

During the time of a worldwide climate crisis caused by pollution and the progressing greenhouse effect, it is essential to search for alternative materials in exchange for plastic and non-renewable natural resources. Along with an increasing awareness concerning ecology, wood utilisation has started to gain more and more interest not only in construction, but also in industry.

Forests cover about 4 billion hectares of land, most of which - 72% - are coniferous. Wood from coniferous trees (softwood) is a valuable natural resource and the process of its biosynthesis removes significant amounts of carbon dioxide from the atmosphere. During the growth of trees, the produced biomass differentiates, which is observed in the deposition of subsequent layers of wood, depending on the season. This leads to the formation of annual rings. The main element of softwood are tracheids - cells which have undergone a programmed death and have a strongly developed secondary cell wall which ensures that the plant has structural resistance and a continuous water flow. The main components of tracheids are various types of polysaccharides which build their cell walls. Depending on the composition of the secondary cell wall, the properties of wood may vary, which has a significant impact on its use in industrial processes. The molecular basis regulating the xylogenesis (the wood formation process) is unknown. Therefore, one of the goals of modern plant biotechnology is to understand the exact basis of the processes leading to the production of wood and its modifications.

Our previous work showed that while the overall composition of cell walls within different parts of an annual ring is uniform, the exact structure and properties of polysaccharides which build them differs. Proteins which initiate the process of xylogenesis and its subsequent modification are transcription factors from the VNS family (VND, NST, SMB), which in *Arabidopsis thaliana*, a widely used model plant, are responsible for the activation of many molecular pathways leading to the formation of secondary cell walls. The subject of our research are conifers widely used in industry, in particular Norway spruce (*Picea abies*), which is characterised by a much greater amount of wood than the *Arabidopsis thaliana*. We discovered three potential VNS in spruce (*PaVNS*) and our biochemical studies provided a preliminary confirmation of how they contribute to wood formation. The aim of the project is to define the exact genetic basis of xylogenesis, its molecular control and comparison of produced wood structure and properties.

We plan to carry out a comprehensive analysis of the *PaVNS* family. Examination with confocal microscopy and yeast system will confirm whether the selected proteins locate to the cell nucleus, and whether, by their capacity to bind DNA, they also affect the activation of genes involved in cell wall development. During our preliminary studies, we noticed that the structure of the resulting cell walls differs depending on the transcription factor, which was used, and that these factors can influence each other. Using bioinformatics techniques, we will identify potential genes involved in secondary cell wall formation in spruce and tobacco, and then compare how individual *PaVNS* affect their activation. Simultaneously, biochemical studies will be conducted in cooperation with international partners to characterise the resulting cell wall. The results will allow us to unambiguously link the created cell wall structure with the activity of selected genes. Due to the different composition of cell walls in gymnosperm (spruce) and dicotyledons (tobacco), the project will enable the study and comparison between the effects of *PaVNS* in native and heterologous plant systems. The VNS counterparts in *Arabidopsis thaliana* can be controlled by changes in the degree of cell oxidation level and post-translational modification of proteins, which has a key influence on their activity. We plan to conduct experiments which will allow us to confirm whether *PaVNS* are sensitive to changes inside the cell and whether it is possible to control their activity in this way. We will conduct our study with the participation of scientists from the Christian-Albrecht University in Kiel, who are eminent researchers in this field.

Accurate characterization of the cell wall from both genetic and biochemical perspective and comparison of results between different types of plants are great advantages of the proposed project. Describing the mechanisms involved in the process of secondary cell wall formation may allow for breakthroughs in targeted breeding of trees making wood with desired properties. In addition, possible control of the xylogenesis process through modulation of proteins which initiate it, may revolutionise and facilitate research into the secondary cell wall. Indication of genes involved in the biosynthesis of coniferous wood as well as methods allowing for their analysis is an invaluable contribution to the development of basic research, enabling other teams to continue research in the field of polysaccharides and plant biology.