Analysis of the primary metabolome in the study of redox processes in plants

Frolova N.[A]*, Tarakhovskaya E.[B], Vereshchagin M.[A], Orlova A.[A], Silinskaya S.[A], Basnet A.[C], Pashkovskij P.[A], Bilova T.[A,B], Frolov A.[A,C]

[A] Laboratory of Biotechnology and Bioanalytical Chemistry, Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Moscow, Russia

[B] Department of Plant Physiology and Biochemistry, St. Petersburg State University, St. Petersburg, Russia,

[C] Leibniz institute of Plant Biochemistry, Halle (Saale), Germany

**E-mail: frolovanadja@yandex.ru*

Metabolomics is a broad-scale and sensitive approach that provides information on the composition and dynamics of the plant metabolome. This information has increasing applications not only for plant phenotyping and chemotyping, but also for understanding the mechanisms underlying plant responses to various environmental stresses. In the adaptation of plants to negative factors, an important role also belongs to the functioning of efficient antioxidant systems capable of providing protection against reactive oxygen species accumulated under stress related to various environmental and biotic factors. Exposure to these factors triggers induction of antioxidant system. Due to this, plants acquire resistance to the stressor. Dynamics of low molecular weight compounds (ascorbic acid, hydrogen peroxide, carotenoids, glutathione, tocopherol, flavonoids), enzymes (peroxidase, catalase, superoxide dismutase, etc.), and photosynthetic pigments might be considered as reliable indicators of the physiological state of plants. These markers represent the "household molecules", i.e. constituents of primary metabolome, which are directly related to adaptive metabolic rearrangements, and dynamics of their contents is highly informative in the context of stress biochemistry. Under stress conditions, compensatory metabolic pathways of plant adaptation involve reorganisation of respiratory metabolism, glycolysis, oxidative pentose phosphate pathway, supplying ATP, intermediates for biosynthesis and biological reducing agents. A complex approach to characterize the dynamics of the plant primary metabolome under stress conditions is established in the Laboratory of Analytical Biochemistry and Biotechnology at the K.A. Timiryazev Institute of Plant Physiology of the Russian Academy of Sciences. Our analytical strategy assumes analysis of primary thermostable and thermolabile metabolites by gas chromatography coupled on-line to the electron ionisation quadrupole mass spectrometry (GC-EI-Q-MS) and ion-pair reversed phase high-performance liquid chromatography, coupled online to tandem mass spectrometry accomplished with a triple-quadrupole mass analyser (RP-IP-HPLC-QqQ-MS/MS). Identification of the primary thermally stable metabolites relies on the spectral similarity search with consideration of the characteristic retention times

(t_Rs), and retention indices (RIs) of analytes in experimental samples and standard substances of known mass spectrometric libraries of NIST (National Institute of Standards and Technology), GMD (Golm Metabolome Database) and in-house library based on defined mixtures of authentic standards. Analysis of the primary thermally labile metabolites relies on multiple reaction monitoring (MRM) experiments and representative panel of relevant authentic standards using MultiQuant™ 3.0.3 software. The resulting metabolite matrices are processed with the online tool MetaboAnalyst 6.0. Thus, analyses of the dynamics of the primary metabolome (including detailed deciphering of the influence of individual metabolic reactions) can provide access to key regulatory pathways and possible mechanisms of redox processes accompanying plant development, senescence, and stress responses, and the involvement of genomics, proteomics, and physiology data can make a significant contribution to understanding the plasticity of the plant metabolome.

The research was supported by RSF (project no.23-14-00266).

Why proteomics is not a silver bullet in glycation research: peptide-based glycation models as a tool to address the mechanisms behind glycation in plants

Frolova N.[A]*, Herfurth U. M.[B], Duc N. V.[C], Soboleva A.[A,C], Birkemeyer C.[D], Bilova T.[C,E], Frolov A.[A,C]

*[A] Laboratory of Analytical Biochemistry and Biotechnology, Timiryazev Institute of Plant Physiology of the Russian Academy of Science, Botanicheskaya Street 35, Moscow, Russia. *E-mail: frolovanadja@yandex.ru*

[B] Department Food Safety, Federal Institute for Risk Assessment (BfR);

[C] Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Leibniz Institute of Plant Biochemistry;

[D] Faculty of Chemistry and Mineralogy, Universität Leipzig;

[E] Department of Plant Physiology ond Biochemistry, St. Petersburg State University, St. Petersburg State University, Universitetskaya Emb.7-9, St. Petersburg, Russia;

Keywords: Advanced glycation end products (AGEs), bottom-up proteomics, GC-MS, LC-MS, model synthetic peptides, protein glycation, peptide-based glycation models.

Protein glycation (often also referred as Maillard reaction of proteins) is usually referred to as an arrow of post-translational modifications targeting side chains of lysine, arginine and, to less extent, cysteine residues in proteins. Carbonyl compounds – sugars, their derivates and α-dicarbonyls, act as potent glycation agents, yielding broad patterns of early and advanced glycation end products (AGEs). To date, the Maillard reaction of proteins is comprehensively described in the food chemistry and clinical aspects. In this context, resulting