

Summary

Abiotic stresses occurring in the natural environment lead to structural and functional changes at the plant cell level, initiating the activation of adaptive mechanisms. The coexistence of various abiotic stresses is often reflected in a greater decrease in plant productivity compared to the separate occurrence of these stresses. Among cereals, barley belongs to one of the best studied species in terms of genetics, and due to the high conservatism of the grass genome, it is a model plant in many studies, including those related to the yielding potential under unfavourable environmental conditions. The aim of the study was a comprehensive analysis of changes in spring barley caused by various abiotic stresses, including qualitative and quantitative determination of metabolites, lipids and sterols in leaves, estimation of *ns-LTP2* gene expression and detection of ns-LTP2 protein in vegetative tissues and components of barley grain along with plant phenotyping. The integration of the obtained data was aimed at providing new knowledge about the molecular adaptation of plants to stressful conditions.

The plant material in the study were spring barley lines and their parental genotypes selected in terms of origin, phenotype and polymorphism of the *ns-LTP2* gene. The MPW lines (*P*: MPS106 × MPS37) with defined polymorphism of the *ns-LTP2*, and recombinant inbred lines (RIL, F₁₀) obtained by the single seed descent technique - MCam (*P*: Maresi × CamB1) were analysed. MCam lines combined the favourable traits of parental genotypes - CamB1 (earliness) and Maresi (semi-dwarfism).

A series of experiments were carried out in which selected genotypes of barley in the tillering stage were subjected to abiotic stresses for a period of 14 days. Phenotyping took place at the time of grain formation and at the stage of full maturity, including 15 traits related to plant architecture, spike morphology, grain weight, and heading date. The experiments were carried out in greenhouse and growing chamber under controlled conditions. Drought (D) was carried out in pots with a moisture of 20% FWC compared to an optimal moisture content of 70% FWC. Salinity (S) was obtained by applying NaCl solution to the pots to obtain a final salt concentration of 250 mM·dm⁻³. In turn, high temperature stress (T) was associated with the temperature in the control conditions 22°C/18°C (day/night), against stress conditions 30°C/10°C (day/night). In addition, the impact of their simultaneous impact (DS, DT, ST, DST) was also assessed.

The influence of abiotic stresses on the metabolome and lipids of the studied genotypes was investigated using GC/MS, GC/FID and Fast-GC/MS techniques. Moreover, the impact of stress conditions on the change of the *ns-LTP2* gene expression level in vegetative tissues and

in barley grains (qRT-PCR) was analysed, but also the level of ns-LTP2 protein accumulation (Western blot) was detected and the impact of abiotic stresses on the level of this protein in the embryonic axes was investigated (ELISA test).

Generally, 384 metabolites have been identified in barley leaves. The analysis of variance revealed 122 metabolites which concentration was changed ($p < 0.05$) due to genotype (76), abiotic stress conditions (41) and its duration (21), as well as interactions between genotype and time point (7), genotype and abiotic stress (29) as well as time point and abiotic stress (30). Different stress response pathways were identified by faster accumulation of amino acids in CamB1, carbohydrates in MPS lines, and efficient nitrogen metabolism was found in Maresi. It was shown that CamB1 is characterized by a faster accumulation of osmoprotectants and antioxidants compared to the European genotypes.

High temperature and its combinations (mainly DT) significantly changed the accumulation level of most classes of lipids and sterols, except for $\Delta 5$ -avenasterol which level increased only under single stress conditions (D or T), which may suggest its unknown and specific function in adapting barley to unfavourable conditions. CamB1, originating from a dry and warm climate, was characterized by the highest levels of campesterol, stigmasterol and $\Delta 5$ -avenasterol compared to other genotypes. On the other hand, MPS lines, differing in the allelic form of the *ns-LTP2* gene, differed from among the studied genotypes in the level of metabolite accumulation, including lipids, which could be an important mechanism of survival in changing environmental conditions.

The *ns-LTP2* gene was not expressed in mature vegetative tissues of barley regardless of stress conditions. Its constitutive expression was detected in aleurone layer and barley embryonic axis. The level of *ns-LTP2* expression differed between genotypes, and abiotic stress (mainly DS) increased its level. In the embryonic axis, the level of total soluble protein increased most under the single stress (S), while the ns-LTP2 protein, regardless of genotype, under simultaneous stresses (ST and DST).

A detailed phenotypic description of the analysed plant material growing under stress conditions was made. The MPS lines were heading at a similar date and had a well-developed main spike, similar to the MPW lines. MPS37 (*ns-LTP2.a*), unlike MPS106 (*ns-LTP2.b*), had the lowest total number of tillers and a high thousand grain weight (similar to Maresi). The MCam lines were characterized by very good parameters of yield-forming traits (similar to one of the parent genotypes - Maresi), and they were heading faster compared to other lines (a characteristic feature of the second parent genotype - CamB1).

A relationship was found between the level of accumulation of metabolites under stress conditions and phenotypic traits, which provided the information about the reaction of plants at the metabolome and phenome level under various environmental conditions. Water deficit was found to be dominant when it occurred with high temperature, while the interaction of drought and salinity was most likely synergistic. On the other hand, the interaction of salinity and high temperature, as well as all three stresses, was unique with the effect significantly different from the stresses occurring individually.

Research on the characteristics and resistance of plants growing in changing environmental conditions is important in the context of progressive climate change. The high plasticity of the CamB1 metabolome, related to the survival of unfavourable conditions and short-term seed production, makes it a good candidate for the development of varieties adapted to changing environmental conditions, but insufficient in the context of high yield. The changes induced by the abiotic stresses in the MPS and CamB1 lines were often similar, and in this reason MPS lines selected in the doctoral dissertation with better parameters of yield forming traits than the Syrian line, seem to be a promising genetic resource for increase the tolerance of barley to abiotic stress.