Winter stresses are among the major environmental factors, which affect plants. Winter-hardiness is a trait, which enables plants to survive winter period, and is associated with plant capacity to overcome a wide spectrum of environmental stresses, such as frost, rapidly changing temperatures, light stress, wind, desiccation, anoxia, ice encasement, mechanical damage, de-acclimation, or various winter-related diseases. Frost tolerance is often thought to be the most crucial trait to determine levels of winter-hardiness, as it usually has the most significant impact on plant survival in winter. Plants from temperate regions acquire frost tolerance in the process of cold acclimation (CA). On the other hand, warmer temperatures trigger de-acclimation (DA) and consequently loss of frost tolerance. However, it has been also recognized that plants may regain frost tolerance during a process of re-acclimation (RA), after the next period of lower temperature.

Unfortunately, not all the crucial components of plant cellular mechanisms associated with tolerance to frost, have been widely studied and precisely recognized in detail to date, especially with respect to the sequence of CA, DA, and RA. Taking into account climate change expectations, including possible winter temperature oscillations, the research on the sequence of processes such as DA and RA in plants, is important. This part of knowledge is still limited and did not attract enough attention of scientists, especially with respect to the species crucial for agriculture in temperature regions.

The main objective of our project is to recognize key physiological and molecular components of plant metabolism involved in acquiring/loss of frost tolerance with respect to the sequence of CA, DA, and RA. The research will be performed using the valuable plant models from a group of *Lolium-Festuca* forage grasses, *F. arundinacea* (tall fescue) and the introgression forms of *L. multiflorum* (Italian ryegrass)/*F. arundinacea* with different levels of winter-hardiness, selected in the variable conditions, including oscillating temperatures. The objectives of the project will be focused on: (1) Analysis of cellular proteome, primary metabolome, and lipidome. (2) Analysis of reactive oxygen species and plant antioxidant capacity. (3) Monitoring of plant photosynthetic capacity and cellular membranes integrity. The particular experiments will be conducted at precisely defined time-points of CA, DA and RA periods and in the control conditions (optimal growth conditions).

The proposed studies demonstrate a high level of novelty. We believe that the results will enable us to draw a comprehensive model of plant response to the sequence of CA, DA, and RA processes in the forage grasses, crucial for forage production in Europe. The results will have also a significant impact on our deeper understanding of plant physiology related to molecular mechanisms of frost tolerance and winter-hardiness in other perennial grass species, especially with respect to the expectations of global climate change, including oscillations of winter temperatures. Furthermore, the research proposed in our project could have also a high level of significance for further development of 'new grass cultivars', improved with respect to their ability to withstand different environmental stresses. This strategy will follow the demands of sustainable agriculture to develop new cultivars of crop plants with a broad spectrum of resistance to various adverse environmental conditions.