

Basic plant organs, such as leaves and flowers, come in range of marvellous shapes and sizes. This beautiful diversity serves many biological purposes, such as attracting pollinators, increasing photosynthetic efficiency or even catching prey in carnivorous plants. Trees are a group of plants showing impressive variation in appearance and shape of one of their most important organs: the leaves. Leaf shape is one of a key characteristic by which we can identify tree species, but the exact mechanism which enables trees to have such a vast array of leaf types remains unknown. The scientific community believes that the primary cell wall (PCW) is one of the key cellular features which control the development of plant organs. The PCWs surround all plant cells and define their size and shape. In order for plant cells to grow, the PCW needs to expand by altering its properties but the basic molecular features responsible for these properties remain unknown. On a chemical level PCW is composed of complex sugar molecules known as the polysaccharides. Important PCW polysaccharides include cellulose and pectin, which for many years were extensively studied for their contribution to the PCW mechanical properties. A recent research article co-authored by the principal investigator of this proposal described that another class of polysaccharides, known as the hemicelluloses, influences growth of the PCW. This interesting discovery was done in a small model plant *Arabidopsis thaliana*. We believe it opens a way for more extensive analysis of the role of hemicelluloses in groups of plants with ecological and economic importance. We thus chose trees as our test platform in which we will analyse the contribution of hemicelluloses to cell growth and shape formation as well as to PCW mechanical properties. Through this project we therefore aim to provide the first holistic overview of hemicellulose function in tree cell and organ development.

To do that we assembled an interdisciplinary team of scientists with biochemists from the Jagiellonian University in Krakow and biophysicists from the University of Silesia in Katowice. Furthermore, we will be assisted by international collaborators from New Zealand Forest Research Institute Scion and the Sainsbury Laboratory Cambridge University. To study the role of hemicelluloses in tree cells we will first investigate their structure using biochemical techniques. We will also use bioinformatics and genetic analyses to identify enzymes, i.e. molecular machines involved in the formation of hemicelluloses in tree PCWs. This understanding, together with genetic engineering tools available at the Jagiellonian University will allow us to transfer tree hemicelluloses into our small model plant *Arabidopsis thaliana*. By using this single test species we will be able to compare the contribution of different hemicellulose structures to mechanical properties of the PCW using instruments such as the atomic force microscope and mechanical testing machine (extensometer) dedicated to small tissue samples, available at the University of Silesia, which enable analysis of structure and mechanical properties of plant cells. In order to investigate the role of hemicelluloses in tree organ formation we will use specific enzymes, known as the glycosyl hydrolases, to break down distinct groups of hemicelluloses in developing organs of different tree species. We will use the excellent microscopy facilities available at the Sainsbury Laboratory to monitor how tree organ development changes when the PCW degrading enzymes, glycosyl hydrolases, specifically digest different types of hemicelluloses in growing tree cells. Finally, through our partners from New Zealand we will gain access to a unique platform for genetic engineering of conifer tree cells. We will use this capacity to completely remove specific types of hemicelluloses from conifer PCW. This will allow us to be the first team in the world to directly study the importance of hemicelluloses for cell and PCW mechanical properties in this hugely important group of trees.

The contribution of hemicelluloses to the mechanics of PCW is poorly understood, despite the critical role of this wall type for the control of plant growth and shape formation. Therefore, by investigating if and how the hemicelluloses control PCW properties our project will tackle one of the main outstanding questions in plant developmental biology. Scientific interest in answering this question is for us one of the main reasons for attempting this research project. We hypothesise that by modifying the structure of PCW hemicelluloses we may be able to induce major changes in tree organ growth, shape and size. The potential importance of these changes and their contribution to forestry, is yet one more significant reason for undertaking this research.

Significance of PCW to tree growth was recently demonstrated in a poplar, where modification of PCW polysaccharide synthesis allowed for major increase in overall plant size. This shows that tree PCW engineering can lead to formation of larger organs with higher biomass content, what may improve capacity of trees to lock the CO<sub>2</sub>. Our work and PCW engineering can therefore be a route to tackling the impending climate emergency. Moreover, PCWs are a main barrier protecting tree cells from pathogen attack. Therefore, identifying mechanisms contributing to the maintenance of PCW properties can allow us to identify breeding targets for improvement of tree resistance to pathogens, which is critical for progress in a range of forestry and agricultural areas. As such our results will not only advance scientific knowledge but may also contribute to safeguarding of global ecosystems for future generations.