

Summary

Increasing climate changes increase the average temperature of the Earth's surface and a higher frequency of extreme weather phenomena, which are a threat to food production. Given the growing human population and declining farmland, there is an urgent need to develop new crops adapted to changing climate. It is possible by developing new plant varieties, either with the use of classical breeding methods or through the use of genetic engineering. The latter approach requires a detailed understanding of the molecular mechanisms underlying various stress responses, particularly temperature stress induced by low and high temperatures. Temperature stress changes photosynthetic efficiency and disturbs redox potential. It also leads to rearrangement of the cell wall cytoskeleton and cell wall remodelling. All these processes are relatively poorly understood. Therefore, this work focused on the response to the temperature stress of leaves of a model species for grasses in the temperate climate zone, *Brachypodium distachyon*.

This study aimed to analyse the response of *Brachypodium distachyon* cell wall proteins in leaves subjected to low (4 °C) and high (40 °C) temperature. Another objective was to obtain mutants with inactivated genes encoding the Fasciclin arabinogalactan protein (*Bradi3g39740*) and pectin methylesterase (*Bradi3g24750*). To characterize the effect of cellular stress on the cell wall proteins, immunocytochemistry with antibodies binding specifically to arabinogalactan proteins and extensins epitopes, analysis of the expression profile of genes encoding arabinogalactan and extensin proteins, as well as analysis of the cell wall proteome were applied. In order to obtain mutants with inactivated genes, site-directed mutagenesis based on CRISPR/Cas9 system was used. In addition, the transformation of the embryogenic callus induced from immature embryos was performed using *Agrobacterium tumefaciens* bacterial cells.

Studies on the distribution of AGP epitopes in leaves of *B. distachyon* revealed their presence mainly in the vascular bundle and extensins in the mesophyll. Differences in the distribution and intensity of signals were demonstrated for the four antibodies recognizing AGP: JIM8, JIM16, LM2, and LM6. At the same time, no differences in the distribution and intensity of signals were observed for antibodies recognizing extensin epitopes. Analysis of the expression profile of the genes encoding AGP and extensin revealed an increase in the expression level, significantly greater in the plants incubated at high temperatures. In turn, proteomic analysis of the

cell wall allowed the identification of 46 proteins with the differentiated presence at high temperature compared to the control. These changes suggest lower protease activity, cell wall lignification and expansion, and changes in the architecture of cell wall polymers, especially pectins. Using the CRISPR/Cas9 system, four mutants with inactivated genes encoding the Fasciclin arabinogalactan protein and pectin methylesterase were obtained. Although the obtained mutants show no change in response to temperature stress, they show slower growth in response to salt stress.