Lecture 7. Metabolomics and Metabolic Profiling

Aim of the lecture. Expected learning outcomes

To introduce the fundamental principles of metabolomics and metabolic profiling, explain modern analytical methods for metabolite extraction, identification, and quantification, and demonstrate the role of metabolomics in investigating metabolic pathways, physiological responses, and molecular mechanisms in biotechnology.

- **Expected learning outcomes**

1. Explain the fundamental principles of metabolomics and its role in postgenomic research.
2. Distinguish between targeted and untargeted metabolomics and their applications.
3. Describe major analytical technologies, including GC–MS, LC–MS, and NMR spectroscopy.
4. Understand the main stages of metabolomic analysis, from sample preparation to metabolite identification and quantification.
5. Apply basic principles of metabolomic data processing, statistical analysis, and metabolic pathway interpretation.
6. Evaluate the applications of metabolomics in plant biotechnology, stress physiology, crop improvement, and systems biology.
7. Explain how metabolomics can be integrated with genomics, transcriptomics, and proteomics to investigate biological processes.

- Key learning outcome: Understanding how metabolomic technologies characterize cellular metabolic states and contribute to functional interpretation of biological processes.

Plan Lecture

1. Introduction to metabolomics: metabolome and its biological significance
1. Major strategies: targeted and untargeted metabolomics
1. Metabolite extraction and sample preparation
1. Analytical technologies: GC-MS, LC-MS, NMR
1. Metabolite identification and quantitative analysis
1. Metabolomic data processing, statistics, and pathway analysis
1. Metabolomics integration and applications in biotechnology

1. Introduction to metabolomics

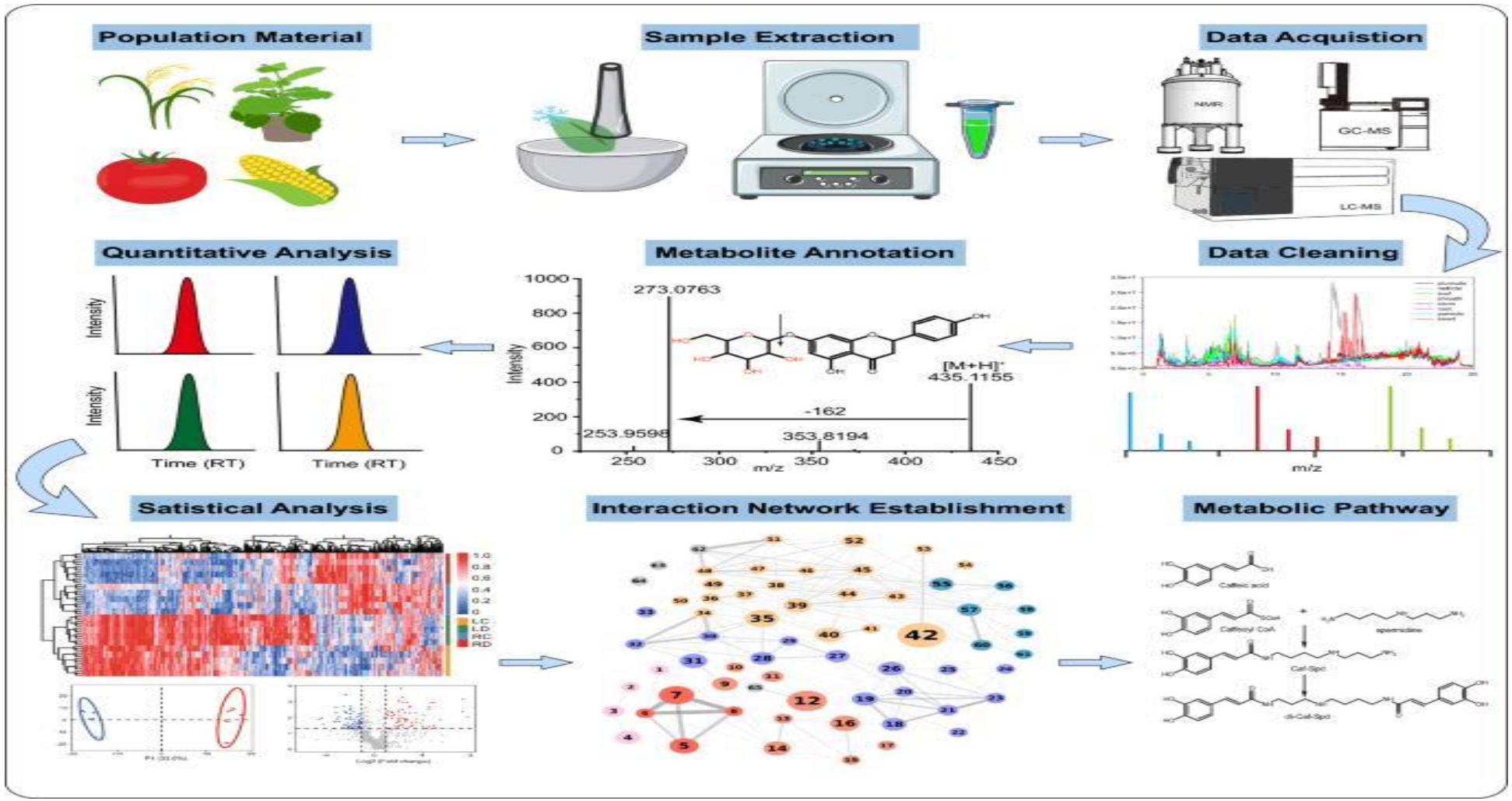
Metabolomics: concept and significance

Metabolomics is the comprehensive study of small molecules (metabolites) present in cells, tissues, organisms, or biological systems under defined conditions.

Metabolomics investigates:

1. Metabolite composition and abundance.
2. Changes in metabolic profiles.
3. Biochemical pathways and metabolic regulation.
4. Responses to developmental and environmental factors.
5. Relationships between metabolism and phenotype.

Key concept: Metabolomics provides a biochemical snapshot of the physiological state of a biological system.


Population Material
Sample Extraction
Data Acquistion
NMR
GC-MS
LC-MS
Quantitative Analysis
Intensity
Time (RT)
Metabolite Annotation
273.0763
[M+H]+
435.1155
-162
253.9596
353.8194
m/z
Data Cleaning
Satistical Analysis
Interaction Network Establishment
Metabolic Pathway

What is the metabolome?

The metabolome is the complete collection of small molecules present in a biological system at a particular time and under specific conditions.

It includes:

1. *Sugars and sugar alcohols.*
2. *Amino acids and organic acids.*
3. *Lipids and fatty acids.*
4. *Nucleotides.*
5. *Plant hormones.*
6. *Phenolic compounds and flavonoids.*
7. *Other primary and specialized metabolites.*

Unlike the genome, the metabolome is highly dynamic and sensitive to environmental conditions.

Position of metabolomics in postgenomic biology

- Genome — genetic information
- Transcriptome — gene expression
- Proteome — proteins and enzymes
- Metabolome — biochemical products and intermediates
- Phenome — physiological and observable traits

These levels are interconnected by regulatory feedback mechanisms, environmental signals, and cellular interactions.

Metabolomics versus other omics

Metabolomics is particularly useful for investigating biochemical consequences of molecular regulation.

Omics	Object of analysis	Main biological question
Genomics	DNA	What is encoded?
Transcriptomics	RNA	Which genes are expressed?
Proteomics	Proteins	Which proteins are present?
Metabolomics	Metabolites	What biochemical changes are occurring?
Phenomics	Phenotypic traits	How are molecular changes manifested?

Main objectives of metabolomics

Metabolomics aims to comprehensively characterize small-molecule metabolites in biological systems and investigate their relationships with cellular functions, physiological processes, and environmental conditions.

Main objectives:

1. Metabolite identification: Detect and characterize metabolites present in cells, tissues, and biological samples.
2. Metabolic profiling: Determine the composition and relative or absolute abundance of metabolites under specific conditions.
3. Comparative metabolomics: Identify metabolic differences between genotypes, developmental stages, treatments, or environmental conditions.
4. Biomarker discovery: Identify metabolites associated with physiological states, stress responses, diseases, or desirable traits.
5. Metabolic pathway analysis: Investigate biochemical pathways and metabolic networks involved in cellular regulation.
6. Functional interpretation: Link changes in metabolite abundance with gene expression, protein activity, and physiological responses.
7. Biotechnological applications: Support crop improvement, metabolic engineering, quality assessment, and optimization of biological production systems.

Example in plant biotechnology: Metabolomic profiling of wheat under drought stress can reveal changes in amino acids, soluble sugars, organic acids, and antioxidant metabolites associated with osmotic adjustment and stress responses.

Key concept: Metabolomics provides a functional overview of cellular metabolism and helps connect molecular regulation with observable biological phenotypes.

Major Metabolomic Analytical Platforms

Metabolomic analysis relies on advanced analytical technologies that enable the separation, identification, and quantification of metabolites in complex biological samples.

Three principal analytical platforms:

1. Gas Chromatography–Mass Spectrometry (GC–MS)

- Separates volatile and thermally stable compounds; many nonvolatile metabolites require chemical derivatization.
- Commonly used to analyze sugars, amino acids, organic acids, and fatty acid derivatives.
- Provides high chromatographic resolution and reproducible metabolite profiling.

2. Liquid Chromatography–Mass Spectrometry (LC–MS)

- Separates metabolites according to their interactions with chromatographic stationary and mobile phases.
- Suitable for a broad range of polar, semipolar, and nonvolatile metabolites.
- Widely applied to phenolics, flavonoids, lipids, phytohormones, and specialized metabolites.
- Offers high sensitivity and supports targeted and untargeted metabolomics.

3. Nuclear Magnetic Resonance (NMR) Spectroscopy

- Detects metabolites based on the magnetic properties of atomic nuclei, commonly hydrogen (^1H).
- Enables identification and quantification of metabolites with minimal sample preparation.
- Provides highly reproducible measurements and structural information.
- Generally has lower sensitivity than mass spectrometry.

Comparison of Metabolomic analytical platforms. Untargeted metabolomics.

Platform	Major strengths	Main limitations
GC–MS	High resolution, established spectral libraries	Derivatization often required
LC–MS	High sensitivity, broad metabolite coverage	Matrix effects and complex data analysis
NMR	Reproducibility, structural information, quantitative analysis	Lower sensitivity
Platform	Major strengths	Main limitations

Untargeted metabolomics aims to detect and compare as many measurable metabolite features as possible without restricting analysis to a predefined compound list.

Characteristics:

1. *Broad metabolic coverage.*
2. *Discovery of unexpected metabolic changes.*
3. *Identification of potential biomarkers.*
4. *Requires extensive data processing and annotation.*

Not every detected feature can be confidently identified as a unique metabolite.

Targeted versus untargeted metabolomics

Important: Both approaches are complementary rather than competing technologies

Feature	Targeted	Untargeted
Objective	Quantify selected metabolites	Broad metabolic discovery
Compounds	Predefined	Not predefined
Coverage	Narrower	Broader
Identification	Usually high confidence	Often requires additional confirmation
Quantification	Often absolute	Frequently relative
Main application	Hypothesis testing	Hypothesis generation

Metabolite extraction and sample preparation

Principles of metabolite extraction

Metabolite extraction aims to recover compounds while minimizing degradation and unwanted metabolic changes.

Main steps:

1. Biological sample collection.
2. Rapid stabilization or quenching of metabolism.
3. Tissue disruption and homogenization.
4. Extraction using suitable solvents.
5. Centrifugation and sample cleanup.
6. Preparation for instrumental analysis.

Extraction solvents and metabolite properties.
Extraction conditions depend on metabolite polarity, stability, sample matrix, and analytical platform.

Solvent system	Typical application
Methanol–water	Many polar and moderately polar metabolites
Acetonitrile–water	Polar metabolite extraction and protein precipitation
Methanol–chloroform–water	Biphasic extraction of polar and nonpolar fractions
Methanol–MTBE–water	Biphasic extraction, particularly useful for lipid-rich samples
Aqueous buffers	Selected targeted assays

Sample preparation for plant metabolomics

Plant tissues contain metabolites with very different chemical properties and concentrations.

Important considerations:

1. Rapid freezing, often using liquid nitrogen.
2. Maintaining consistent sampling time.
3. Controlling developmental stage and tissue type.
4. Preventing enzymatic degradation.
5. Using biological replicates and quality-control samples.
6. Including appropriate internal standards.

Example: Leaf metabolomics under drought requires careful control of sampling time because metabolite levels vary with circadian rhythms and water status

Analytical technologies

Major metabolomic analytical platforms

The three principal technologies are:

1. Gas chromatography–mass spectrometry (GC-MS)
2. Liquid chromatography–mass spectrometry (LC-MS)
3. Nuclear magnetic resonance spectroscopy (NMR)

Each platform detects different groups of metabolites and provides complementary information.

Gas chromatography–mass spectrometry (GC-MS)

GC-MS separates volatile and thermally stable compounds before mass-spectrometric detection.

Many nonvolatile metabolites require chemical derivatization.

Applications:

1. *Sugars.*
2. *Amino acids.*
3. *Organic acids.*
4. *Fatty-acid derivatives.*
5. *Selected primary metabolites.*



Gas chromatography–mass spectrometry (GC-MS) workflow

1. Metabolite extraction
2. Derivatization, when required
3. Gas chromatographic separation
4. Mass spectrometric detection
5. Spectral comparison and metabolite identification

GC-MS is especially valuable for reproducible profiling of many primary metabolites.

Liquid chromatography–mass spectrometry (LC-MS)

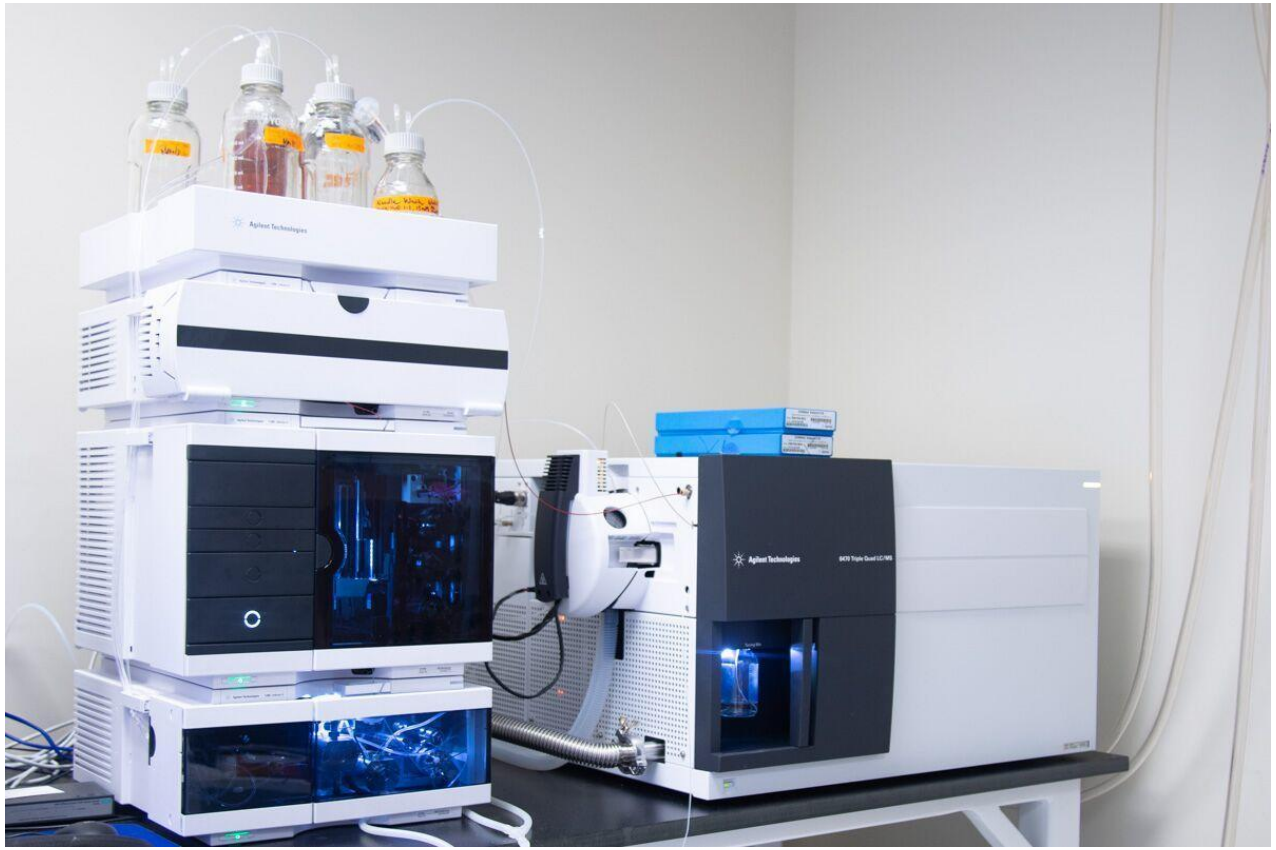
LC-MS combines liquid chromatographic separation with mass-spectrometric detection.

Advantages:

1. *Suitable for many nonvolatile or thermally unstable compounds.*
2. *Broad coverage of plant specialized metabolites.*
3. *High analytical sensitivity.*
4. *Compatible with targeted and untargeted workflows.*

Applications include flavonoids, phenolic acids, lipids, phytohormones, and other metabolites.

Liquid chromatography–mass spectrometry (LC-MS)



LC-MS/MS in metabolite identification

Tandem mass spectrometry provides structural information through fragmentation of selected precursor ions.

Metabolite ion → precursor selection → fragmentation → product-ion spectrum → structural interpretation

MS/MS spectra can be compared with spectral libraries and authentic standards.

However, structurally similar metabolites and isomers may require additional chromatographic or reference-standard evidence.

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectroscopy investigates molecules through the magnetic properties of atomic nuclei.

Advantages:

1. Highly reproducible measurements.
2. Structural information.
3. Minimal sample preparation in many workflows.
4. Potential for absolute quantification.
5. Generally nondestructive analysis.

Limitations:

include lower sensitivity than many MS-based approaches and overlapping signals in complex mixtures.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Metabolite identification and Quantitative metabolomics

Metabolite identification

Metabolite identification uses combinations of:

1. *Accurate mass.*
2. *Isotopic pattern.*
3. *Retention time or retention index.*
4. *MS/MS fragmentation spectra.*
5. *Spectral libraries.*
6. *Authentic chemical standards.*

Identification confidence should be reported appropriately; a database match alone does not necessarily confirm metabolite identity.

Two principal forms of Quantitative metabolomics are:

1. **Absolute quantification**: *reports concentrations or amounts using validated calibration procedures and suitable standards.*
2. **Relative quantification**: *compares normalized signal intensities or abundances between samples.*

- Internal standards, calibration curves, and quality-control procedures improve analytical reliability.

Quality control in metabolomics. Principal component analysis (PCA)

Reliable metabolomics requires:

1. *Biological replication.*
2. *Extraction blanks.*
3. *Pooled quality-control samples.*
4. *Internal standards.*
5. *Randomized injection order.*
6. *Monitoring of instrument stability.*
7. *Evaluation of analytical variation and batch effects.*

PCA is an unsupervised multivariate statistical method used to reduce the dimensionality of complex datasets.

In metabolomics, PCA helps:

1. Visualize similarities and differences between samples.
2. Identify major sources of variation.
3. Detect potential outliers.
4. Evaluate quality-control sample clustering.

- A PCA plot does not, by itself, establish that groups differ significantly.

Metabolomic data analysis
Metabolomic data-processing workflow

Raw chromatograms/spectra

↓

Peak detection

↓

Peak alignment

↓

Feature grouping and annotation



Normalization and quality control



Statistical analysis



Biological interpretation

Differential metabolite analysis

Differential analysis identifies metabolites or features whose abundance differs between experimental groups.

Typical steps:

1. *Calculate abundance differences or fold changes.*
2. *Apply appropriate statistical tests.*
3. *Correct for multiple comparisons.*
4. *Visualize results using heatmaps or volcano plots.*
5. *Interpret changes in biological context.*

Challenges and Future Perspectives in Metabolomics

Metabolomics is rapidly advancing through improvements in analytical technologies, computational methods, and integration with other omics approaches.

Major challenges:

- Metabolome complexity: Large chemical diversity and differences in metabolite concentrations.
- Metabolite identification: Limited availability of authentic standards and reference spectra.
- Analytical variability: Effects of sample preparation, instrument performance, and matrix interference.
- Data processing: Complex requirements for peak detection, normalization, statistical analysis, and metabolite annotation.
- Biological interpretation: Difficulty distinguishing causal metabolic mechanisms from correlated changes.

Emerging research directions:

- Single-cell metabolomics: Investigation of metabolic heterogeneity among individual cells.
- Spatial metabolomics: Mapping metabolite distributions within tissues and organs.
- Metabolic flux analysis: Investigation of metabolic pathway activity using stable isotope tracers.
- AI-assisted metabolomics: Application of machine learning to metabolite identification and biomarker discovery.
- Multi-omics integration: Combining metabolomics with genomics, transcriptomics, proteomics, and phenomics.

Key concept: Advances in analytical sensitivity, computational methods, and multi-omics integration are expanding the role of metabolomics in systems biology and biotechnology.

Conclusion

- Metabolomics provides comprehensive information about small-molecule metabolites and their relationships with cellular functions.
- Targeted and untargeted metabolomics offer complementary strategies for investigating metabolic composition and biological responses.
- GC–MS, LC–MS, and NMR spectroscopy are major analytical platforms used for metabolite identification and quantification.
- Metabolomic workflows include sample preparation, analytical measurement, data processing, statistical analysis, and biological interpretation.
- Metabolic profiling reveals biochemical changes associated with development, environmental stress, and genetic variation.
- Integration with genomics, transcriptomics, and proteomics supports the identification of metabolic pathways and regulatory networks.
- Emerging technologies, including spatial metabolomics and AI-assisted analysis, provide new opportunities for biotechnology.

Overall conclusion: Metabolomics connects molecular regulation with biochemical and physiological phenotypes, providing essential tools for understanding biological systems and improving biotechnological applications.

Control Questions

1. What is metabolomics, and what are its main objectives?
2. How do targeted and untargeted metabolomics differ?
3. What are the major classes of metabolites investigated in metabolomic studies?
4. What are the principles and applications of GC–MS, LC–MS, and NMR spectroscopy?
5. What are the major stages of a metabolomic analysis workflow?
6. Why are metabolite extraction and sample preparation important for reliable analysis?
7. What methods are used for metabolite identification and quantification?
8. How are multivariate statistical methods, including PCA and PLS-DA, applied in metabolomics?
9. How does metabolomic profiling contribute to understanding plant responses to environmental stress?
10. What are the major challenges and future perspectives of metabolomics in biotechnology?

Recommended Literature

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