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Basic Information:
Course Title: "Methods for the analysis of plant proteins. Proteomic analysis"

Lecturer: Lyudmila Simova-Stoilova, PhD, Institute of Plant Physiology and Genetics, BAS
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Total Teaching Hours: 30 hours

Annotation
The course is intended for a wide range of specialists working in the field of plant sciences (biologists, biochemists, biotechnologists, agronomists), and provides a methodological basis for practical work with plant proteins. Both classical biochemical methods and modern systems approaches such as proteomics are presented. It consists of two parts. The first one discusses methods for extraction, fractionation, quantification, labeling, electrophoretic, immunochemical techniques for identification and quantification of proteins, with emphasis on the specificity of the plant material to work with. The second part provides basic knowledge about proteomics. The entire analysis process is followed from the preparation of plant samples, separation techniques (two-dimensional electrophoresis/liquid chromatography), data analysis and processing, mass spectrometric identification of proteins, analysis of post-translational modifications, quantification, accepted standards, single-cell proteomics, and application of artificial intelligence. Despite its focus on plant proteins, the course examines fundamental issues and would be useful for other specialists interested in proteomics.

Course content (brief description by topics)

Part I Classical biochemical methods

Topic 1: Features of proteins as an object of study. Extraction of proteins from different types of plant tissues. Fractionation of samples and enrichment in specific proteins.

Topic 2: Methods for quantitative determination of proteins - advantages and limitations.

Topic 3: Electrophoretic separation of proteins under non-denaturing and denaturing conditions. Development of enzymatic activities after electrophoretic separation.

Topic 4: Basic techniques for purification and concentration of proteins.

Topic 5: Immunoblotting and other immunochemical techniques for identification and quantitation.

Topic 6: Protein labeling and use of ligand interactions. Heterologous expression of plant proteins.

Part II Systems Methods - Proteomic Analysis

Topic 7: Basic concepts, necessary equipment, workflow. Gel-based and liquid chromatography-based analyses (first and second generation proteomics) - differences.

Topic 8: Preparation of plant samples for proteomic analysis. Removal of interfering impurities and reduction of the proportion of dominant proteins

Topic 9: Gel-based methods of protein separation. Isoelectrofocusing. Two-dimensional electrophoresis and DIGE as a variant. Image analysis and processing.

Topic 10: Separation of peptides by liquid chromatography coupled with mass spectrometry.
Topic 11: Types of mass spectrometers. Interpretation of mass spectra. Protein identification using PMF and MS/MS. De novo sequencing and database search.

Topic 12: Data analysis. Qualitative and quantitative proteomics.

Topic 13: Post-translational modifications (PTMs) - biological significance, types, MS analysis, neutral loss and diagnostic ions. Phosphorylation and glycosylation as the most common PTMs.
Topic 14: Single cell proteomics – approaches, limitations, perspectives.

Topic 15: Application of artificial intelligence in proteomics. Multiomic approaches.

Teaching and assessment methods
The course is conducted in a face-to-face or distance learning format, in Bulgarian or English. Assessment is through tests – one for each of the two parts, taking into account the interest and activity shown during the training.

Competencies acquired as a result of training (3–5 points)

1. Acquisition of methodological competence for biochemical work with plant proteins.
2. Basic knowledge of proteomics – advantages and limitations, new perspectives.
3. Additional knowledge, enabling professional adaptation to new scientific fields.

Literature:

1. Protein Purification: Principles and Practice. R. K. Scopes. Springer NY 1994.3rd ed.
2. Plant biochemistry. Hans Walter Heldt. Elsevier Acad. Press 2005.
3. Proteomics in practice. R. Westermeier, T. Naven. 2002 Wiley –VCH Verlag GmbH
4. Plant Proteomics - Methods and Protocols. Methods in Molecular Biology 1072, 2014. Eds Jorrin-Novo, J.V., Komatsu, S., Weckwerth, W., Wienkoop, S. Humana Press.
5. Advances in Plant Omics and Systems Biology Approaches. 2021 Ed. F.V.Winck, Springer Nature

Additional information
Basic prior knowledge of biochemistry is required.