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Advancements in Plant Proteomics and Metabolomics: Tools and Techniques

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ABSTRACT

Advancements in plant proteomics and metabolomics have revolutionized our understanding of plant biology by providing comprehensive insights into molecular mechanisms governing growth, development, and stress responses. Proteomics enables large-scale protein profiling through advanced extraction techniques, mass spectrometry-based approaches, and bioinformatics tools, elucidating protein interactions and functions. Similarly, metabolomics explores metabolic pathways via cutting-edge separation techniques and mass spectrometry, offering crucial insights into plant metabolism and stress adaptation. Integrating these 'omics' approaches has facilitated discoveries in plant stress biology, crop improvement, and plant-microbe interactions. Despite challenges in data integration, coverage, and reproducibility, future developments, including artificial intelligence and multi-omics integration, promise to enhance plant science applications in agriculture, biotechnology, and environmental sustainability.

Keywords: Plant proteomics, Plant metabolomics, Mass spectrometry, Bioinformatics tools, Crop improvement

Introduction

Plants are unique and multi-organisms that have developed sophisticated biological structures to survive in a changing environment. From the tropical rainforest to the hot desert, there are elaborate structures that show the ways that plants detect, signal, and operate in different environments. The detailed knowledge of molecular processes controlling plant growth, development, and interactions with biotic and abiotic factors is essential for improving our knowledge of plant biology and agriculture. Mastering the subtlety of how plants grow, develop, and respond to stress factors in terms of their molecules is important to enhance plant science and agriculture. More recently, there have been two areas that have provided great insights into molecular aspects of plant biology: plant proteomics and plant metabolomics.[1,2]

Studies of protein complement and small-molecule profiles have emerged in recent decades as significant approaches for the elucidation of plant molecular biology. Studying plants at the proteomic level identifies aspects of the proteome, or the total complement of proteins, shedding light on the constant and complex state of the functional activity of cells, tissues, and the whole organism. Plant proteomics, a global profiling of proteins on a large scale, serves to understand the nature, interaction, and function of cells, tissues, and the entire organism.[3]

Conversely, metabolomics centers on the large-scale quantification of small molecular weight metabolites, which are the final products of cellular processes and are bioinformatic markers for specific metabolic conditions.[4] The use of these synergistic "omics" technologies has given investigators a broader view of plant systems and has contributed to breakthroughs in several subtopics of plant biology and welfare, such as plant metabolisms, stress response mechanisms, as well as crop enhancement.[5,6]

The present article will give a brief account of the existing sophisticated tools and methods of plant proteomics and metabolomics, along with the recent advancements in the field that have helped to enhance the existing knowledge of plants and the possible potential for future uses in plant proteomics and metabolomics.

Proteomics in Plant Science

Protein Extraction and Separation Techniques

Confidentiality in plant proteomics studies is pegged on the ability to extract and separate proteins from plants' tissues. Some of the classical procedures that were applied to extract plant proteins include phenol extraction and TCA/acetone precipitation.[7,8] However, these techniques can be time-consuming and may not enrich all protein subsets, especially low-abundance or membrane-associated proteins. To overcome these issues, a series of changes in the extraction techniques have been proposed that include the incorporation of detergents, chaotropic agents, and organic solvents to increase the yield of the extracted proteins.[9,10] Moreover, subcellular fractionation procedures, including organelle purification and membrane proteins, are also possible, which allows for the selective analysis of certain proteins within plant cells.[11,12]

After preparation, the plant proteins can be purified by the two-dimensional gel electrophoresis (2D-GE), which classifies the proteins according to both the isoelectric point and molecular weight.[13] They have been described as useful for the identification of proteins that are expressed differentially under given experimental conditions. Despite this, some problems are observed when using 2D-GE, namely the low sensitivity of the method to detect low-concentration proteins and hydrophobic proteins, including membrane proteins.

To overcome these limitations, researchers have lately shifted focus toward liquid chromatography (LC)-based separation techniques, including high-performance liquid chromatography (HPLC), and ultra-HPLC that afford high resolution, sensitivity, and throughput.[14,15] Such techniques, when used in conjunction with mass spectrometry (MS), remain the most popular approaches to the comprehensive analysis of plant proteomes (Table 1).

MS-Based Proteomics

It has come to overshadow other techniques in contemporary plant proteomics thanks to its tremendous sensitivity, selectivity, and, most importantly, the capacity to analyze thousands of plant proteins at once.[16,17] The most commonly used MS-based proteomics approaches in plant research are:

1. Shotgun Proteomics: It is also called "bottom-up" proteomics as protein samples are first proteolytically digested into peptides, then separated by LC and identified by MS.[18] Shotgun proteomics holds the possibility to identify and quantify a high number of proteins in a single experiment, and has been used intensively in plant research.
2. Targeted Proteomics: This approach aims at selectively and accurately measuring specific pre-defined targeted proteins or peptides through methods such as targeted selected reaction monitoring (SRM) and parallel reaction monitoring.[19,20] Targeted proteomics is best applied for the identification and quantification of biomolecular targets of interest, which is essential while validating biomolecules discovered through the discovery approach of proteomics research.
3. Top-Down Proteomics: in this approach, proteins are directly extracted and deposited into the mass spectrometer without being digested.[21] Top-down proteomics can give data about protein changes, varieties, and proteoforms, which might not be achieved from the bottom-up strategy.
4. Quantitative Proteomics: Label-free quantification, stable isotope labeling (SILAC, iTRAQ, TMT, etc.), and data-independent acquisition (DIA) can be considered as the main approaches to quantitative proteomics in order to compare protein expression levels in different samples.[22,23] They have been useful in determining proteins that are up- and down-regulated under different plant treatments or in specific environmental conditions.

Table 1 | Comparison of proteomics techniques in plant science

Technique	Primary Characteristic	Advantages	Limitations
2D-GE	Protein separation by isoelectric point and molecular weight	Useful for differential protein expression	Low sensitivity for low-abundance and hydrophobic proteins
Shotgun Proteomics	Bottom-up approach with protein digestion	High protein identification capacity	Loss of protein-level information
Targeted Proteomics	Selective measurement of specific proteins	High accuracy and quantification	Limited to pre-defined proteins
Top-Down Proteomics	Direct protein analysis without digestion	Captures protein variants and modifications	Lower throughput compared to a bottom-up approach
Quantitative Proteomics	Comparative protein expression analysis	Allows comprehensive protein-level comparisons	Requires advanced computational methods

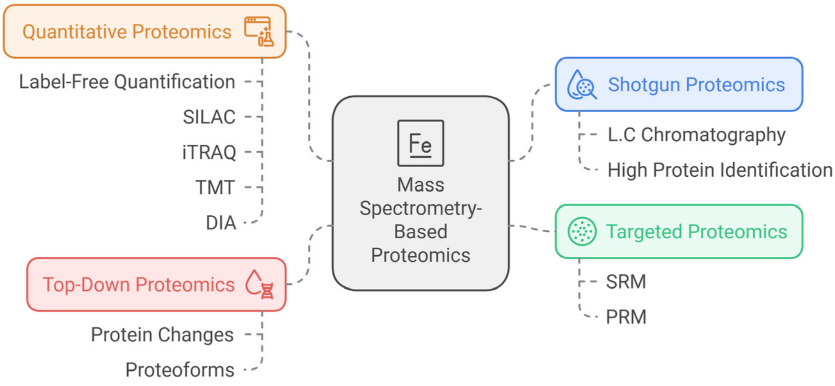

Fig 1 | Mass-spectrometry-based proteomics

As a result of these MS-based proteomics techniques and approaches, there has been a revolutionary change in how plant biologists perceive plants. For instance, proteomics has been invaluable in establishing how proteins are synthesized in plant organs, how plants respond to stress, and metabolic regulation in plants. In addition, proteomics has facilitated the discovery of new proteins and modified proteins that are important for plant function (Figure 1).[24,25]

Bioinformatics and Data Analysis in Plant Proteomics

Proteomic experiments produce massive amounts of data, and therefore, the subsequent processing, analysis, and interpretation of the data necessitate the use of complex bioinformatics tools and computational methods.[26] This includes the following key steps:

1. Protein Identification: The peptide mass spectra derived from the MS analysis are then searched against a protein sequence database, for example, UniProt, to obtain the corresponding proteins.[27] Protein identification in this method is facilitated by search algorithms and scoring systems.
2. Protein Quantification: The analysis of quantitative proteomics can be defined as the comparison between the protein amounts in different samples and under various experimental conditions, which apply statistical procedures and computational methods.[28]
3. Functional Annotation: Described proteins are assigned information regarding their biological roles, cell compartmentalization, and participation in certain pathways or processes, usually in reference to public databases and computational tools.[29]
4. Protein-Protein Interaction and Network Analysis: Computer simulations and modeling are used to forecast and explain protein interactions that may possibly reveal the structure and function of protein assemblies and signaling pathways of plant cells.[30]
5. Integrated Omics Analysis: Proteomics integrated with other omics like genomics, transcriptomics, and metabolomics may provide a deeper understanding of the plant systems and the regulatory processes.[31]

These bioinformatics tools, together with the improvement of the computational methods and the emergence of a great amount of plant genomic and proteomic databases, have played a crucial role in the development of plant proteomics research that allows investigators to explore the challenging molecular basis of plant biology (Figure 2).

Metabolomics in Plant Science

Metabolite Extraction and Separation Techniques

A massive field that qualitatively describes plant physiology with respect to metabolomics is based on the detection and identification of small-molecule metabolites.[32] As in proteomics, extraction and isolation of hp metabolites played a central role in the success of plant metabolomics studies.

Several techniques, including solvent extraction, supercritical fluid extraction, and microwave-assisted extraction, have been used for the extraction of metabolites from plant tissues.[33,34] The selection of the extraction procedure is based on the type of plant sample, the metabolites of interest, and the analytical technique employed for the identification of metabolites.

After extraction, plant metabolites are usually isolated by chromatographic techniques such as GC and LC as well as in combination with MS for identification and quantification of metabolites.[35,36] GC-MS is suitable for analysis of thermally stable volatile metabolites while LC-MS as is a more generalized technique suitable for the analysis of both polar and non-polar metabolites.[37,38] (Table 2).

MS-Based Metabolomics

MS is the dominant technique employed in plant metabolomics, as it provides extremely high sensitivity and selectivity and allows the identification and quantification of numerous metabolites.[39] The most common MS-based approaches in plant metabolomics include:

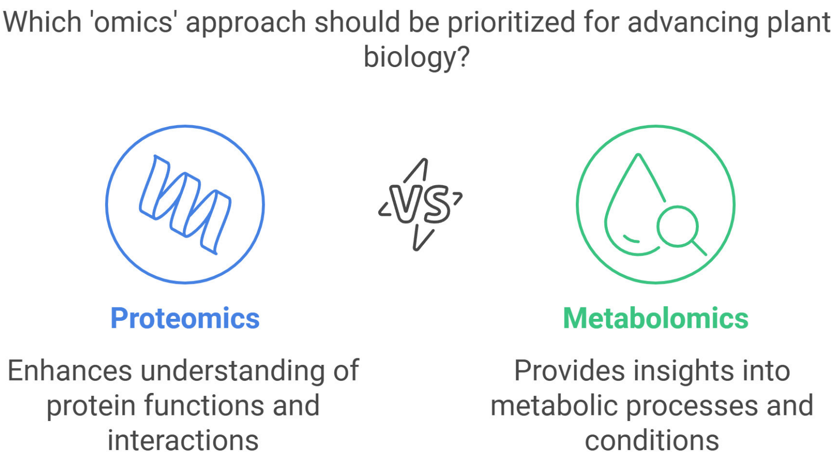

Fig 2 | Which omics approach should be prioritized for advancing plant biology

Table 2 | Comparison of metabolomics techniques in plant science

Technique	Primary Focus	Key Strengths	Analytical Approach
Untargeted Metabolomics	Comprehensive metabolite identification	Discovers unknown metabolites	High-resolution MS with broad screening
Targeted Metabolomics	Specific metabolite quantification	High sensitivity for known metabolites	SRM
Lipidomics	Lipid profiling	Detailed lipid molecular species analysis	LC-MS with high selectivity
Isotope-Labeled Metabolomics	Metabolic pathway tracing	Dynamic metabolic process understanding	Stable isotope precursor tracking

1. Untargeted Metabolomics: This approach of plant metabolomics employs a non-targeted identification and quantification, which seeks to identify and quantify all the metabolites within the plant without prior information regarding target metabolites.[40] Targeted and untargeted metabolomics data can be obtained using high-resolution MS techniques like QTOF or Orbitrap MS, and data-dependent acquisition or DIA spectra are collected and matched against a metabolite database for identification.
2. Targeted Metabolomics: In this approach of study, the work is carried out based on a chosen number of standard or prior known metabolites, and this may employ SRM or multiple reaction monitoring methods on triple quadrupole mass spectrometers.[41] With targeted metabolomics, valuable qualitative and quantitative data on selected metabolites can be obtained with high sensitivity.
3. Lipidomics: Metabolomics is a wider concept in which lipidomics occupies its specific niche; lipids participate in signaling processes in plants as well as in the formation of membranes and energy storage.[77] LC-MS is the most common approach to lipid profiling as it offers the needed high sensitivity in combination with selectivity.[42]
4. Isotope-Labeled Metabolomics: When plants are grown on stable isotope-labeled precursors, like 13C-enriched carbon source, it is possible to analyze the flow of metabolites and to determine the pathways in a plant.[43] In this regard, this approach offers dynamic data on metabolic processes and could shed light on the mechanism of plant metabolic regulation.

Some of the MS-based metabolomics techniques have even enhanced our understanding of plant metabolism signaling and stress response. For instance, using metabolomics, researchers have characterized the metabolites that are essential in the plant defense system and discovered additional secondary metabolites that can be used for medical as well as industrial purposes, establishing the changes that occur in plant metabolism consequent to environmental pressures.

Bioinformatics and Data Analysis in Plant Metabolomics

The data analysis and interpretation in plant metabolomics involve a series of computational and bioinformatics steps, including:

1. Metabolite Identification: The obtained mass spectra are then compared with spectral reference databases or libraries, including METLIN, KEGG, and MassBank, to obtain the related metabolites.[45–47] This process involves correct measurements of mass, fragmentation, and the right data.

2. Metabolite Quantification: Metabolite quantification includes comparing the intensity of specific-, medium- or low-molecular-weight metabolites under various treatment groups or between sample types.[47]
3. Metabolic Pathway and Network Analysis: To identify proposed metabolic relations and regulatory interactions, individual metabolites are fit to metabolic pathways and networks present in plant populations.[48]
4. Multivariate Data Analysis: Non-supervised and supervised statistical analyses, including principal component analysis, partial least square discriminant analysis, and clustering, are used to determine latent features, trends, and discriminative metabolites of plant metabolomes.[49]
5. Integrated Omics Analysis: Using metabolomics data in conjunction with other omics data, including genomics, transcriptomics, and proteomics, helps to unravel the interactions between different molecular layers of plant biology (Figure 3).

These bioinformatics strategies, together with the emergence of novel metabolite databases, analytical methods, and powerful computational platforms, have significantly contributed to the advancement of plant metabolomics studies with regard to the elucidation of the complex regulatory networks and mechanisms underlying plant metabolic processes as well as the responses to biotic and abiotic stresses.

Applications of Plant Proteomics and Metabolomics

As further noted, due to these discoveries that have taken place in proteomics and metabolomics of the plant, there has been a high achievement in a number of areas of plant and crop sciences. Some of the key applications include:

1. Plant Development and Physiology: Functional proteomics and metabolomics have also helped to define the molecular basis of plant growth regulation, development, and the ability to acclimate to the environment, including an understanding of proteins and metabolites produced during seed germination, root formation, and stress in plants.[51,52]
2. Plant Stress Biology: Screening of proteomics and metabolomics has helped to understand multiple pathways and metabolic changes in plants to adapt and overcome biotic (pathogen) and abiotic (drought, salinity, heat) stresses, resulting in the development of biomarkers and regulatory sections.[53,54]
3. Crop Improvement: These omics techniques have been applied to discover features of interest to agriculture, including yield, nutritional quality, and resistance to biotic concerns like pests and pathogens.[55,56]
4. Plant-Microbe Interactions: Both proteomics and metabolomics have been used for the investigation of interconnectivity between the plant system and its microbiota, MIAs, identification of microbial effectors, PTI, and effector-triggered immunity, and metabolic crossover in the multipartite symbiosis.[57,58]
5. Secondary Metabolism and Natural Products: Genomics and proteomics have helped study mechanisms to elaborate and control secondary metabolites with pharmaceutical or industrial value, also known as natural products that can be used in medicine, agriculture, and biotechnology.[59,60]
6. Food and Nutrition: Protein profiling and metabolite profiling have been used to evaluate the quality, safety, and nutritional quality of crop plants and point out changes that happened in plant-derived food products during processing and storage.[61,62]
7. Precision Agriculture: The incorporation of proteomics and metabolomics technologies with other technologies, including remote sensing, precision farming, and big data analytics, has the potential to improve the form of agriculture.[63,64]

The emerging technologies in plant proteomics and metabolomics and their uses, as well as improvements in the tools and algorithms for data analysis, computation, and informatics, have provided strategic positioning of these omics fields as critical endeavors in enhancing our comprehension and utilization of the

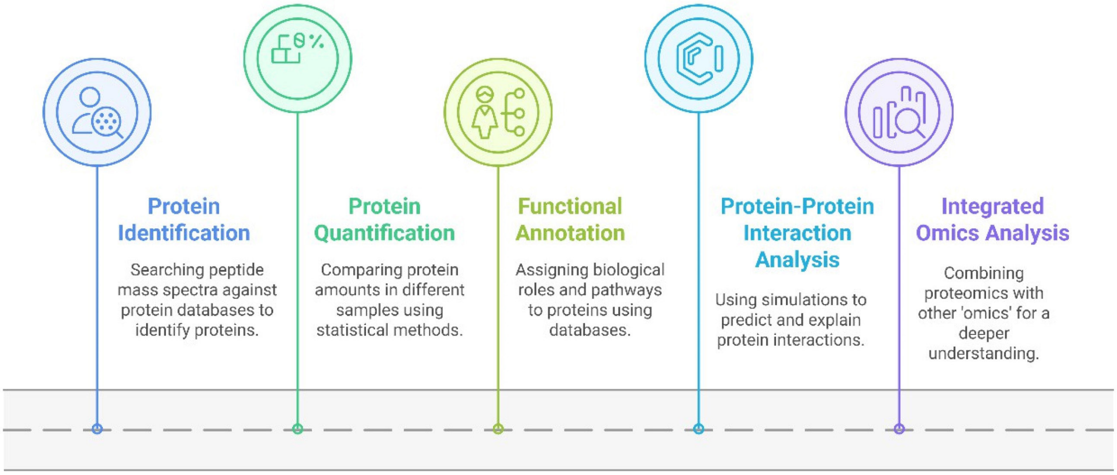

Fig 3 | Bioinformatics and data analysis in plant proteomics

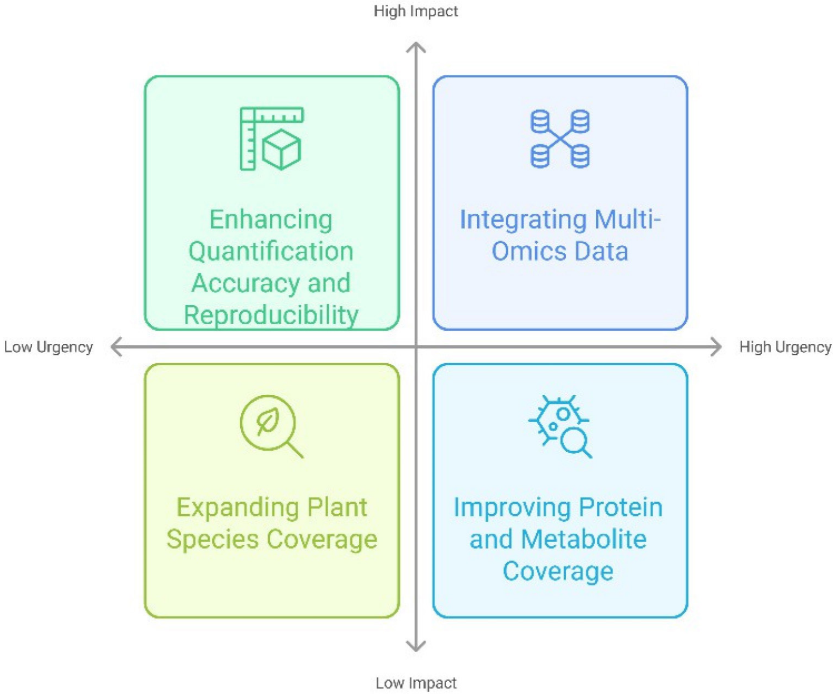

Fig 4 | Prioritizing challenges in plant proteomics and metabolomics

broad aspects of plant biology for the improvement of the well-being or human beings (Figure 4).

Challenges and Future Directions

While plant proteomics and metabolomics have made significant strides, there are still numerous challenges and opportunities for further advancements in these fields:

1. Improving Protein and Metabolite Coverage: In spite of the significant advancements in the developments of analytical tools, the tasks of the identification and quantification of the entire set of proteins and metabolites in plants, called, respectively, proteome and metabolome, is still an unresolved problem, especially in case of low-abundance and membrane-associated proteins; as well as by poorly ionized metabolites.
2. Enhancing Quantification Accuracy and Reproducibility: The identification and relative quantification of proteins and metabolites in one or more samples is often mandatory for a correct biological interpretation. Further advancements in standardization, data normalization, and many other quality control activities are required to counter these challenges.
3. Integrating Multi-Omics Data: Proteomics and metabolomics, besides other omics information like genomics, transcriptomics, and epigenomics, can be trained on plants, allowing a better understanding of the system. There is, therefore, a need to develop improved bioinformatics and computational methods that enable the endeavoring and integrating of these big, big datasets.
4. Advancing Spatial and Temporal Resolution: The use of innovative tools of imaging MS and other high-throughput time series analysis will allow a more comprehensive understanding of how and where the different molecular processes of plants occur and when they are regulated.
5. Translating Findings to Applied Research and Biotechnology: It is still a daunting task to link the basic omics study of plants to solving problems in, for instance, breeding programs or natural product discovery, as well as precision farming, though progress has been made.
6. Enhancing Data Accessibility and Sharing: Plant proteomics and metabolomics databases will be useful in covering extensive information about plants, and standards and guidelines will ensure that data is easily portable and understandable within the plant science community.
7. Expanding Plant Species Coverage: Most proteomics and metabolomics investigations were made with model plants, including the Arabidopsis plant, as well as crops of economic importance. Applying these omics studies toward a broader array of plant taxa, particularly lesser-studied or non-model species, will help the plant science community better understand the extensive plant phylogenetic and genomic diversity that exists in the world today.
8. Artificial Intelligence and Machine Learning Integration: New AI and machine learning tools let scientists study complex proteomics and metabolomics at unprecedented levels to generate better insights into plant science.[65,66]
9. Climate Change Adaptation Research: Plants use proteomics and metabolomics research to study their molecular reactions to fast environmental changes and find ways to adapt genetically into climate-resistant plant types.[67,68]

With the development of plant proteomics and metabolomics, overcoming these challenges and exploiting new potential connected to new technologies and better computations will be important for the higher development of these omics methods in plant science research.

Conclusion

Plant proteomics and metabolomics have become highly essential tools in carrying out current plant science research as they give detailed information on a molecular basis that dictates plant growth, development, and response to stimuli. The development of new methods, such as MS-based technology, in combination with the constant advancements in the field of bioinformatics and computational facilities, has greatly contributed to the advancement of plant biology.

The use of proteomics and metabolomics data alongside other approaches has given researchers better and more interconnected models of plant systems and molecular layers. The use of combinations of omics data has proved very useful in constructing detailed and integrated overviews of plant systems and how distinct molecular layers interact with one another. These systemic views have resulted in the discovery of new proteins, metabolites, and signaling mechanisms that are important for plant resilience and yield.[3]

With the advancement in plant science in the future, the expansion of the plant proteomics and metabolomics facilities and approaches will stay crucial in discussion with the real issues involving the agricultural industry, which includes augmenting yield rates, stress tolerance, and the advancement of sustainable production programs. In addition, "omics" approaches will be key for the identification of wider plant natural products and their biotechnological potential to unlock healing and new industrial and environmental applications.

Moving forward, the combination of plant proteomics and metabolomics with novel approaches and techniques like machine learning and artificial intelligence, along with high-throughput phenotyping, will bring continued progress toward the application of the discoveries made in basic and applied research toward improving plant science and beneficial impact on the quality of human life, food security, and the environment.

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