Review

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Review

Progress in Transcriptomics and Metabolomics in Plant Responses to Abiotic Stresses

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Abstract: Abiotic stress is a significant factor restricting the normal growth and development of plants. Under abiotic stress, plants maintain their life and continuous growth by reconfiguring transcriptional regulation and metabolic networks. In recent years, with the development of sequencing technology and mass spectrometry technology, transcriptomics and metabolomics have emerged as new disciplines following genomics and proteomics, which are conducive to identifying metabolites and regulatory genes in plants under abiotic stress. In this review, we mainly analyzes the technical characteristics, advantages, and disadvantages of transcriptomics and metabolomics, focusing on reviewing the research progress in the field of plant responses to abiotic stress (temperature stress, salt stress, water stress, and heavy metal stress) using transcriptomics and metabolomics both domestically and internationally in recent years. It also provides a prospective view on current issues, which helps accelerate the understanding of the mechanisms of plant responses to abiotic stress and provides new ideas for breeding stress-resistant varieties in the future.

Keywords: plants; abiotic stress; transcriptomics; metabolomics; progress

1. Introduction

With the advent of the omics era, transcriptomics and metabolomics have become important branches of omics in addition to genomics and proteomics, and are integral parts of systems biology [1]. Transcriptomics can reveal the expression profile of the entire genome at the transcriptome level under stress conditions, qualitatively and quantitatively analyze the expression differences of various mRNA genes, and help understand the complex regulatory networks related to plant adaptation and tolerance to stress, as well as screen for functional genes related to plant resistance. It is also essential in mapping transcriptional profiles, searching for polymorphic markers, deeply mining new genes, identifying gene families, reconstructing transcriptional regulatory networks, and revealing specific biological molecular mechanisms [2]. Metabolomics is a discipline that studies the quantitative and qualitative analysis of all metabolites in specific developmental stages, organs, tissues, and cells of organisms. It mainly focuses on low-molecular-weight metabolites with relative molecular masses less than 1000 in metabolic cycles. As a powerful tool for functional genomics and systems biology, metabolomics can not only elucidate gene functions but also unbiasedly identify and quantify all metabolites in biological systems. With the development of mass spectrometry technology, metabolomics can target a wider range of metabolites, providing reliable means for us to study metabolic reprogramming in plants under abiotic stress [3]. Currently, transcriptomics and

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metabolomics have been widely applied in research on stress tolerance in crops such as maize, rice, soybean, and barley [4–7].

Abiotic stress refers to any adverse effects on plants caused by non-biological factors in a specific environment, including extreme temperatures, salinity, drought, flooding, heavy metals, etc. These stress factors often interact or combine with each other. With the continuous and complex changes in the global environment, plants frequently encounter abiotic stress during their growth and development, severely affecting their normal growth, resulting in yield reduction, quality decline, and even plant death [8]. When plants are under abiotic stress, they have evolved mechanisms to sense these environmental challenges, reconfigure transcriptional regulation and metabolic networks to maintain homeostasis within the plant, and adopt new steady states to adapt to the environment for survival and reproduction [9]. In recent years, with the rapid development of multi-omics technologies, especially transcriptomics and metabolomics, they have been widely used to uncover these biological processes. This article combines relevant literature to review the basic research progress of transcriptomics and metabolomics in plant responses to abiotic stress, and looks forward to the application prospects of using multi-omics technologies to study plant stress tolerance, laying a theoretical foundation for future breeding of high-quality and stress-tolerant plant varieties.

2. Transcriptomics

Transcriptomics is a discipline that studies gene transcription and transcriptional regulation in cells at the overall level. It was first proposed by Velculescu in 1997 and first used in a scientific paper [10]. In a broad sense, the transcriptome refers to the total sum of all RNAs in a cell, tissue, or organism under a specific physiological condition or environment, including messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), and no-coding RNA. In a narrow sense, it refers to the total sum of all mRNAs in the cell that participate in protein translation, and the transcriptomics research mentioned is mainly mRNA. Transcriptomics studies the function and structure of genes from the transcriptional level and reveals the molecular mechanisms of specific biological processes. By the end of the 20th century, with the rapid progress and development of science and technology, transcriptomics has become a discipline widely applied in various research fields [11,12].

2.1. Comparative Analysis of Transcriptomics Research Technologies

Based on the different principles of transcriptomics technology, the research technologies used in transcriptomics are mainly divided into two categories: one is hybridization-based technologies, such as microarray technology, and the other is sequencing-based technologies, including expressed sequence tag (EST), serial analysis of gene expression (SAGE), massively parallel signature sequencing (MPSS), RNA sequence technology, and Single cell transcriptome sequencing technology (scRNA-seq). Microarray and EST technologies were developed earlier, while transcriptome sequencing technology is currently developing rapidly. With its advantages of speed, accuracy, high throughput, and low cost, it has been widely used in transcriptomics research and gradually become the main transcriptome sequencing technology. As research progresses, scRNA-seq as an emerging technology can perform qualitative and quantitative analysis of transcriptome activity at the single-cell resolution, revealing the heterogeneity of gene expression between individual cells, which helps gain a deeper understanding of the metabolic networks and regulatory mechanisms of different cell types during development [13]. These technologies each have their unique characteristics, overlapping, and competition with each other, and the emergence of new technologies is constantly driving the further. In practical applications of transcriptomics, the advantages and disadvantages of various technological platforms for transcriptomics can be comprehensively considered, along with the specific needs of the research work, to select a suitable transcriptomics technology for study (Table 1).

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Table 1. Comparative analysis of transcriptomics technologies.

Technology	Theory	Advantage	Limitation
Microassay	Hybrid	1. Fast speed 2. Low cost 3. Simple sample preparation 4. Flexible analysis range	1. The sensitivity for detecting low-expression genes is insufficient 2. The sensitivity of hybridization technology is limited 3. The prerequisite work requires a high level of foundation 4. It is difficult to detect abnormal transcription products
EST	Sanger	1. The detection range is wide 2. The accuracy is high 3. Improve the efficiency of gene isolation	1. The sequencing read length is short 2. The error rate is high 3. The sequencing throughput is low
SAGE	Sanger	1. High-throughput detection 2. It can quantitatively evaluate gene expression levels 3. Gain a comprehensive understanding of gene expression regulation mechanisms	1. High cost 2. Complex data processing 3. Relying on known gene databases, there are certain limitations in identifying unknown genes
MPSS	Sanger	1. High throughput 2. Quantitatively display the expression of genes within cells	1. High cost 2. Complex operation 3. Difficulties in bioinformatics processing
RNA sequencing	High throughput sequencing	1. High throughput 2. High accuracy 3. Wide detection range 4. Low cost	1. The sample preparation is cumbersome. 2. It cannot reveal the heterogeneity of expression among single cells. 3. The bioinformatics analysis tools are limited.
scRNA-seq	High throughput sequencing	1. High accuracy and specificity 2. Clarify cell function and localization	1. High requirements for sample quality 2. High cost 3. Difficulties in data analysis/interpretation

2.2. Application of Transcriptomics in Plant Stress Resistance Research

Abiotic stress can prompt plants to alter their physiological morphology and molecular cellular levels to adapt to adverse living environments. Studying plants' response mechanisms to different abiotic stresses allows for the screening of differentially expressed genes that respond to stress factors, revealing the connection between key functional genes and resistance. Transcriptomics, which studies gene function and structure at the overall level, can rapidly predict defense-related factors for stress responses, which is significant for understanding plant stress resistance mechanisms and improving plant stress resistance. The main research focus of transcriptomics includes transcriptome sequencing and analysis, detection of low-abundance transcripts, discovery of polymorphic markers, in-depth mining of new genes, transcriptome mapping, gene family identification, regulation of alternative splicing, and evolutionary analysis. It has been widely applied in various crop stress resistance studies (Table 2).

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Table 2. The list of key genes of plants in response to abiotic stresses.

Gene	Crops	Abiotic stress	Gene Function	References
OsWRKY87	Rice	Drought, salt stress	*OsWRKY87* functions as a transcriptional activator	[14]
OsSEH1	Rice	Cold stress	*OsSEH1* regulates the expression and metabolite accumulation of genes related to phenylpropanoid and flavonoid biosynthesis, mediating ABA expression levels in response to cold stress	[15]
OsCSLD4	Rice	Salt stress	*OsCSLD4*, a cell wall polysaccharide synthase, responds to salt stress through ABA-induced osmotic stress	[16]
OsNAC5	Rice	Drought stress, cold stress	It enhances stress tolerance by upregulating the expression of *OsLEA3* gene	[17]
ZmHsf01	Maize	Heat stress	Plays an important role in heat shock signal transduction and downstream gene expression	[18]
ZmNAC3	Maize	Cold stress, salt stress	*ZmNAC3* encodes a nuclear-targeted protein with a highly conserved NAC domain at its N-terminus	[19]
ZmICE1	Maize	Cold stress	*ZmICE1* regulates the expression of *DREB1* gene, inhibits the expression of *ZmAS*, reduces Glu/Asn biosynthesis, thus alleviating the production of reactive oxygen species	[20]
TdSHN1	Wheat	Heavy metal stress	Enhances cadmium tolerance by increasing the activity of superoxide dismutase and catalase	[21]
ZmCAO1	Maize	Waterlogging stress	Mutation of *ZmCAO1* leads to downregulation of key photosynthetic genes, increased reactive oxygen species, and sensitivity to waterlogging	[22]

3. Metabolomics

Metabolomics originated from the term "metabonomics" proposed by British scientist Nicholson and German scientist Fiehn. It is defined as a science that conducts qualitative and quantitative analysis of all low-molecular-weight (generally referring to molecular weights <1000) metabolites in a certain organism, tissue, or cell. It is one of the essential components of systems biology [23]. Compared to other omics, metabolomics reflects the actual events occurring in cells under specific conditions, which are the result of the combined effects of genes and environmental factors within the organism. It is closer to the phenotype of organisms, and minor changes in the genome and proteome can be reflected and amplified at the metabolomic level [24]. Meanwhile, the greatest advantage of metabolomics is that it can be applied to any species without prior knowledge of its biochemical or genetic composition, and it has been widely used in plant metabolite research [25,26].

3.1. Comparative Analysis of Metabolomics Research Techniques

Currently, the commonly used detection techniques in metabolomics mainly include nuclear magnetic resonance (NMR) technology, gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and capillary electrophoresis-mass spectrometry (CE-MS). NMR is one of the earliest techniques applied in metabolomics research. It is a spectroscopic technique based on the principle of nuclear energy absorption and re-emission caused by changes in external magnetic fields. Its disadvantage lies in its low sensitivity and simple sample processing, mainly used for the analysis of metabolite structures [27]. GC-MS technology is mainly used to analyze thermally stable, volatile, and gasifiable small-molecule metabolites, with high resolution and sensitivity. Samples require derivatization and are mainly used to detect volatile substances [28]. However, LC-MS technology does not require volatility or thermal stability of the detected

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substances, generally does not require complex pre-treatment such as derivatization, and has high resolution and sensitivity, mainly used for the detection of non-volatile substances [29]. CE-MS technology achieves the separation of metabolites based on the difference in the migration speed of charged molecules in an electric field, combining the advantages of capillary electrophoresis and mass spectrometry analysis. It is widely used in the separation and analysis of strongly polar metabolites, especially charged metabolites [30]. Different detection technology platforms have their advantages and disadvantages in terms of detection objects, sensitivity, separation efficiency, analysis speed, and accuracy. A comparative analysis of the advantages and disadvantages of metabolomics research techniques is shown in Table 3.

Table 3. Comparative analysis of metabolomics technologies.

Technology	Targets	Advantage	Limitation
NMR	Most compounds in metabolites	1. Small sample size required 2. No sample preprocessing required 3. Accurate provision of metabolite structure information	1. Low detection sensitivity and resolution 2. Difficult to detect low-abundance metabolites 3. High requirements for sample preparation
GC-MS	Volatile, gasifiable, or small molecules	1. High resolution and sensitivity 2. Ability to identify metabolite structures 3. Easy qualitative analysis of metabolites	1. Unable to separate macromolecules 2. Cannot analyze thermally unstable and non-gasifiable substances 3. Complex and time-consuming derivatization preprocessing procedures
LC-MS	High boiling point, non-volatile, non-derivatizable, macromolecules	1. High detection sensitivity 2. Fast analysis speed 3. Ability to separate metabolites with similar structures	1. Limited database size 2. Limited types of metabolites analyzed 3. Not all metabolites can be accommodated by the same column material
CE-MS	Trace, complex samples	1. High detection sensitivity 2. Fast analysis speed 3. Small sample size required 4. Wide coverage of metabolites	1. High requirements for equipment and devices 2. Small sample size, poor reproducibility of separation 3. Narrow linear range for quantitative analysis 4. Limited quantitative analysis due to the narrow linear range

3.2. Application of Metabolomics in Plant Stress Resistance

In nature, plant metabolites are considered the ultimate products of gene and protein activity, divided into primary metabolites and secondary metabolites. Primary metabolites are mainly involved in plant growth and development, while secondary metabolites primarily participate in plant signal transduction, plant-environment interactions, and plant defense. They also play an extensive role in abiotic stress processes. The changes in metabolites under abiotic stress are the result of the combined effects of genetic and environmental factors, directly reflecting the physiological phenotype and biochemical levels within the organism. Studying and analyzing the accumulation patterns of metabolites in different species, different tissues of the same species under various abiotic

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stresses provides a reference for discovering plant-specific accumulated metabolites and stress-resistant marker metabolites that respond to stress. Currently, metabolomics has been widely applied to understanding plants' responses to abiotic stress, from the perspective of elucidating the regulatory mechanisms of plants against abiotic stress (Table 4).

Table 4. The list of metabolites of plants in response to abiotic stresses.

Abiotic Stress	Crops	Metabolite	Change	References
Heat stress	Maize	Tryptophan, Threonine, Histidine, Raffinose, Galactitol, Lactitol	Upregulated	[31]
Heat stress	Wheat	N-based Amino Acids, ABA, IAA-conjugates	Upregulated	[32]
Cold stress	Maize	Guanosine 30, 50-Cyclic Monophosphate, Sophoroside-7-O-Glucoside, L-Lysine, L-Phenylalanine, L-Glutamine, Shanenol, Feruloyl Tartaric Acid	Upregulated	[33]
Cold stress	Maize	Trans-aconitate, Coumaroyl Hydroxycitrate, Geranyl Glucosyl Rhamnoside Rhamnoside, Caffeoylquinate, Ferroylquinate, (Iso)Vitexin, DIBOA-Glucoside	Upregulated	[34]
Cold stress	Maize	Chlorophyll, Glucose-6-Phosphate Dehydrogenase, Sucrose to Starch Ratio	Upregulated	[35]
Cold stress	Canola seed	Amino Acids, Organic Acids, Sugars	Upregulated	[36]
Drought stress	Wheat	1-Aminocyclopropane-1-Carboxylic Acid, Asn, 5-HT, GABA, Cystine, Deoxyuridine, Tryptamine, Putrescine	Upregulated	[37]
Drought stress	Mulberry tree	Galactolipids, Phospholipids, Flavonoids, Cinnamic Acid, Amino Acids, Carbohydrates, Benzenoids, Organic Heterocyclic Compounds	Upregulated	[38]
Drought stress	Barley	Amino Acids, Sugars, Abscisic Acid, Jasmonic Acid, Ferulic Acid	Upregulated	[39]
Salt stress	Barley	Aminoacyl-tRNA Biosynthesis, Glycine, Serine, and Threonine Metabolism, Glyoxylate and Dicarboxylate Metabolism, Porphyrin and Chlorophyll Metabolism	Upregulated	[40]
Salt stress	Wheat	Amino Acids and Derivatives, Flavonoid Compounds, Organic Acids and Derivatives, Nucleotides and Derivatives, Lipids	Upregulated	[41]
Salt stress	Blueberry	Glycine, Malic Acid, Octadecanoic Acid, L-Threonic Acid	Upregulated	[42]
Heavy metal stress	Rice	Lipids, Eicosanoids	Upregulated	[43]
Heavy metal stress	Purple sweet potato	Glutathione, Tryptophan	Upregulated	[44]

4. Studies on Transcriptomics and Metabolomics of Plant Responses to Different Abiotic Stresses

Abiotic stress factors such as high temperature, cold injury, drought, flooding, salinity, and heavy metal stress are ubiquitous in nature, and these adverse environments have severely constrained plant growth and development, affecting crop yield and quality [45]. In response to abiotic stress, plants develop an active defense mechanism to resist the adverse environmental effects, involving a series of complex reaction mechanisms involving multiple genes, signaling pathways,

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and metabolic processes [46]. Transcriptomics can reveal differential gene expression and complex regulatory networks under different conditions, but it is difficult to accurately identify key genes, pathways, and regulatory genes. Meanwhile, metabolites, as the final products of gene transcription and protein modification, can be revealed by metabolomics to elucidate the changes in organisms under genetic or environmental influences. However, due to the vast and complex nature of plant metabolites, metabolomics data can only cover a portion of the plant metabolome. Therefore, combining transcriptomics and metabolomics can comprehensively achieve a "cause-to-effect" approach, simultaneously exploring various biological issues in plants from two directions and validating each other, contributing to a systematic and comprehensive understanding of plant information transmission processes and functional formation mechanisms. In recent years, the combined analysis of transcriptomics and metabolomics has played an important role in identifying key genes and metabolites involved in plant responses to abiotic stress, exploring the molecular mechanisms of plant growth, development, and stress responses.

4.1. Studies on Transcriptomics and Metabolomics of Plant Responses to Temperature Stress

Temperature is one of the critical environmental factors for plant growth and development, and only under suitable temperature conditions can plants normally conduct material transport and energy exchange. When plants are subjected to temperature stress, it can cause a series of physiological changes, leading to irreversible damage to cellular homeostasis, degradation of functional proteins, disruption of metabolic pathways, affecting crop quality and yield, and even causing plant death.

4.1.1. Heat Stress

Global warming has increased the frequency of extreme high temperatures, and heat stress is a major abiotic stress factor limiting plant growth and yield. Under heat stress, plants primarily respond by regulating antioxidant substances, osmolytes, heat shock proteins, starch, amino acids, protein structure, and other aspects [47]. Wang et al. used transcriptome and metabolome analysis to study the response mechanism of sweet corn to heat stress, revealing that both phenylpropanoid compounds and photosynthesis-related pathways were affected by heat stress. Additionally, the expression levels of various alkaloids and flavonoid compounds were upregulated, with RNA-dependent RNA polymerase 2, UDP-glucosyltransferase 73C5, *LOC103633555*, and tetrachloride interactive domain 7 identified as four hub genes under heat stress [48]. Hu et al. performed transcriptome and metabolome analysis on two tall fescue varieties with different temperature sensitivities, finding that the transcriptional abundance of 12 enzyme-related genes in the carbohydrate metabolic pathway increased significantly under heat stress, and the contents of sugars and lipids accumulated with increasing heat treatment time [49]. Wang conducted transcriptome and metabolome analysis on two pepper varieties with different heat sensitivities, identifying 5754 and 5756 differentially expressed genes in heat-tolerant and heat-sensitive varieties, respectively, along with 94 and 108 differentially accumulated metabolites. Specifically, changes in amino acids, organic acids, flavonoid compounds, and sugars emphasized the complex reaction mechanisms involved in pepper heat tolerance, and glutathione metabolic pathways also played a crucial role in pepper's response to heat stress [50]. Guo et al. investigated the development and starch deposition changes in waxy corn kernels after pollination under different temperature treatments. Through transcriptome and metabolome analysis, they found that IAA, ABA, and SA signaling pathways underwent significant changes during the heat stability response, and the signaling pathways mediated by coenzymes, abscisic acid, and salicylic acid were more active in response to heat stress. These hormones play an important role in grain development [51]. Xiang et al. utilized combined transcriptome and metabolome analysis to show that high temperature stress significantly reduced the contents of zeatin, salicylic acid, jasmonic acid, and auxin. The upregulation of ARR-B genes enhanced zeatin signaling transduction, enhancing maize heat tolerance [52].

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4.1.2. Cold Stress

Cold stress can lead to frost damage, not only limiting plant growth and development, affecting crop quality and yield, but also affecting plant species distribution. Cold stress triggers changes in plant hormone signaling, photosynthesis, osmolytes, synthesis and metabolic pathways, and related gene expression to respond to the adverse environment [53]. Guo et al. studied the response mechanism of maize to cold stress and found that the content of endogenous ABA increased under cold stress, indicating that ABA, The genes identified could potentially become the target for cultivating cold-resistant waxy corn [54]. Xu et al. conducted transcriptome sequencing and metabolomic analysis on tobacco under low-temperature stress treatment, identifying 6,905 differentially expressed genes (DEGs) that are primarily involved in signal transduction, carbohydrate metabolism, and phenylpropanoid biosynthesis. Additionally, 35 protective metabolites were detected, participating in the cold stress response process, including amino acids, carbohydrates, intermediates of the tricarboxylic acid (TCA) cycle, and phenylpropanoid-related substances [55]. Li et al. compared the physiological activities of pumpkin inbred lines under different temperature conditions. Transcriptome and metabolome analyses showed that cold stress significantly increased the contents of malondialdehyde, relative conductivity, soluble proteins, sugar content, and catalase activity. Moreover, the transcription of genes involved in plant hormone signal transduction pathways was activated, along with transcription factor families such as AP2/ERF, bHLH, WRKY, MYB, and HSF [56].

4.2. Transcriptomic and Metabolomic Studies of Plant Responses to Water Stress

Water is an essential environmental factor for plant life activities, and water availability is the primary factor affecting plant growth, yield, and quality formation. Water deficiency leads to slow plant development, significantly inhibiting seed germination and seedling growth, and in severe cases, can cause significant crop yield reductions. Excessive water leads to a reduction in plant root activity, preventing normal respiration, while under hypoxic stress, plant photosynthesis decreases, severely inhibiting related physiological metabolism and growth, ultimately leading to cell death [57]. Research shows that plants can adapt to adverse living environments by changing their physicochemical properties under water stress. The main molecular mechanism of plant response to water stress is through the perception and transmission of stress signals by cells to regulate different metabolic pathways and signal transduction pathways, making responses at the transcriptional and translational levels, thus altering the expression of corresponding genes [58].

4.2.1. Drought Stress

Drought is one of the most significant factors limiting plant production and threatening food security [82]. Drought stress causes many physiological changes in plants. Under drought stress, crops maintain the osmotic pressure balance inside and outside cells through small molecules such as sugars and amino acids, thereby responding to drought stress and controlling plant resistance and growth [59]. Mathan et al. found that drought stress increased the sucrose content in leaves, root tissues, and phloem sap of *Oryza sativa* L. varieties, maintaining sugar balance within rice plants to cope with drought stress [60]. Li et al. used a combined transcriptome and metabolome analysis to show that drought stress reduced the activity of sucrose synthase genes *sus1* and *SH-1*, inhibiting sucrose conversion to UDP-glucose, resulting in decreased pollen vitality [61]. Wei et al. performed a transcriptome analysis on two drought-tolerant maize lines and two drought-sensitive lines, identifying a drought-resistant gene *ZmbHLH124*, which can activate the transcription of the drought-related *ZmDREB2A* gene, thereby enhancing maize drought tolerance [62]. Ackah et al. conducted a metabolome analysis on mulberry Yu-711 under drought stress treatment, finding significant changes in the levels of total lipids, galactolipids, and phospholipids, accounting for 48% of the total differentially expressed metabolites. Among them, fatty acyl-based substances were significantly reduced by 73.6%. Additionally, other metabolites, including polyphenols (flavonoids and cinnamic acid), organic acids (amino acids), carbohydrates, phenyl compounds, and organic heterocyclic

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substances, also showed dynamic response trends to drought stress, revealing that mulberry responds to drought stress through differential accumulation of metabolites [38]. Wang et al. conducted transcriptome sequencing analysis on drought-stressed sweet sorghum seedlings, identifying 40 drought stress-responsive genes. GO enrichment analysis and KEGG analysis of differentially expressed genes indicated that endoplasmic reticulum processing and spliceosome metabolic pathways are related to drought stress responses. Sweet sorghum enhances osmotic adjustment ability by activating drought stress-related proteins and carbohydrate-related gene expression to further respond to drought stress. Li et al. subjected two maize inbred lines, si287 (drought-tolerant) and X178 (drought-sensitive), to drought stress treatment. Using transcriptome and metabolome analysis techniques, they found significant differences in pathways related to glycolysis/gluconeogenesis, flavonoid biosynthesis, starch and sucrose metabolism, and amino acid biosynthesis. Furthermore, proline, tryptophan, and phenylalanine are key amino acids for maize to cope with drought stress [63]. Additionally, ABA is the hormone that has the greatest impact on drought stress. Plants can accumulate large amounts of ABA in their bodies, triggering ABA signal transduction and inducing the expression of stress-related genes, thereby enhancing plant drought tolerance [64]. Xiong et al. conducted a transcriptome analysis on soybean plants under drought stress, revealing that 26 *GmPP2A-B00* members within the soybean *PP2A-B00* family genes responded to drought. Since Protein Phosphatase 2A plays a crucial role in regulating cellular ROS signaling, the results indicated that GmPP2A-B genes can promote drought resistance in soybeans by regulating ROS signal transduction [65].

4.2.2. Waterlogging Stress

Waterlogging stress has become a major abiotic stress affecting crop growth, development, and yield, affecting about 12% of the global land area and causing up to 20% crop yield loss. The most direct impact of waterlogging stress on plants is the impairment of aerobic respiration, limiting a series of processes such as energy metabolism, growth and development, and physiological metabolism [66]. Plants adapt to adverse environments by regulating morphological structure, energy metabolism, endogenous hormone biosynthesis, and signal transduction [67]. Under waterlogging stress, oxygen supply in the soil is greatly limited, and hypoxia stimulates and exacerbates the accumulation of reactive oxygen species (ROS), ultimately leading to cell death and plant senescence. To cope with oxidative stress, plants develop an antioxidant defense system to maintain the dynamic balance of ROS in the body, mitigate ROS-induced cellular damage, and promote cell survival [68]. Luan et al. compared the waterlogging tolerance between barley varieties Franklin and TX9425 and discovered 124 differentially expressed genes (DEGs) involved in ROS scavenging through transcriptome analysis. These DEGs were involved in the synthesis of glutathione S-transferase, peroxidase, catalase, and L-ascorbate peroxidase, most of which were related to peroxidase. The results also revealed that alcohol dehydrogenase plays an important role in coping with waterlogging stress [69]. Feng et al. compared the waterlogging tolerance of two sweet corn varieties D120 and D81. The transcriptome results showed that 2492 and 2351 differentially expressed genes were identified under waterlogging stress, respectively. In the waterlogging-tolerant genotype D120, genes involved in ROS balance were significantly expressed, enhancing ROS scavenging ability. In addition, the *ZmERF055* gene was identified on chromosome 9, which can enhance plant waterlogging tolerance and maintain ROS balance [70]. Hong et al. subjected two rapeseed inbred lines G230 and G218 to waterlogging stress and conducted a combined transcriptome and metabolome analysis. The results showed that the differentially expressed genes and metabolites were mainly enriched in metabolic pathways, biosynthesis of secondary metabolites, biosynthesis of flavonoids, and vitamin B6 metabolism [71]. Multiple studies have shown that plant hormones also play a key role in responding to waterlogging stress. Ethylene is crucial in the adaptation of plants to hypoxia and metabolic adaptation during flooding, and it can adapt to hypoxic environments by enhancing the stability of the ethylene response factor family VII (ERF-VIIs) [72,73]. For instance, Yuan Cheng conducted a transcriptome and metabolome analysis on winter wheat grains under flooding stress. The results indicated that improving crop waterlogging

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tolerance was achieved by regulating the expression of ethylene, abscisic acid, and jasmonic acid-related synthesis genes. Pathways such as the ascorbate-glutathione cycle and sugar metabolism jointly resist flooding stress. The study by Sreeratree et al. also showed that ethylene and jasmonic acid play an important role in enhancing waterlogging tolerance [74]. Wu et al. subjected CM37 and cmh15 seedlings to waterlogging stress and found through transcriptome sequencing analysis that differentially expressed genes were mainly enriched in photosynthesis, photosystem pathways, and glycolysis/gluconeogenesis pathways [75]. Owusu et al. used transcriptome analysis to identify several key genes for cotton waterlogging tolerance, including *PER1*, *PRX52*, *PER64*, *ADH*, *PDC*, *MT1*, *XTH*, and *SUS*. These genes are related to the antioxidant system, and transcription factors (WRKY, AP2/ERF, and MYB) also play a crucial role in the waterlogging tolerance mechanism. Metabolome analysis revealed that waterlogging stress significantly induced pathways such as phenylpropanoid biosynthesis, galacturonate synthesis, valine, leucine, and isoleucine biosynthesis, purine metabolism, and galactose metabolism. The accumulation of these amino acids has a significant effect on enhancing plant waterlogging tolerance [76]. In summary, the regulatory mechanisms of plants responding to waterlogging stress are highly complex. Integrating transcriptome and metabolome analysis provides a powerful and efficient method for identifying waterlogging tolerance genes and exploring related metabolites.

4.3. Research on Transcriptomics and Metabolomics of Plant Responses to Heavy Metal Stress

Heavy metal stress is one of the most destructive abiotic stresses, significantly affecting the growth, development, yield, and quality of crops. It poses a major challenge to sustainable agricultural development. Heavy metals refer to a group of biologically toxic metals and metalloids, such as cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), etc. Even in small amounts, they can cause physiological and morphological disorders in plants, exerting adverse effects on plant growth through osmotic stress, ion imbalance, oxidative stress, membrane disorders, cell toxicity, and metabolic disturbances, and even leading to plant death in extreme cases [77].

Studying plant responses to heavy metal stress through transcriptomics and metabolomics is conducive to comprehensively analyzing the complex regulatory network associated with heavy metal stress response, and serves as an important means for screening and breeding heavy metal-tolerant plants. Zhou et al. investigated the response mechanism of wheat under cadmium stress and discovered 1561 differentially expressed genes (DEGs) in L17 and 297 DEGs in H17 through transcriptome analysis. These genes are primarily involved in terpenoid backbone biosynthesis, phenylalanine metabolism, photosynthesis, ABC transporters, and glutathione metabolism in response to cadmium stress [78]. Dubey et al. identified 1138 upregulated genes and 1610 downregulated genes in rice roots under chromium stress. Most of the genes expressing differently under the two chromium stress conditions are related to glutathione metabolism, transport, and signal transduction pathways, indicating that glutathione plays a crucial role in the detoxification process during chromium stress [79]. Mwamba et al. employed metabolomics techniques to examine changes in metabolite contents in Brassica napus under different cadmium stress conditions. They found that lignin was highly expressed in high cadmium-accumulating Brassica napus, suggesting that lignin can hinder cadmium entry into plants. Plant sterols, monoterpenes, and carotenoids were also induced by cadmium. In cadmium-tolerant Brassica napus, unsaturated fatty acids, lipoproteins, and glycerophospholipids accumulated significantly, and inositol-derived signaling metabolites were induced. These changes can rapidly trigger detoxification mechanisms in plants [80]. Lai et al. used metabolomics analysis to show that under the mutual induction of uranium and cadmium, the expression of antioxidant substances in purple sweet potato cells increased significantly, thereby enhancing the plant's tolerance to heavy metals [81]. Wei et al. employed metabolomics and transcriptome sequencing to study the metabolic and transcriptional response mechanisms of Kochia scoparia to cadmium stress. Cadmium stress affected the accumulation and transport of cadmium in plants, increased the content of soluble sugars, enhanced the activities of ascorbate peroxidase and peroxidase, and reduced the activity of superoxide dismutase, thereby affecting plant growth and development. Glutathione metabolism and lignin biosynthesis were the key metabolic pathways [82].

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These studies suggest that analyzing and comparing the differences in transcription levels between tolerant and sensitive genotypes under heavy metal stress from the perspective of transcriptomics can identify stress tolerance-related genes. Meanwhile, plant responses to heavy metal stress from the perspective of metabolomics mainly involve reducing heavy metal absorption, heavy metal chelation, antioxidant defense, and scavenging free radicals [83].

4.4. Research on Transcriptomics and Metabolomics of Plant Response to Salt Stress

Soil salinity stress poses a significant limitation to stable yield and income of crops, posing a huge threat to agricultural production and being one of the major abiotic stresses faced by plants [84]. High salt stress can cause osmotic stress in plants, leading to water deficiency, physiological imbalance, and metabolic obstruction [85]. At the same time, it can cause ion toxicity, excessive accumulation of reactive oxygen species (ROS) in the body, resulting in oxidative damage to organelles and membrane components, direct damage to proteins and photosynthesis, impairing plant growth, and ultimately leading to plant death. To reduce the harmful effects of salt stress on plants, plants can enhance their tolerance to salt stress through osmotic adjustment, ion balance, scavenging of ROS, and hormonal regulation [86].

The use of transcriptomics and metabolomics to study the response mechanisms of crops to salt stress and to identify key genes and metabolites regulating biological processes has been widely applied in plants such as maize, rice, barley, soybean, and tobacco. Xu et al. studied the adaptive response of oat cultivars to salt stress. Metabolomic analysis revealed 201 metabolites, including sugars, amino acids, organic acids, and secondary metabolites. Salt stress interfered with the biosynthesis, energy consumption, and sugar metabolism of BY2 and BY5. The different defense abilities of these two oat cultivars to salt stress were due to their differences in energy consumption strategies, energy material synthesis, and root ion transport [87]. Lu et al. performed transcriptomic and metabolomic analysis on grapevines under saline-alkali stress. Saline-alkali stress induced signal transduction and metabolic processes, with significant increases in the content of ascorbic acid, glutathione, most phenolic acids, flavonoids, and alkaloids. The biosynthetic pathway of flavonoids plays a crucial role in grape's response to salt stress. The results showed that plant response to saline-alkali stress is closely related to the ability of the antioxidant system to scavenge ROS [88]. Han et al. performed metabolomic and transcriptomic analysis on cotton under salt stress treatment, revealing that salt stress increased the content of amino acids, sugars, and ABA, while reducing the content of vitamins and terpene compounds. Among them, the accumulation of cysteine, ABA, isopentenyl adenine-7-N-glucoside, and tulipose is crucial for cotton's salt tolerance mechanism [89]. Jin et al. studied the salt tolerance mechanism of three soybean cultivars (JD19, LH3, and LD2) with different salt tolerance. Transcriptomic analysis showed that compared with LD2, salt stress increased antioxidant metabolism, stress response metabolism, glycine metabolism, serine metabolism, and gene expression related to transcription and translation in JD19 and LH3. Metabolomic analysis revealed that amino acid metabolism and the TCA cycle are important metabolic pathways for soybean to cope with salt stress [90]. Shu et al. studied the mechanism of Brassica napus response to salt stress, indicating that abscisic acid and jasmonic acid are key factors in the process of responding to salt stress. Meanwhile, some metabolites, such as N-acetyl-5-hydroxytryptamine, L-cysteine, and L-(+)-arginine, play a crucial role in maintaining ROS balance [91]. These results indicate that salt stress can induce the establishment of plant defense signaling networks by affecting the homeostasis of plant hormones.

5. **Perspectives and Conclusions**

In summary, transcriptomics and metabolomics, as significant components of systems biology, have provided reliable research tools for rapidly predicting stress-related defense factors, revealing the relationships between metabolic pathways, signal transduction, and defense responses, identifying the types of metabolites and their changing patterns, and uncovering various changes in plants under abiotic stress. These methods contribute significantly to improving our understanding of plant stress resistance and the mechanisms behind it. Particularly, the combined analysis of

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transcriptomics and metabolomics plays a vital role in elucidating the genetic basis of plant response and adaptation to abiotic stress, fostering the cultivation of stress-resistant varieties, and supporting the stable yield of crops. While related genes involved in plant stress-resistant metabolic pathways have been cloned and their molecular mechanisms gradually elucidated, our understanding of plant stress resistance remains limited. Further research is needed to explore these pathways and other synergistic effects.

Plant response to adversity stress is a complex process involving the perception of stress signals, signal transduction, and plant defense mechanisms, with intricate metabolic networks. When plants encounter various stresses in nature, cross-effects may exist. Understanding the specific and cross-shared signal transduction and metabolic pathways of plants, as well as how to efficiently mine functional genes and precisely identify metabolites, are crucial in plant stress resistance gene research.

Currently, the rapid development of high-throughput technologies has made data acquisition in transcriptomics and metabolomics more economical and efficient, enabling researchers to gain a deeper understanding of the molecular regulation mechanisms and metabolic regulatory networks of different crops in response to adversity stress. However, further exploration is needed to effectively screen and utilize core data from vast amounts of information. Although the integrated analysis of transcriptomics, metabolomics, and genome-wide association studies is currently in-depth and systematic, there are issues such as insufficient integration with other omics technologies and in-depth data mining. Therefore, it is necessary to further integrate transcriptomics, metabolomics, and other omics technologies to develop more efficient bioinformatics analysis techniques to meet the needs of data collection, storage, and computational analysis. Revealing the overall mechanism of plant response to abiotic stress from genes to traits will provide a robust foundation for better elucidating the molecular mechanisms of biological processes. With the continuous development of multi-omics technologies, more genes related to resistance will be discovered, revealing the essence of plant stress resistance in a more comprehensive manner.

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