superoxide dismutase (MnSOD) and catalase (CAT) in comparison to the wild-type plants. Changes in the ROS metabolism were positively correlated with significantly decreased tracheid average size and numbers, increased cell wall thickness and increased non-photochemical quenching (NPQ). In contrast, we did not observe any significant changes in the transpiration rate. The presented results suggest that the *P. tremula* x *tremuloides PAD* gene, homologous to the *AtPAD4*, is involved in the regulation of cellular ROS homeostasis and in cell death that is associated with wood development processes, therefore, it optimises tree growth and development. We concluded that the poplar homologues gene to *AtPAD4* have similar and a new reported here wood-specific developmental functions.

We suggest the following hypothesis: 1) Tree growth and development of secondary xylem (wood) result from two antagonistic processes; regulation of the cell cycle and CD, the ultimate end of the cell cycle. CD in xylem tissues is also dependent on the molecular regulators such as LSD1/EDS1/PAD4. Regulatory action of these molecular regulators depends on NPQ and PsbS. Therefore, in order to change cell wall composition, thickness and wood production we need to reduce the NPQ- and photorespiratory-dependent CD, by the simultaneous deregulation of LSD1, EDS1 and PAD4 in a proper combination together with a simultaneously increased NPQ capacity (PsbS overexpression).

Biotechnological improvement of poplar biomass productivity and cell wall composition, 30 months. Task 1.1. Generation of transgenic plants. Poplar sp. will be transformed with multigene constructs. Generally, two types of constructs have been considered: Simultaneous inhibition of *PAD4 or PAD4 and LSD1* in combination with overexpression of PsbS. These constructs are expected to increase photosynthetic efficiency and reduce NPQ- and photorespiration-dependent CD in the xylem therefore change growth and cell wall compozition.

Task 1.2. Selection of transgenic lines. The transgenic plants generated in task 1.1. will be assigned. Firstly, we will test biochemical, molecular and biophysical traits of the cell wall in different lines in (e.g. a combination of drought, high temperature and excess light) with the use of our high throughput patented system (PCT/EP2011/059682, WO2011/154522). This will allow us to select the best lines for field experiments. Selected plants will be further characterised in field and laboratory conditions.

Milestone after 2 years for tasks 1.1 and 1.2 will be: selection of at least one transgenic poplar line with altered CD and cell wall composition.

This proposal and experimental job is planned for one Ph.D. students.

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