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Research Advances in MALDI-MSI and Its Applications in *In-situ* Characterization of Endogenous Molecules in Plants

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Abstract This paper provides a systematic review of Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry Imaging (MALDI-MSI), encompassing its technical principles, experimental workflows, matrix optimization strategies, and recent advancements in plant science applications. It highlights the method's groundbreaking applications in spatial mapping of plant metabolites, dynamic hormone monitoring, and functional studies of tissue microdomains, while offering critical insights into current technical limitations and future research directions.

Key words Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry Imaging (MALDI-MSI), Plants, Endogenous molecules, Metabolomics, Research advances

0 Introduction

Since its emergence in the 1990s, Mass Spectrometry Imaging (MSI) has undergone transformative development, evolving from animal tissue analysis to plant tissue applications. Early MSI research predominantly focused on spatial distribution mapping of proteins and small molecule metabolites in tumor tissues. With breakthroughs in instrumental sensitivity and matrix technology, Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) has emerged as a cornerstone technique for deciphering complex metabolic networks in plants. The unique structural features of plant tissues, including cuticles, cell walls, and multilamellar membrane structures, pose significant challenges for conventional metabolomics approaches. MALDI-MSI overcomes these limitations through its non-destructive surface sampling and *in situ* ionization capability, offering novel perspectives for plant physiology, developmental biology, and ecotoxicological research. In recent years, MSI has established itself as an indispensable tool for molecular spatial mapping in biological systems, owing to its exceptional sensitivity (< ppm level), high spatial resolution (cellular to subcellular scale), and label-free detection capacity. As a representative MSI platform, MALDI-MSI enables *in situ* visualization of endogenous metabolites (such as lipids, proteins, carbohydrates, and phytohormones) in plant tissues through its matrix-assisted laser desorption/ionization process, achieving micrometer-level spatial resolution while preserving sample integrity.

1 Technical advantage and scientific value

The unique advantages of MALDI-MSI are reflected in the following aspects: simultaneous detection of hundreds of metabolites without labeling; spatial resolution reaching 5 μm, meeting subcellular-level analytical requirements; broad dynamic range (fmol to pmol levels) suitable for trace plant hormone detection; compatibility with diverse molecular types (lipids, peptides, carbohydrates, *etc.*). In plant research, MALDI-MSI has been successfully employed to elucidate lipid dynamics during leaf development of *Arabidopsis thaliana*, map spatiotemporal distributions of secondary metabolites in rice disease resistance responses, and unravel starch accumulation mechanisms in the aleurone layer of maize kernels, providing critical data support for plant metabolic engineering and molecular breeding strategies.

2 Technical principle and experimental process of MALDI-MSI

2.1 Technical principle MALDI-MSI achieves soft ionization by utilizing matrices (*e.g.*, DHB, CHCA) to absorb laser energy and transfer it to target molecules. Its core mechanisms include: co-crystallization of the matrix with analytes to enhance laser energy utilization; collision-induced dissociation (CID) generating characteristic fragment ions for structural identification; and time-of-flight mass spectrometry (TOF) enabling high mass accuracy (<5 ppm) detection. A typical workflow involves: tissue sectioning → matrix coating → mass spectrometry scanning → data reconstruction, with the entire process conducted under vacuum conditions to ensure efficient ion transmission.

2.2 Detailed explanation of experiment process

2.2.1 Matrix Selection and Optimization. Matrix performance directly influences imaging quality. Traditional organic matrices (*e.g.*, DHB, SA) often generate fragment interference, whereas novel matrices (*e.g.*, HNTP, 2-MBT) significantly reduce background noise by introducing electron-withdrawing groups such as nitro and trifluoromethyl. Optimization strategies include: screening optimal solvent ratios via orthogonal experimental design $L_9(3^4)$; assessing matrix crystallization uniformity using UV absorption spectroscopy; evaluating matrix-assisted laser desorption efficiency through vacuum stability tests. For example, He *et al.* improved the signal-to-noise ratio of lipid imaging in *A. thaliana* leaves by threefold through optimized CHCA concentration (0.5 -

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2.0 mg/mL).

2.2.2 Sample pretreatment. Plant tissues require specialized handling due to their high water content and susceptibility to secondary metabolite degradation. Key procedures include: rapid freezing (liquid nitrogen quenching) to prevent metabolic conversion; cryosectioning (10 – 20 μm thickness) to preserve tissue structural integrity; vacuum drying to eliminate residual moisture. Studies have demonstrated that ITO-coated slides reduce electrostatic interference and improve matrix coating uniformity. For instance, Huang *et al.* [26] achieved *in situ* capture of rice root exudates using ITO-coated slides combined with a matrix sprayer (GET-Sprayer).

2.2.3 Mass spectrometry and data analysis. The MALDI-TOF/TOF mass spectrometer serves as the primary analytical platform, where parameter optimization (*e. g.*, laser frequency at 200 Hz, scanning step size of 100 μm) directly impacts imaging quality. The data processing workflow encompasses mass spectral peak extraction and deconvolution, molecular formula prediction (via database matching such as Mascot), and spatial distribution visualization (using software like BioMap). Notably, high-abundance pigments in plant tissues (*e. g.*, chlorophyll) often induce ion suppression effects, necessitating interference mitigation through chemical derivatization (*e. g.*, methylation) or background signal subtraction.

3 Matrix development and technological innovation

3.1 Limitations of traditional matrix and improvement strategies Traditional matrices (*e. g.*, DHB, SA) exhibit significant limitations in plant research: DHB tends to form needle-like crystals that degrade spatial resolution; SA shows insufficient sensitivity in negative ion mode; uneven co-crystallization between the matrix and metabolites induces ion suppression. To address these issues, researchers have developed multiple improvement strategies, including nanomatrices (*e. g.*, gold nanoparticles, graphene) to enhance laser absorption, hybrid matrices (*e. g.*, DHB + CHCA) to balance sensitivity and selectivity, and surface-assisted laser desorption/ionization (SALDI) to reduce matrix consumption.

3.2 Research and development progress of new matrix In recent years, two categories of novel matrices have garnered significant attention: synthetic organic matrices such as 2-hydroxy-5-nitro-3-trifluoromethylpyridine (HNTP), whose strong electron-withdrawing capacity markedly reduces lipid ion suppression effects, as validated in imaging of wheat caryopsis oil bodies; and biocompatible matrices like 4-hydroxy-3-nitrobenzonitrile (HZN), which demonstrates compatibility with both polar and nonpolar metabolites, successfully applied in volatile organic compound detection in tomato fruits. Table 1 compares the key performance metrics of traditional and novel matrices.

Table 1 Comparison of key performance indicators of traditional and new matrices

Matrix type	Sensitivity//fmol	Spatial resolution//μm	Degree of matrix interference	Applicable molecule type
DHB	10	10	High	Polypeptides, lipids
CHCA	5	5	Intermediate	Proteins, oligosaccharides
HNTP	2	8	Low	Polar metabolites
HZN	8	7	Low	Small molecule metabolites

4 Innovative application of MALDI-MSI in plant science

4.1 Analysis of spatial distribution of metabolites MALDI-MSI has expanded its application in plant metabolomics from single-metabolite imaging to multi-omics joint analysis. For instance, the dynamic distribution of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in *A. thaliana* leaves has elucidated the coupling mechanism between photosynthesis and membrane lipid metabolism; the spatiotemporally specific expression of strigolactones in secretory vesicles of rice roots provides new insights into symbiotic nitrogen fixation research; the localization of starch synthase in the aleurone layer of maize kernels clarifies the carbon metabolic regulatory network during the grain-filling stage.

4.2 *In-situ* detection of plant hormones The spatial resolution of plant hormones (*e. g.*, ABA, JA) was historically constrained by their low abundance (pg/g level) and susceptibility to degradation. Novel matrices such as 2,4-dihydroxy-5-nitrobenzoic acid have significantly improved detection sensitivity by enhancing ionization efficiency and suppressing matrix interference. For example, the spatiotemporal-specific expression of ethylene biosynthesis enzymes (ACS) during cucumber fruit development has unveiled regulatory mechanisms of fruit ripening, while the strong correlation between jasmonic acid accumulation patterns and pathogenesis-related (PR) gene expression in *A. thaliana* disease resistance responses has provided molecular targets for disease-resistant breeding.

4.3 Study on the function of tissue microregion The combination of MALDI-MSI with histological staining (*e. g.*, hematoxylin-eosin staining) enables morphology-function correlation analysis. Representative cases include: metabolic reprogramming of callose deposition in phloem induced by tobacco mosaic virus infection; spatial heterogeneity of proline accumulation in *A. thaliana* root tips under salt stress; and the gradient distribution of cellulose synthase in the elongation zone of cotton fibers, which provides new insights for fiber quality improvement.

5 Technical bottlenecks and future prospects

5.1 Existing challenges Standardization of sample preparation: the high heterogeneity of plant tissues and the absence of unified pre-processing protocols hinder reproducibility; iIntegration of

multimodal imaging: Current MALDI-MSI systems face limitations in real-time combination with fluorescence imaging or Raman spectroscopy; complexity of data analysis: bioinformatic interpretation of massive mass spectrometry datasets remains predominantly dependent on manual annotation.

5.2 Future development directions Intelligent matrix spraying: developing AI-driven matrix spraying robots to achieve nanoscale precision control; *in-situ* metabolic flux analysis: integrating stable isotope labeling (*e. g.*, $^{13}C_6$-glucose) to track carbon metabolic pathways; single-cell resolution breakthroughs: leveraging nanoscale secondary ion mass spectrometry (NanoSIMS) to achieve subcellular metabolic imaging; field-based *in-situ* detection: developing miniaturized MALDI-MSI devices to advance field plant phenomics.

6 Conclusions

MALDI-MSI technology, with its high sensitivity and spatially resolved *in situ* analysis capabilities, is reshaping the paradigm of plant science research. As matrix technology continues to innovate, multimodal imaging platforms become integrated, and artificial intelligence algorithms are deeply applied, MALDI-MSI will play an indispensable role in deciphering plant stress responses, secondary metabolic regulation, and crop molecular design. It is poised to provide core technological support for sustainable agricultural development and the exploitation of biological resources.

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