Summary

Frost tolerance, an important component of winter-hardiness, is a trait induced under conditions of cold acclimation. This multistage process is associated with many physiological and molecular alterations in plant cells. The main objective of this scientific work was to recognize selected cellular mechanisms associated with the process of cold acclimation and the induction of frost tolerance in *Lolium multiflorum*/*Festuca arundinacea* introgression forms.

The plant materials used in the research involved two tetraploid BC_5 *L. multiflorum/F. arundinacea* introgression forms, distinct in their levels of frost tolerance – the high frost tolerant and the low frost tolerant form. All the physiological and molecular analyses were conducted at the selected time-points of three week period of cold acclimation, and in the control conditions (after one week of pre-hardening). The integrity of biological membranes (with respect to the levels of electrolyte leakage and lipid peroxidation), contents of superoxide anion and hydrogen peroxide, parameters of chlorophyll fluorescence and gas exchange, accumulation of Cor14b, accumulation and activities of the selected enzymes of the Calvin cycle and antioxidant system, furthermore, accumulation of transcripts for the genes, coding these enzymes, were analyzed. Moreover, proteomes of both introgression forms using 2-D electrophoresis and mass spectrometry, were analyzed and proteins differentially accumulated under cold acclimation between two introgression forms, identified.

It was demonstrated that the high frost tolerant introgression form was characterized by a higher integrity of biological membranes, lower contents of reactive oxygen species, and a higher efficiency of enzymatic antioxidant system in low temperature. The analysis of proteome revealed five isoforms of fructose-1,6-bisphosphate aldolase with distinct accumulation levels under cold acclimation between both analyzed introgression forms. Furthermore, analysis of CO_2 assimilation, an activity of fructose-1,6-bisphosphate aldolase, and chlorophyll fluorescence, including maximum quantum yield of primary PSII photochemistry, indicated that the high frost tolerant introgression form was able to acclimate its photosynthetic apparatus in low temperature. This introgression form was also characterized by higher accumulation of Cor14b protein in these conditions.

The obtained results demonstrated that the ability to cold acclimate of photosynthetic apparatus and to maintain a relatively high activity level of enzymatic antioxidant system under cold acclimation process, are the important attributes of cellular metabolism, which could have a significant impact on the increase of frost tolerance in *L. multiflorum/F. arundinacea* introgression forms. The obtained and presented results may be a good starting point for further studies to recognize the mechanisms of winter-hardiness in the *Lolium-Festuca* group of forage grasses, also with respect to their resistance to de-acclimation and their ability to re-acclimate.