Nordic Forest Research
Cooperation Committee - SNS

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Project number: 107

FINAL PROJECT REPORT

Please notice that the size of text sections in the form can be adjusted if needed.
The length of the final report should not exceed 5 pages.

1. Projekt titel	**Xylan modification for added value wood products**
2. Title of project	**Modifikation av xylan för bättre ved produkter**
3. Project leader /coordinator (name, address, telephone, telefax. e-mail)	Prof. Ewa Mellerowicz Department of Forest Genetics and Plant Physiology Swedish University of Agricultural Sciences (SLU) S901-83 Umea Sweden ewa.mellerowicz@genfys.slu.se tel: 46 (0)90 786 8367
4. Time schedule	The project started 01 Jan 2009 and ended 31 Dec 2011
5. Project cost	SNS-grant: 150 000 EU Total project cost: 474 280 EU
6. The purpose of the project/main problems/hypotheses addressed	Xylan is the principal hemicellulose in hardwoods and grasses. This project addresses the hypothesis that the structure of xylan determines important cell wall, wood and lignocellulose properties. To test this hypothesis, we have modifyied xylan structure *in planta* using transgenic approach, and analyzed effects of the different xylan modifications on plant growth and development, on cell wall properties, and on the properties of wood and lignocellulose obtained from the transgenic plants. By precisely modyfying xylan structures and studying the effects of these modifications we can further our understanding of cell wall assembly and architecture, important for deployment of lignocellulose.

7. Brief description of the research plan and of possible larger deviations from the plan	The project involved four different parts that were carried out in parallel in four laboratories of the SNS partners: ***1. Production of transgenic poplars*** ***2. Chemo-enzymatic xylan structure analysis*** ***3.* In situ *studies of xylan*** ***4. X-ray structural studies*** All four parts were carried out and a progress had been achieved in all four areas. No larger deviations from the plan were made, only small adjustments were implemented as needed to efficiently progress towards the main objectives. These included abandononing of using the inducible promoter for wood modification and concentrating instead on the wood-specific promoter evaluation, and postponing the CoMPP analyses of transgenic wood until more information is available on succesfull xylan modifications in different lines.
8. Results (max 2 pages)	***1. Production of transgenic poplars*** Strategies for targeting of xylan-modifying enzymes to the appoplast were developed and expected changes in xylan structure were obtained. A new wood-specific promoter for expressing transgenes in developing secondary-walled tissues was identified. The efficiency of the identified wood-specific promoter in inducing desired wood modifications has been compared to the efficiency of the commonly used promoter. The wood-specific promoter was proven better in a wide study involving over 50 transgenic lines grown in the greenhouse and tested for wood chemical changes (Ratke et al., in preparation). Different types of transgenic lines were obtained carrying proven xylan structure modifications. These include the lines with shorter xylan chain length and the lines with decreased xylan branching. We also have obtained lines in which we have targeted putative xylan-lignin link, and we have first indications that this strategy was working. These lines constitute a valuable resource to address many important questions on cell wall architecture, wood development, wood and lignocellulose proterties and we have obtained some interesting results in these areas already. Based on the greenhouse experiments, we have determineed which types of xylan modifications are well tolerated by plants, and which are causing detrimental effects on growth and development. Some modifications resulted in better plant growth and higher biomass production. We are presently trying to understand the physiological basis of these responses of plants to modifications of xylan. We have also obtained lines with clearly improved mechanical strength and the lines with improved saccharification. The lines with substantially improved saccharification were the subjest of patent application filed in Sweden and in US (Mellerowicz et al, 2012). ***2. Chemo-enzymatic xylan structure analysis*** A non-destructive way for the analysis of glucuronoxylan structures in wood secondary wall has been developed by combining xylanase hydrolysis of powdered wood samples and mass spectrometry (AP-Maldi combined with Ion Trap Mass Spectrometer) anaysis of the products (Chong et al., 2012). The relative abundances of xylooligosaccharides carrying different degrees of acetyl groups and MeGlcA substitutions in wild type and transgenic poplar were compared. The method is especially useful in following acetylation degree and pattern of xylan substitution. The method is applied to characterize xylan in previously described Arabidopsis xylan biosynthetic mutants (Chong et al., in preparation).

	purified as antigens. Mouse monoclonal cell lines that produce antibodies against these oligosaccharides were successfully obtained. Specificity of UX and XUX antibodies has been characterized using different methods. These two antibodies were found to recognize differet structures in xylan, anti-UX reacts specifically with MeGlcA substituents wereas anti-XUX recognizes xylan backbone. In addition, CoMPP experiments showed that xylan acetylation prevents the antibodies from binding to xylan-containing cell walls. The results were published (Koutaniemi et al., 2012). **3. In situ *studies of xylan*** Immunolabelling of plant tissue sections was carried out using UX and XUX antibodies both at light and transmission electron microscopy. The two antibodies were found to label plant cell walls. Several plant species, including poplar xylem, Arabidopsis stem, wheat straw and maize internodes were studied (Koutaniemi et al., 2012). The UX antibody labelling patterns, and *in situ* FTIR analyses were applied to study chemical changes induced by fungal glucuronyl esterase enzyme expressed in Arabidopsis (Tsai et al., in press). ***4. X-ray structural studies*** Hierarchical study of the cell wall structure of wood in the wild type and in transgenic lines of hybrid aspen was carried out using wide and small angle x-ray scattering. The size of cellulose crystallites, the microfibril angle (MFA) and the crystallinity i.e. the volume fraction of crystalline cellulose in the sample, were determined in the three different classes of transgenic aspen. Analyses revealed novel effects of matrix modification on cellulose structure (Derba-Maceluch et al., in preparation; Biswal et al, manuscript; Takahashi et al., in revision). The data open a new perspective on the regulation of cellulose microfibril architecture in wood fibers. Never-dried wood samples were used and structural changes were monitored during drying. Microtomography was used to reveal the three-dimentional cellular structure in the wood (Svedström et al., 2012). Similar studies of cellulose structure in genetically modified plants were initited in Arabidopsis. Data on cellulose crystallite dimentions and cellulose MFA were obtained for the basal stem. The same constructs were made in aspen, and the two model species will thus be compared allowing for unique comparisons between herbaceous and woody species.
9. What advantages has been gained by the Nordic collaboration (i.e. by the cooperating partners, use of the project results)	This project required the aceess to specialized techniques available in the laboratories of the different consortium partners to take a full advantage of having the transgenic lines generated by partner 1. Thanks to the SNS collaboration, a battery of different techniques was applied which allowed progress further in our understaning of the molecular structure of cell wall. In particular, the involvement of Prof. Ritva Serimaa's team was facilitated by SNS funding and provided new technology in the project. In addition, all partners developed further their techniques in their respective fields thanks to the SNS collaboration. Partner 1 developed new very efficient promoter and signal sequence for efficient cell wall targeting. Partner 2 developed new techniques for xylan analysis and new antibodies, which have been used in this project and will be used for many other projects. Partner 3 and partner 1 are currently developing a technique to detect acetylation in different polymers by CoMPP. Thus all the partners gained form participation in the SNS projects. This is also reflected in the common published papers and the manuscrps in preparation.

10. Publications and other communication activities (please list scientific reports, more popular reports and other communication activities)	**Refereed scientific papers from this project:** Tsai AY, Canam T, Gorzsás, A, Mellerowicz, EJ, Campbell, MM, Master, ER Constitutive expression of a fungal glucuronoyl esterase in Arabidopsis reveals altered cell wall composition and structure. Plant Biotechnology Journal, in press. Svedström K, Lucenius J, Van den Bulcke J, Van Loo D, Immerzeel P, Suuronen J-P, Brabant L, Van Acker J, Saranpää P, Fagerstedt K, Mellerowicz E, Serimaa R (2012) Hierarchical structure of juvenile hybrid aspen xylem revealed using x-ray scattering and microtomography. Trees, in press Chong S-L, E Battaglia, PM Coutinho, B Henrissant, M Tenkanen, RP de Vries (2011). The –glucuronidase of *Schizophyllum commune* is a member of a novel glycoside hydrolase family (GH115). Applied Microbiology and Biotechnology, in press. Chong SL, Nissilä T, Ketola RA, Koutaniemi S, Derba-Maceluch M, Mellerowicz EJ, Tenkanen M & Tuomainen P. (2011) Feasibility of using atmospheric pressure matrix-assisted laser desorption/ionization with ion trap mass spectrometry in the analysis of acetylated xylooligosaccharides derived from hardwoods and Arabidopsis thaliana. Anal Bioanal Chem 401: 2995-3009 Leppanen, K ; Bjurhager, I ; Peura, M ; Kallonen, A ; Suuronen, JP ; Penttila, P ; Love, J ; Fagerstedt, K ; Serimaa, R . 2011. X-ray scattering and microtomography study on the structural changes of never-dried silver birch, European aspen and hybrid aspen during drying. **Refereed reviews related to this project:** Gorshkova T, Brutch N, Chabbert B, Deyholos M, Hayashi T, Lev-Yadun S, Mellerowicz EJ, Morvan C, Neutelings G, Pilate G. (2012) Plant fiber formation: state of the art, recent and expected progress, and open questions. Crit Rev Plant Sci. 31:201–228 Mellerowicz EJ and Gorshkova T . (2012) Tensional stress generation in gelatinous fibres: a review and possible mechanism based on cell wall structure and composition. J Exp Bot, 63: 551-565 **Manuscripts in preparation:** Chong, S.-L., Koutaniemi, S., Virkki, L., Pynnönen, H., Tuomainen, P. & Tenkanen, M., Quantitation of 4-O-methylglucuronic acid from plant cell wall, submitted to Carbohydr. Polymers, under revision. Takahashi J, Awano T, Lucenius J, Ratke C, Kontro I, Winzéll A, Kallas Å, Derba-Maceluch M, Gorzsás A, Lesniewska J, Gouget A, Elhasi T, Berthold F, Immerzeel P, Teeri TT, Ezcurra I, Serimaa R, and Mellerowicz EJ Suppression of PtxtXyn10A affects plant growth and secondary wall microfibril angle. Plant Physiol., under revision. Biswal A, Soeno K, Latha Gandla M, Immerzeel P, Leucenius J, Pawar PM, Pattathil S, Hahn MG, Serimaa, R, Moritz T, Jönsson L, Israelsson M and Mellerowicz EM. Aspen PL1 family pectate lyase *Ptxt*PL1-27 mobilizes matrix polysaccharides from woody tissues and improves saccharification yield. Manuscript prepared for PNAS. Pawar PM, Tenkanen M, Mellerowicz EJ. Prospects for *in planta* alteration of lignocellulose acetylation. Invited manuscript prepared for the Frontiers in Plant Biotechnology special issue on lignocellulosic biofuels.

11. Project summary (about 1/3 page) with main emphasis on results for possible use in the News & Views section of Scandinavian Journal of Forest Research	Xylan is one of most abundant polymers in plant cell wall. It is the main hemicellulose in the wood of decidous trees and in grasses, and an important component in the wood in conifers. Xylan is thought to interact with cellulose by hydrogen bonding and with lignin, by covalent linkages. Thus it is thought to have an important role in overall cell wall architecture. Xylan structure consists of a backbone made of repeating xylobiose units, and short side chains of different nature, which are thought to impact on interaction of xylan with different cell wall components. To investigate these interactions between xylan, lignin and cellulose, we have modified all possible side chains of xylan, and its backbone, in plants, by introducing fungal genes encoding xylan-modyfying enzymes to their genomes. By applying different technologies for studying cell wall physical and chemical characteristics through the Nordic cooperation between the laboratories in Umea, Helsinki and Copenhagen, we have been able to pinpoint the types of xylan modifications that determine different cell wall characteristics. For example, we have found that xylan chain length determines cellulose microfibril angle, which is the major determinant of fiber mechanical strength. We also discovered that xylan acetylation plays a key role in determining lignocellulose saccharification, directly affecting biofuel production. We predict that several other wood and lignocellulose properties are affected in the xylan-modified lines, in particular, the properties important for wood mechanical strength, and for lignocellulose pulping and biofuel production.
12. Date and signature	Date: Signature of project leader/coordinator