

AIM OF THE PROJECT The main goal of the project is characterization of lipocalins family and recognition of chloroplastic lipocalins function in protection of photosynthetic apparatus against oxidative stress in *Festuca glaucescens*. Among lipocalins two groups can be distinguished: true lipocalins and lipocalin-like proteins. True lipocalins include chloroplastic lipocalins and temperature-induced lipocalins. Lipocalins have the ability to hydrophobic ligand binding and their expression is regulated through abiotic factor like: drought, low temperature and heat. It suggests that they can play the protective function for photosynthetic apparatus during oxidative stress. The detailed goals are as follows: (i) identification of the genes encoding lipocalins (including true lipocalin and lipocalin-like proteins) within *F. glaucescens* genome, (ii) cellular function analysis of true lipocalins in the wild-type plants, (iii) generation of transgenic plants with *CHL* gene/genes knockout, (iv) analysis of photosynthesis activity in the wild-type plants and *CHL*/knock-out transgenic plants exposed to combined drought and high light treatment, (v) analysis of thylakoid membrane lipidome in the wild-type plants and *CHL* gene/genes knock-out transgenic plants exposed to combined drought and high light treatment.

RESEARCH DESCRIPTION Planned studies include: *de novo* sequencing and assembly of *F. glaucescens* genome with use of Next-Generation Sequencing (NGS) technology; annotation of the genes encoding TIL and *CHL* lipocalins; sub-cellular localization of *CHL* lipocalins; identification of ligands that bind to TIL and *CHL* lipocalins; generation of *F. glaucescens* transgenic plants with *CHL* gene/genes knock-out (gene editing with CRISPR/Cas9 use); combined drought and high light treatment of wild-type and *CHL*/knock-out transgenic plants to evoke oxidative stress; lipid peroxidation and reactive oxygen species content measurement; functioning of antioxidant system analysis including: activity of catalases, peroxidases and dismutases measurement in the wild-type plants and knockout mutants exposed to combined drought and high light treatment; functioning of Calvin cycle analysis including: chlorophyll fluorescence, gas exchange, expression and activity of chloroplastic aldolase measurement in the wild-type plants and knockout mutants exposed to combined drought and high light treatment; lipidome analysis of thylakoid membrane in the wild-type plants and knockout mutants exposed to combined drought and high light treatment.

JUSTIFICATION FOR TACKLING SCIENTIFIC PROBLEMS Genome sequence of *F. glaucescens* is not recognized what significantly limits scientific areas that can be performed with use of this species, as well as different species from *Festuca* genus with big economic importance. Hence knowledge about molecular basis of *F. glaucescens* abiotic stresses tolerance, including lipocalin contribution, is insufficient. Gene editing with CRISPR/Cas9 use and *Agrobacterium tumefaciens*-mediated grass transformation is not developed in Plant Molecular Physiology and Cytogenetics Team.

THE MOST IMPORTANT EXPECTED EFFECTS The most important effects include: (i) whole-genome sequence of *F. glaucescens* obtainment; (ii) characterization of lipocalin family in *F. glaucescens*; (iii) recognition of chloroplastic lipocalins function in abiotic stress tolerance through knock-out of selected lipocalin gene/genes in mutants.