

The aim of the project is to explore whether the LTP2 gene expression is differentially regulated by abiotic stresses, resulting in the modification of the lipidome and phenome. The level of protein (reported as the strong allergen) determined by the expression of the LTP2 gene will be studied in the project to verify the hypothesis about the increased biosynthesis of this putative allergen under the abiotic stresses.

Barley lines previously obtained from the backcross, selected by 954-1377 SNP marker polymorphism mapped in chromosome 4H in the MLOC\_53422 and annotated by Ensembl Plants as the nsLTP2 gene (non-specific lipid transfer proteins 2), are unique plant material to the proposed research. Additionally recombinant inbred lines (RIL F8/ MCam) derived from Maresi and Cam/B1/CI08887/CI05761 (CamB1) cross combination will be also analyzed. CamB1 is a Syrian inbred line adapted to the water deficiency conditions. The stability analysis in conjunction with genotypic and phenotypic data allow for lines selection with advantageous both parental genotypes alleles: the earliness from the Syrian line, the semi-dwarfness from cv. Maresi with simultaneous stable grain yield under water deficit conditions (G x E interaction analysis, Sergen;  $P < 0.01$ ). BC lines and RILs have the same parental genotype (Maresi), but on the other hand, CamB1, which is in the MCam lines pedigree, determines the adaptation to the water scarcity conditions. The planned experiments with such plant material allow answer the question how the level of the LTP2 gene expression, the protein synthesis and the lipid content will change in RILs adapted to stress conditions compared to BC lines.

Phenotypic observations will be conducted both in the greenhouse and in the growth chamber in three replications. In the project selected barley lines will be subjected to abiotic stresses in the tillering stage for 14 days, and phenome evaluation will be conducted at the full maturity stage. Phenotyping will be related to 20 characteristics associated among others with the spike morphology, plant architecture, grain and straw yield, as well as dates of achieving the main development stages. Additionally, the qualitative and quantitative lipids composition will be examined, with special emphasis on sterols by LC-MS. The LTP2 gene transcript level will be also observed using Real Time PCR. In turn, LTP2 protein detection will be performed using the Western Blot. All measurements will be executed on biological samples obtained under control and abiotic stresses conditions (drought, high temperature, salinity). The results will be analyzed in terms of studied barley lines variation and the effects of genotype-environment interaction will be also interpreted.

Barley (*Hordeum vulgare* L.) is one of the first crop to be domesticated and is the fourth most important cereal, after maize, rice and wheat, covering approximately 10% of the worldwide acreage of cereals. In past decades, the barley genome has been extensively analyzed. The result of the project undertaken by the International Barley Genome Sequencing Consortium represents a powerful resource for enhanced the barley genome analyses and provides a significantly larger number of well characterized genetic stocks. This may enable a capacious research of any region of the barley genome. Hence, barley has been considered as a model plant for grasses and can be a convenient genome to examine and understand many different genetic mechanisms including the response to abiotic stresses. Increased salinization, drying environment and climate warming are forecasted in the nearest future. Drought, salinity and high temperature have been reported to be interrelated and may affect similar cells response. It is known that the osmotic stress is resulted from the water and salt stress. Also the salinity, drought and high temperature have been accompanied by the oxidative stress and stimulated similar cell response, among others molecules denaturation, protein accumulation, lipids modifications.

The mature barley seed proteome is dominated by proteins involved in stress response including Pathogenesis-Related proteins (PRs). Currently, they are classified into 17 families, based on primary sequence, immunological relationship and biological activity. Group 14 comprises a lipid transfer protein. The term “non-specific lipid-transfer protein” (nsLTP) is used in reference to the apparent lack of specificity for the various phospholipids. LTPs have been recognized to be involved in several biological processes. But little is known about their role in response to abiotic stresses, especially in relation to barley. Lipid transfer proteins have become the great of importance due to their significance in industrial applications and human health. They have been reported to be presented in barley malt and contributed to beer quality and foam formation. Moreover, they have been identified as a one of the main human allergens. Based on the molecular weight (Mw) of the mature proteins, plant nsLTPs can be classified into two main types, nsLTP1 (9 kDa) and nsLTP2 (7 kDa). They exhibit low overall amino acid sequence similarity (30% identity). LTP2 protein have a higher melting temperature (over 100°C) and the detergents resistance than LTP1. This may suggest the potent allergenic even at a lower concentration. Moreover, LTP2 has the ability to bind not only linear lipid molecules (as in the case of LTP1), but also sterols (of a great importance to human, including the total cholesterol and LDL reduction, without HDL decrease). Due to its role in lipid transfer the LTP2 may be crucial in lipidome modification in response to abiotic stress.

Recently, limited information is reported about the LTP2, especially in the research area of barley. A novel scientific knowledge about LTP2 will be provided by the integrative approach of the project. The coordinated analysis of the gene expression, protein and qualitative and quantitative lipids composition, in conjunction with phenotypic data will fill the gap of the LTP2 role in response to abiotic stresses. Backcrossed lines characterized by the closely coincident genetic background and selected due to the LTP2 gene seems to be the unique and adequate research material to realize the objectives of the project. Our results will reveal important information for functional annotation of barley genome that is becoming a model for cereal plants, due to the recent publication of its sequence. The integrated research and collected knowledge will serve as a basis for the methods of barley and other crop plants improvement in the future.