Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

MULTI-OMICS-GUIDED PLANT–BENEFICIAL MICROBE INTERACTIONS FOR SUSTAINABLE CROP PROTECTION, DISEASE SUPPRESSION, AND ABIOTIC STRESS RESILIENCE

Arshad Ali[1], Ambreen Zain*[2], Muhammad Abid[3], Muhammad Atiq ur Rehman[4]

[1]*Islamia College Peshawar*
[*2]*University of Agriculture Faisalabad, Pakistan, Institute of Horticulture Sciences*
[3]*Gomal University, Dera Ismail Khan*
[4] *Senior Scientist, Provincial Reference Fertilizer Testing Laboratory Raiwind Lahore Pakistan*

[1]2022-5185@icp.edu.pk, [*2]ambreenzain2023@gmail.com, [3]muhammadabid7139@gmail.com
[4]sardaratiq971@gmail.com

DOI: https://doi.org/10.5281/zenodo.23657224

Keywords
Endophytic bacteria; multi-omics; abiotic stress tolerance; plant holobiont; single-cell omics; spatial transcriptomics; dual RNA-seq; synthetic communities; bioinoculants; climate-smart agriculture.

Article History
Received: 01 January 2026
Accepted: 17 March 2026
Published: 31 March 2026

Copyright @Author
Corresponding Author: *
Ambreen Zain

Abstract

Global agricultural productivity is increasingly threatened by climate change, environmental degradation, and shrinking arable land, with abiotic stressors such as drought, salinity, thermal extremes, and heavy metal toxicity reducing potential crop yields by up to 70%. Plants mitigate these challenges through intimate associations with endophytic bacteria, forming a functional holobiont in which bacterial endophytes colonize internal plant tissues and act as key regulators of nutrient acquisition, systemic signaling, and stress adaptation. This review provides a comprehensive synthesis of multi-omics approaches spanning genomics, metagenomics, transcriptomics including dual RNA-sequencing, proteomics, metabolomics, and spatially resolved single-cell platforms that have transformed our understanding of plant–endophytic bacterial interactions. We dissect the molecular mechanisms underlying endophyte-mediated growth promotion and abiotic stress mitigation, including nitrogen fixation, phosphate and potassium solubilization, siderophore production, phytohormone modulation, ACC deaminase activity, osmotic adjustment, ROS scavenging, and biological priming. Special emphasis is placed on spatially resolved and single-cell omics technologies, such as scRNA-seq, MERFISH, STARmap, MALDI-MSI, and LAESI-MSI, which overcome the limitations of bulk tissue averaging by resolving cellular heterogeneity and localized host–microbe interactions within the endosphere. Through case studies on drought, salinity, temperature extremes, and heavy metal stress, we illustrate how integrated multi-omics pipelines reveal mechanistic insights into crop stress mitigation. We further discuss bioinformatic integration frameworks, including MOFA2, DIABLO, WGCNA, and genome-scale metabolic modeling, alongside translational challenges in developing synthetic communities and commercial bioinoculants. This review highlights how multi-omics-driven dissection of plant–endophyte interactions can accelerate the development of climate-smart agricultural strategies for sustainable crop production.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

1. INTRODUCTION

Global agricultural productivity faces unprecedented pressure from climate change, severe environmental degradation, and the contraction of arable land. Abiotic stressors predominantly drought, elevated salinity, thermal fluctuations (extreme heat and cold), and heavy metal toxicity represent the primary environmental constraints limiting crop yield and performance worldwide, causing reductions in potential agricultural output estimated at up to 70% (Oshunsanya et al., 2019). These environmental constraints trigger osmotic shock, cellular dehydration, membrane destabilization, reactive oxygen species (ROS) hyper-accumulation, and profound metabolic dysregulation in plant tissues (Rathod & Verma, 2023).

To survive unfavorable edaphic and atmospheric conditions, plants rely not only on their intrinsic genetic programs but also on intimate, dynamic associations with surrounding and internal microbial communities. This host-microbe ecological continuum forms a functional entity known as the plant holobiont. Among the functional components of the phytobiome, endophytic bacteria hold a crucial position (Dubey et al., 2025). Bacterial endophytes are defined as microorganisms that colonize internal plant tissues such as the root endodermis, stem vascular bundles, and foliar mesophyll without causing disease symptoms or structural damage to the host. Displaying phylogenetic diversity across phyla such as Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, bacterial endophytes establish intimate physiological communication networks with host cells, acting as vital regulators of plant nutrient acquisition, systemic signaling, and environmental stress adaptation (Kuo et al., 2025). Historically, investigations into plant–endophyte interactions relied on reductionist, culture-dependent isolation techniques, morphological characterizations, and target-gene expression assays. While these foundational approaches identified core plant growth-promoting (PGP) traits such as hormone production, inorganic nutrient solubilization, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity they were inherently limited in their capacity to resolve the systems-level molecular cascades that govern host-symbiont co-adaptation under abiotic stress (Muluneh, 2021).

The development and integration of high-throughput multi-omics technologies spanning genomics, metagenomics, transcriptomics (including dual RNA-sequencing), proteomics, metabolomics, and spatially resolved single-cell platforms have transformed plant microbiome research. By moving beyond simple microbial inventory profiling, integrated multi-omics allows researchers to dissect the complex metabolic networks, regulatory circuits, and inter-species signaling mechanisms that operate within the plant endosphere (Matondo et al., 2026). This review delivers an exhaustive synthesis of multi-omics approaches used to elucidate plant–endophytic bacterial interactions, detailing molecular mechanisms of abiotic stress mitigation, single-cell and spatial breakthroughs, bioinformatic integration pipelines, and translational strategies for climate-smart agriculture (Salem & Ibrahim, 2024).

2. Molecular Mechanisms of Endophytic Bacterial-Mediated Plant Growth and Stress Mitigation

Endophytic bacteria employ an array of direct and indirect physiological mechanisms to promote crop growth and confer tolerance against abiotic environmental stressors. These benefits are delivered through biochemical transformations, hormonal tuning, osmotic protection, and systemic physiological priming within host plant tissues (Mametja et al., 2025).

2.1 Direct Growth Promotion and Nutrient Mobilization

Nutrient availability frequently limits crop development, particularly under stress conditions that restrict root system expansion and membrane transport activities. Endophytic bacteria enhance plant nutrient uptake directly within host tissues, bypassing soil-root interface barriers. Biological nitrogen fixation is executed by endophytic diazotrophs expressing nitrogenase enzyme complexes (encoded by conserved *nif* gene

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

clusters), converting atmospheric nitrogen (N_2) directly into plant-assimilable ammonium (NH_4^+) inside the host vascular system (Richardson et al., 2009).

Simultaneously, bacterial endophytes facilitate phosphate and potassium mobilization through the excretion of low-molecular-weight organic acids, such as gluconic, citric, and succinic acids. These organic acids lower localized micro-environment pH and chelate cations ($Ca^{2+}, Al^{3+}, Fe^{3+}$), liberating bound insoluble mineral phosphates ($HPO_4^{2-}, H_2PO_4^-$) and potassium ions for direct host assimilation. Under iron-limiting or metal-polluted conditions, endophytes produce high-affinity ferric ion chelators (siderophores) that sequester bioavailable Fe^{3+} and deliver it to host root cells while suppressing opportunistic vascular pathogens (Ain et al., 2024).

2.2 Phytohormone Modulation and ACC Deaminase Activity

Endophytic bacteria continuously adjust host growth trajectories and stress responses through the biosynthesis of phytohormones and the enzymatic cleavage of hormone precursors. Bacterial endophytes synthesize indole-3-acetic acid (IAA) via tryptophan-dependent or independent pathways, promoting cell division, vascular differentiation, and lateral root architecture, which increases the root surface area available for water and nutrient absorption. Furthermore, microbial synthesis of cytokinins and gibberellins enhances shoot development, maintains photosynthetic pigment concentration, and delays stress-induced leaf senescence (Gamalero et al., 2023).

A major biochemical mechanism for abiotic stress alleviation is the enzymatic regulation of plant stress ethylene levels. Under severe environmental conditions, plants upregulate endogenous ethylene production via a two-step biosynthetic pathway:

$$\text{S-adenosyl-L-methionine (SAM)} \xrightarrow{\text{ACC synthase}} \text{1-aminocyclopropane-1-carboxylate (ACC)} \xrightarrow{\text{ACC oxidase}} \text{Ethylene}$$

Elevated stress ethylene concentrations lead to root growth inhibition, chlorosis, and premature senescence (Jaroszuk-Ściseł et al., 2019). Endophytic bacteria harboring the *acdS* gene synthesize the enzyme ACC deaminase, which cleaves host-derived ACC exudates inside plant tissues into α-ketobutyrate and ammonia. By acting as a metabolic sink for ACC, endophytic bacteria lower systemic ethylene concentrations, preserving root cell division and elongating functional root systems under extreme environmental stress (Glick, 2020).

2.3 Osmotic Adjustment and Reactive Oxygen Species (ROS) Scavenging Systems

Abiotic stressors induce cellular dehydration and trigger the overproduction of toxic reactive oxygen species, including superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$). ROS hyper-accumulation induces oxidative stress, damaging structural proteins, degrading nucleic acids, and causing membrane lipid peroxidation (Khan et al., 2019).

Endophytic colonization strengthens host defenses against osmotic and oxidative stress via dual pathways. First, endophytes induce host accumulation of compatible organic osmolytes, including proline, glycine betaine, trehalose, myo-inositol, and soluble carbohydrates (Jiang et al., 2013). These compounds maintain cytosolic turgor, stabilize macromolecular structures, and protect cell membranes against desiccation. Second, endophytes upregulate host enzymatic ROS-scavenging systems inducing elevated activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) while expanding non-enzymatic antioxidant pools like glutathione and ascorbate. This enzymatic upregulation quenches reactive radicals, preserves chloroplast integrity, and reduces malondialdehyde (MDA) levels,

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

preventing structural membrane decay (Hossain & Dietz, 2016).

2.4 Priming and Systemic Acclimation

Endophytic bacteria induce biological priming, pre-conditioning host physiological and transcriptional networks to respond more effectively to environmental challenges. Endophyte-inoculated plants maintain baseline molecular preparedness; upon subsequent exposure to drought, temperature extremes, or salinity, primed host plants initiate defense gene activation, stomatal adjustments, and osmoprotectant synthesis more rapidly and intensely than un-inoculated controls (Suzuki et al., 2013).

3. The Multi-Omics Toolkit in Plant–Endophyte Systems

Elucidating the multi-layered communication networks operating between plant hosts and bacterial endophytes requires an integrated suite of molecular technologies. Each omics modality targets a distinct layer of biological organization, transforming static genetic data into functional models of host-microbe co-adaptation (Shishodia et al., 2025).

Genomics and metagenomics provide the functional blueprint of plant-associated bacterial endophytes. High-throughput sequencing of 16S rRNA gene amplicons and shotgun metagenomic DNA resolves taxonomic composition and reveals functional gene pools across endospheric micro-environments. Metagenomic assembly algorithms reconstruct Metagenome-Assembled Genomes (MAGs) directly from uncultivated tissue samples, exposing the metabolic traits of non-culturable endophytes (Kaur et al., 2025). Genome mining algorithms, most notably antiSMASH (Antibiotic and Secondary Metabolite Analysis Shell), scan bacterial genomes to identify Biosynthetic Gene Clusters (BGCs). These BGCs encode secondary metabolite machinery including Non-Ribosomal Peptide Synthetases (NRPS), Polyketide Synthases (PKS), terpenes, and siderophore complexes responsible for niche colonization, anti-pathogen biopesticides, and biostimulant production (Chen et al., 2022).

Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

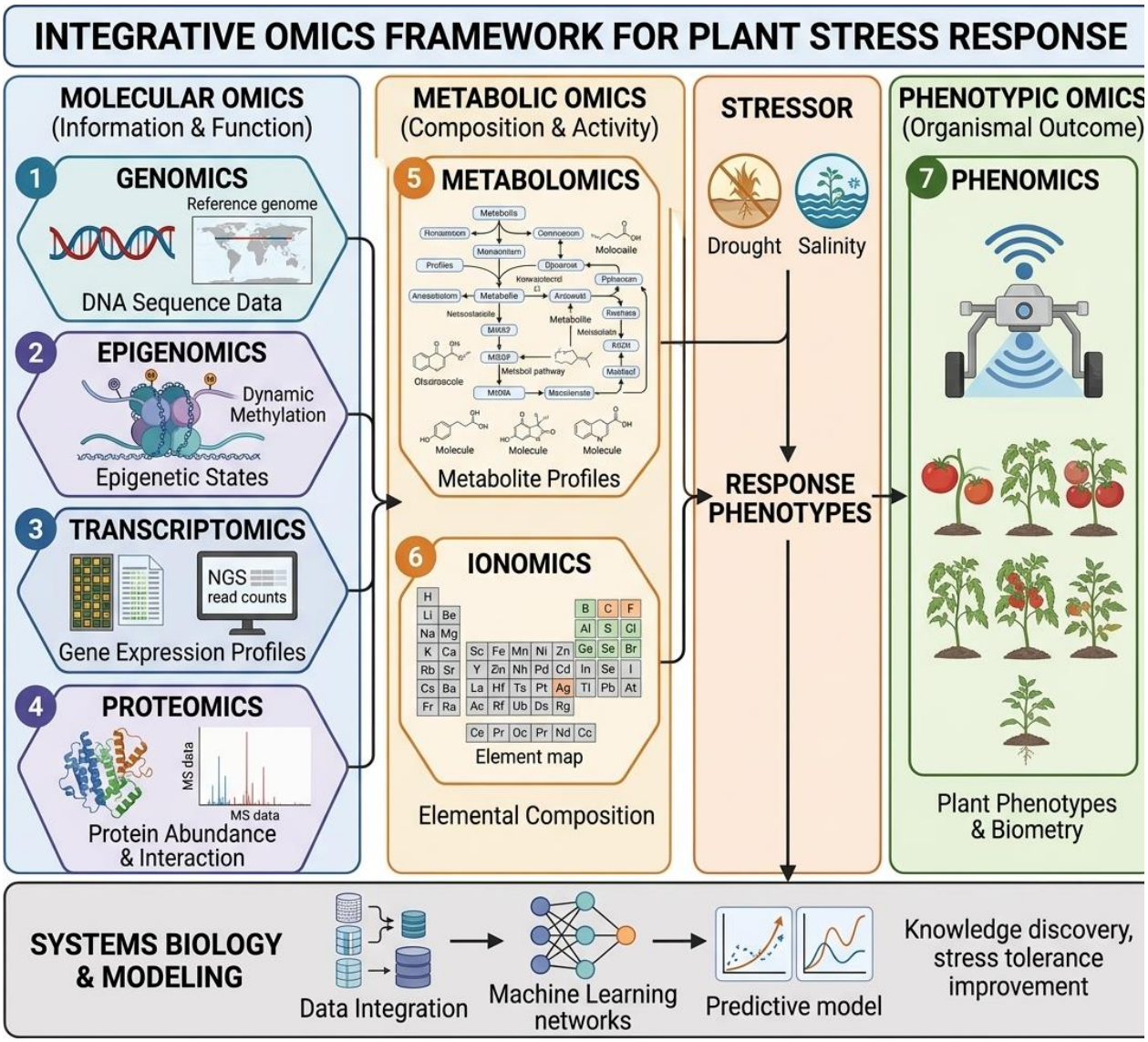


Figure 1: Integrative multi-omics framework and systems biology modeling workflow for dissecting plant environmental stress responses.

Table 1: Multi-Omics Modalities for Profiling Plant–Endophytic Bacterial Interactions

Omics Modality	Biological Level Targeted	Key Methodologies & Tools	Primary Insights Generated
Genomics & Metagenomics	Whole genome sequence, metagenomic DNA	Shotgun sequencing, 16S rRNA profiling, MAG assembly, antiSMASH, MiBIG	Strain taxonomy, core endosphere microbiome, BGC identification, nutrient mobilization gene potential
Transcriptomics & Dual RNA-Seq	Host and endophyte mRNA transcripts	Illumina/Nanopore RNA-seq, Dual RNA-seq, DESeq2, NetGenerator	Simultaneous host-symbiont gene profiling, cross-kingdom molecular dialogue, transcriptional reprogramming

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Proteomics & Metaproteomics	Expressed host and microbial proteins	LC-MS/MS, TMT/iTRAQ labeling, Label-Free Quantification (LFQ)	Functional translation, ACC deaminase synthesis, photosystem protection, enzyme kinetics
Metabolomics & Volatilomics	Primary and secondary small molecules	LC-MS/MS, GC-MS, NMR, Headspace-SPME	Osmolyte profiling, root exudate dynamics, microbial VOC identification, bioactive metabolites
Spatial & Single-Cell Omics	Cellular heterogeneity and spatial localization	scRNA-seq, MALDI-MSI, LAESI-MSI, MERFISH, STARmap	Microenvironment-specific expression, in situ metabolite mapping, cell-type-specific symbiosis

4. Spatially Resolved and Single-Cell Omics: Unraveling Microenvironment Dynamics

Conventional bulk multi-omics techniques isolate RNA, proteins, or metabolites from entire plant organs, effectively averaging molecular signals across millions of distinct host cells and heterogeneous bacterial niches. These spatial averaging masks cellular heterogeneity and obscures localized host-microbe interactions. The advent of spatially resolved and single-cell omics technologies resolves these limitations, enabling sub-cellular, cell-type-specific dissection of the plant endosphere. (Du, 2025)

Single-Cell RNA Sequencing (scRNA-seq) and single-nucleus RNA sequencing (snRNA-seq) allow high-throughput transcriptomic profiling of individual host plant cells and associated microbial endophytes. By performing enzymatic protoplasting or nuclear isolation, single cells are microfluidically barcoded and sequenced, revealing distinct transcriptomic identities across cell layers (Wu et al., 20214). In root-endophyte systems, scRNA-seq distinguishes the molecular responses of epidermal cells (initial contact sites) from cortical cells (colonization zones) and vascular endodermal cells (systemic transit gateways). Single-cell approaches reveal how host sub-populations selectively downregulate pattern-triggered immunity (PTI) pathways to permit beneficial endophyte entry while simultaneously activating hormone signaling networks within localized tissues (Pattabiram et al., 2025).

Because cell dissociation in scRNA-seq destroys native tissue architecture, spatial transcriptomics (ST) platforms preserve histological context by profiling transcripts directly within intact tissue sections. Modern spatial transcriptomics relies on multiplexed Fluorescence In Situ Hybridization (FISH) or In Situ Sequencing (ISS). Multiplexed FISH technologies such as Multiplexed Error-Robust FISH (MERFISH) and Sequential FISH (seqFISH) utilize combinatorial fluorescent probe barcoding and sequential imaging to map thousands of host and microbial transcripts in situ at sub-micron resolution (Liu & Zhang, 2025). Alternatively, ISS platforms like Spatially-resolved Transcript Amplicon Readout Mapping (STARmap) and BaristaSeq execute direct RNA sequencing within fixed, hydrogel-cleared plant tissues. These spatial techniques allow researchers to map bacterial endophyte micro-colonies while simultaneously quantifying localized host defense, sugar transporter expression, and ROS-scavenging activation immediately adjacent to bacterial clusters (Nguyen et al., 2026).

Spatial metabolomics complements transcriptomic mapping by visualizing the exact distribution of small molecules, osmolytes, and secondary metabolites across tissue micro-zones without requiring chemical extraction. Mass Spectrometry Imaging (MSI) represents the core analytical engine of spatial metabolomics. Matrix-Assisted Laser Desorption/Ionization (MALDI-MSI) uses a focused laser beam directed at matrix-coated tissue sections to map small osmolytes, lipids, and secondary phytometabolites at micro-meter spatial resolution (Cheng et al., 2024. Laser Ablation Electrospray Ionization (LAESI-MSI) provides ambient ionization, utilizing mid-infrared laser pulses to excite endogenous water molecules

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

in ambient tissues, permitting spatial profiling of hydrated plant organs without extensive vacuum processing. For sub-micron spatial resolution, Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) resolves metabolite exchange across single plant cell walls and apoplastic boundaries. Spatial metabolomics has uncovered localized metabolic gradients, showing how endophytes drive the concentrated accumulation of protective osmolytes and antioxidants specifically within vascular and cortical root tissues under abiotic stress (Cheng et al., 2025).

5. Case Studies: Multi-Omics Integration in Specific Abiotic Stresses

Integrating multi-omics pipelines across model plant hosts and beneficial endophytes has yielded detailed mechanistic insights into crop stress mitigation (Saleem et al., 2025).

5.1 Drought Stress Mitigation

Drought stress restricts soil water availability, inducing stomatal closure, photosynthetic decline, ROS generation, and severe cellular dehydration. Integrated transcriptomics and metabolomics demonstrate that endophytic bacteria mitigate drought damage through physiological rewiring. Dual RNA-seq profiling reveals that endophytes upregulate host abscisic acid (ABA)-dependent and ABA-independent signaling pathways, inducing expression of dehydration-responsive element-binding (DREB) transcription factors, Late Embryogenesis Abundant (*LEA*) proteins, and plasma membrane aquaporin genes (*PIPs* and *TIPs*) to optimize cellular water retention (Bittencourt et al., 2022). Concurrently, metabolomic profiling shows elevated concentrations of proline, GABA (γ-aminobutyric acid), myo-inositol, and raffinose family oligosaccharides in drought-stressed, endophyte-colonized crops. Bacterial synthesis of exopolysaccharides (EPS) forms a physical bio-gel within root apoplastic spaces, retaining moisture and sustaining root hydraulic conductivity during soil drying (Duruflé & Déjean, 2023).

Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

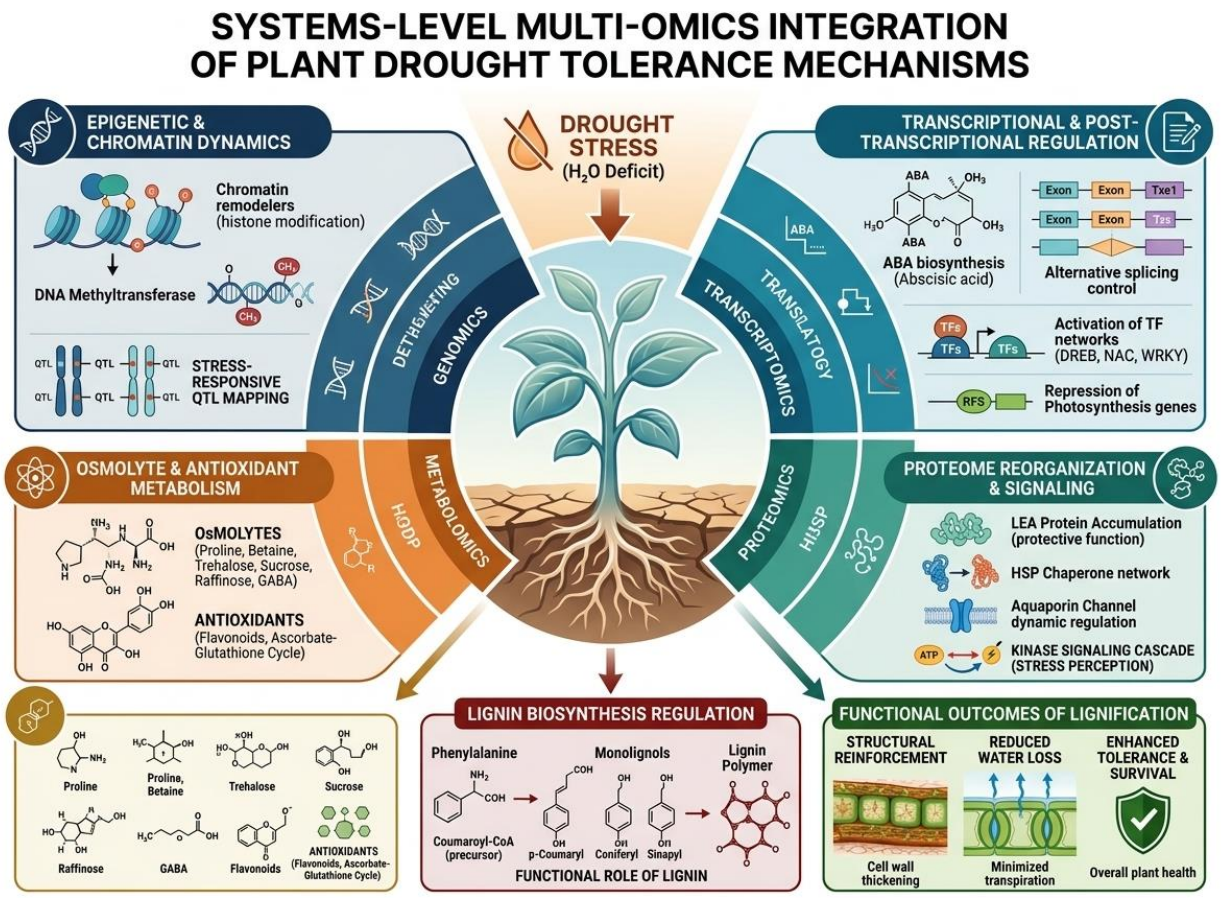


Figure 2: Systems-level multi-omics molecular cascades governing plant drought stress perception, signaling networks, and physiological adaptation mechanisms.

5.2 Salt and Saline-Alkali Tolerance

Soil salinity imposes osmotic stress and ion toxicity due to excessive intracellular Na^+ and Cl^- accumulation. High cytosolic Na^+ displaces essential K^+, inactivates photosynthetic enzymes, and destabilizes cell membranes. Multi-omics dissections of endophyte-inoculated crops under salt stress demonstrate systemic ion homeostasis restoration. Endophyte inoculation upregulates host high-affinity potassium transporters ($HKT1$) and plasma membrane Na^+/H^+ antiporters ($SOS1$), while stimulating vacuolar Na^+/H^+ exchangers ($NHX1$) (Liu et al., 2026). This sequesters excess Na^+ into vacuoles and maintains a high cytosolic K^+/Na^+ ratio. Proteomic and transcriptomic profiling of salt-stressed crops inoculated with ACC deaminase-producing endophytes reveals suppression of stress-ethylene signaling cascades, stabilization of Calvin cycle enzymes (Rubisco, fructose-1,6-bisphosphatase), and reductions in toxic MDA alongside increases in protective SOD, POD, and CAT activities (Liu & Wang, 2021).

5.3 Temperature Extreme Resilience (Cold and Heat)

Thermal stress destabilizes plant structural integrity: cold temperatures induce plasma membrane rigidification and ion leakage, whereas heat stress induces protein denaturation, lipid bilayer fluidization, and photosynthetic collapse. A prominent model illustrating microbial cold-stress mitigation is *Burkholderia phytofirmans* strain PsJN in grapevine (*Vitis vinifera*) and cereal crops.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Integrated multi-omics investigations reveal that strain PsJN pre-conditions host cold tolerance via carbohydrate metabolic priming (Su et al., 2025). Transcriptomics and targeted metabolomics show that PsJN inoculation modulates plant carbohydrate pathways prior to chilling exposure, triggering accumulation of soluble sugars specifically sucrose, glucose, fructose, and raffinose which act as cryoprotectants stabilizing cell membranes. Under chilling exposure (4°C), PsJN-inoculated plants exhibit accelerated expression of cold-responsive defense genes, rapid ROS scavenging, and preservation of Photosystem II quantum yield (Φ_{PSII}), preventing cold-induced photoinhibition (Hazel & Williams, 1990).

5.4 Heavy Metal Detoxification and Phytoremediation

Heavy metal accumulation ($Cd^{2+}, Pb^{2+}, Cr^{6+}, As^{3+}$) disrupts root membrane permeability, replaces metal cofactors in essential enzymes, and induces severe oxidative damage. Multi-omics analyses of metal-resistant bacterial endophytes demonstrate multi-tiered phytoremediation mechanisms. Metal-resistant endophytes chemically transform toxic metal ions within host tissues; for example, chromium-resistant endophytes reduce highly toxic Cr^{6+} into less toxic, lower-solubility Cr^{3+} inside the endosphere (Patra et al., 2026). Simultaneously, multi-omics datasets reveal endophyte-induced upregulation of host metallothionein (MT) and phytochelatin synthase (PCS) genes, which chelate metal ions and sequester them into root cell vacuoles, preventing systemic translocation to shoot tissues and edible grain organs.

Table 2 highlights representative endophytic bacterial strains and insights gained from integrated multi-omics studies under abiotic stress conditions (Singh et al., 2026).

Table 2: Representative Endophytic Bacterial Strains and Multi-Omics Insights in Abiotic Stress Alleviation

Bacterial Endophyte Strain	Host Plant Species	Target Abiotic Stress	Integrated Omics Applied	Key Mechanistic Discoveries
***Burkholderia phytofirmans* PsJN**	*Vitis vinifera*, *Triticum aestivum*, *Zea mays*	Cold / Chilling stress, Drought, Salinity	Transcriptomics, Metabolomics	Defense pathway priming; sucrose and raffinose accumulation; Photosystem II quantum yield preservation
Endophytic Strain S4	*Oryza sativa* (Rice)	Saline-Alkali stress	Transcriptomics, Metabolomics	Upregulation of SOD/POD/CAT enzymes; osmotic balance via proline/soluble sugars; Na^+/Cl^- homeostasis
***Bacillus subtilis* strain 168T / ALN strains**	Model crops (*Arabidopsis*, Cereals)	Abiotic / Pathogenic stress	Genomics, Genome Mining (antiSMASH)	Identification of core BGCs coding for lipopeptides, siderophores, and osmoprotectant molecules
ACC Deaminase-producing endophytes (*Serratia* sp., *Burkholderia* sp.)	*Oryza sativa*, *Triticum aestivum*	Salt / Salinity stress	Proteomics, Transcriptomics	Enzymatic cleavage of ACC to suppress stress ethylene spikes; proteomic stabilization of Calvin cycle enzymes
Paenibacillus peoriae	*Zea mays* (Maize)	Fungal infection & Abiotic stress	Dual RNA-Seq	Simultaneous upregulation of host sugar transporters (SWEET) and bacterial

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

				microaerobic respiration genes

6. Bioinformatic Pipelines and Systems Biology Integration

The accumulation of multi-omics datasets presents considerable computational challenges in data normalization, cross-platform integration, and biological network inference. Systems biology integration framework tools convert disparate omics data into unified regulatory models (Kant et al., 2025).

To derive biological meaning from multi-layer omics datasets, bioinformaticians employ specialized statistical integration algorithms. Multi-Omics Factor Analysis (MOFA2) serves as an unsupervised framework using Bayesian factor analysis to decompose heterogeneous omics datasets into latent factors, identifying global variance drivers across host and endophyte tiers. Data Integration Analysis for Biomarker discovery using Latent cOmponents (DIABLO), implemented within the mixOmics R package, provides a supervised integration method that selects key multi-omics feature subsets (genes, proteins, metabolites) discriminating host stress outcomes (Svinin et al., 2025). Weighted Gene Co-expression Network Analysis (WGCNA) constructs network topologies to group co-expressed host and microbial genes into functional modules, correlating these modules with physiological traits (Na^+/K^+ ratio, leaf water potential, MDA content) to pinpoint driver genes. Additionally, Two-Way Orthogonal Partial Least Squares (O2PLS) isolates joint variation shared between omics layers from system-specific orthogonal noise, clarifying bi-directional host-microbe interaction effects (Polu, 2026).

Processing dual RNA-seq datasets requires specialized bioinformatic workflows. Initial quality control and decontamination steps utilize tools such as FastQC and Trimmomatic to remove low-quality reads, adapter fragments, and ribosomal RNA (rRNA) sequences. Processed reads undergo parallel alignment: spliced aligners such as STAR or HISAT2 map host plant reads against eukaryotic reference genomes, while unmapped reads are routed through Bowtie2 to align against bacterial endophyte genomes (Lv & Fan, 2026). Disambiguation algorithms eliminate ambiguous reads mapping to conserved domain regions shared across kingdoms. Feature quantification via featureCounts or HTSeq yields raw count matrices, which are normalized using DESeq2 or edgeR. Finally, inter-species gene regulatory network inference platforms, such as NetGenerator, utilize time-series transcriptomic data to model direct transcriptional influences, predicting which bacterial regulatory factors activate or repress corresponding host defense and metabolic genes (Kumar, 2026).

Genomic sequences mined via antiSMASH are integrated with metabolic pathway databases such as KEGG, BioCyc, and SEED/RAST to reconstruct Genome-Scale Metabolic Models (GEMs). Applying Flux Balance Analysis (FBA) to GEMs simulates carbon, nitrogen, and energy exchange between host plants and bacterial endophytes under varying abiotic stress scenarios. These computational models predict metabolic fluxes, identify nutrient trade-off bottlenecks, and guide the rational design of bioinoculant formulations optimized for stress mitigation (Kim & Tagkopoulos, 2018).

7. Translational Challenges, Synthetic Communities, and Future Outlook

Despite multi-omics breakthroughs in characterizing plant–endophytic bacterial interactions under controlled conditions, translating laboratory discoveries into scalable agricultural applications presents complex challenges (Mametja et al., 2025).

A major bottleneck in bioinoculant deployment is the performance gap observed between controlled greenhouse trials and variable field environments. Strains displaying strong plant growth-promoting traits and stress mitigation in vitro often fail to establish persistent populations in field crops due to variable soil temperatures, moisture extremes, competitive exclusion by native microbiomes, and host genotype specificity. Furthermore, endophytic communities display high functional

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

redundancy, where taxonomically distinct species execute similar ecological functions. Identifying true "keystone taxa" endophytes that exert outsized influence on microbiome stability and host stress resilience remains a core research priority (Kumar et al., 2026).

To address single-strain bioinoculant instability, researchers leverage multi-omics datasets to construct Synthetic Communities (SynComs). SynComs are rationally designed multi-species bacterial consortia built to mimic natural microbiome functionality while maintaining low complexity. Multi-omics-guided SynCom construction relies on three core design principles (Kumar & Nautiyal, 2026). First, functional complementarity ensures member strains provide synergistic benefits such as combining an ACC deaminase-producing endophyte with a nitrogen-fixing diazotroph and a phosphate-solubilizing strain. Second, metabolic compatibility, modeled via GEM flux balance analysis, prevents destructive substrate competition among inoculant strains. Third, niche occupancy and keystone inclusion ensure stable colonization across diverse root cell layers and environmental gradients (Li et al., 2026).

Deploying endophytic inoculants at commercial scales requires navigating biosecurity regulations and establishing standardized quality control frameworks. High-throughput whole-genome sequencing must verify the complete absence of human pathogenicity factors, toxin BGCs, or antibiotic resistance genes prior to field release (Manna et al., 2025). Standardizing endosphere tissue preparation, RNA/metabolite extraction protocols, and computational pipelines is necessary to ensure reproducibility across study systems. Finally, advancing bio-formulation technologies such as microencapsulation, biochar carriers, and liquid seed-coating formulations will protect microbial viability during commercial storage and field application, supporting the adoption of microbiome-centric biostimulants in modern agriculture (Mishra et al., 20222).

Conclusion

Multi-omics technologies have fundamentally reshaped our understanding of plant–endophytic bacterial interactions, moving the field beyond reductionist, culture-dependent approaches to systems-level models of host–microbe co-adaptation. By integrating genomics, metagenomics, transcriptomics, proteomics, metabolomics, and spatially resolved single-cell platforms, researchers can now dissect the complex metabolic networks, regulatory circuits, and inter-species signaling mechanisms that operate within the plant endosphere under abiotic stress. This review has synthesized the molecular mechanisms by which endophytic bacteria promote crop growth and confer tolerance to drought, salinity, thermal extremes, and heavy metal toxicity, including nutrient mobilization, phytohormone modulation, ACC deaminase activity, osmotic adjustment, ROS scavenging, and biological priming. The advent of single-cell and spatial omics has been particularly transformative, enabling cell-type-specific and microenvironment-resolved dissection of host–microbe interactions that were previously obscured by bulk tissue averaging. Case studies across model and crop systems demonstrate that integrated multi-omics pipelines, coupled with sophisticated bioinformatic frameworks such as MOFA2, DIABLO, WGCNA, and genome-scale metabolic modeling, can identify key driver genes, metabolic bottlenecks, and keystone taxa governing stress resilience. Despite these advances, significant translational challenges remain, including the performance gap between controlled and field environments, functional redundancy within endophytic communities, regulatory and biosecurity considerations, and the need for standardized protocols. Multi-omics-guided synthetic community design, incorporating principles of functional complementarity, metabolic compatibility, and niche occupancy, offers a promising path toward stable and effective bioinoculants. Future research should prioritize the identification of true keystone taxa, the development of robust bio-formulation technologies, and the integration of artificial intelligence and predictive modeling to accelerate the translation of multi-omics discoveries into scalable, climate-smart agricultural solutions that

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

enhance crop productivity and food security under increasingly adverse environmental conditions.

REFERENCES

Ain, Q. U., Hussain, H. A., Zhang, Q., Maqbool, F., Ahmad, M., Mateen, A., ... & Imran, A. (2024). Coordinated influence of Funneliformis mosseae and different plant growth-promoting bacteria on growth, root functional traits, and nutrient acquisition by maize. *Mycorrhiza*, *34*(5), 477-488.

Bittencourt, C. B., Carvalho da Silva, T. L., Rodrigues Neto, J. C., Vieira, L. R., Leão, A. P., de Aquino Ribeiro, J. A., ... & Souza Jr, M. T. (2022). Insights from a multi-omics integration (MOI) study in oil palm (Elaeis guineensis Jacq.) response to abiotic stresses: part one salinity. *Plants*, *11*(13), 1755.

Chen, X. L., Sun, M. C., Chong, S. L., Si, J. P., & Wu, L. S. (2022). Transcriptomic and metabolomic approaches deepen our knowledge of plant–endophyte interactions. *Frontiers in Plant Science*, *12*, 700200.

Cheng, X., Cao, Y., Liu, X., Li, Y., Li, Q., Gao, D., & Yu, Q. (2024). Single-cell and spatial omics unravel the spatiotemporal biology of tumour border invasion and haematogenous metastasis. *Clinical and translational medicine*, *14*(10), e70036.

Cheng, X., Peng, T., Chu, T., Yang, Y., Liu, J., Gao, Q., ... & Wei, J. (2025). Application of single-cell and spatial omics in deciphering cellular hallmarks of cancer drug response and resistance. *Journal of Hematology & Oncology*, *18*(1), 70.

Du, L., & Yang, H. (2025). Spatial omics in 3D culture model systems: decoding cellular positioning mechanisms and microenvironmental dynamics. *Journal of translational medicine*, *23*(1), 1356.

Dubey, A., Singh, P. K., Singh, S., Manzoor, U., Sahoo, C., & Saini, A. (2025). Impact of climate change and land degradation on agriculture. In *Eco-resilience: Climate change, land degradation and sustainable solutions* (pp. 49-95). Cham: Springer Nature Switzerland.

Duruflé, H., & Déjean, S. (2023). Multi-omics data integration in the context of plant abiotic stress signaling. In *Plant Abiotic Stress Signaling* (pp. 295-318). New York, NY: Springer US.

Gamalero, E., Lingua, G., & Glick, B. R. (2023). Ethylene, ACC, and the plant growth-promoting enzyme ACC deaminase. *Biology*, *12*(8), 1043.

Glick, B. R. (2020). Modulating phytohormone levels. In *Beneficial plant-bacterial interactions* (pp. 139-180). Cham: Springer International Publishing.

Hazel, J. R., & Williams, E. E. (1990). The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Progress in lipid research*, *29*(3), 167-227.

Hossain, M. S., & Dietz, K. J. (2016). Tuning of redox regulatory mechanisms, reactive oxygen species and redox homeostasis under salinity stress. *Frontiers in plant science*, *7*, 548.

Jaroszuk-Ściseł, J., Tyśkiewicz, R., Nowak, A., Ozimek, E., Majewska, M., Hanaka, A., ... & Janusz, G. (2019). Phytohormones (auxin, gibberellin) and ACC deaminase in vitro synthesized by the mycoparasitic Trichoderma DEMTkZ3A0 strain and changes in the level of auxin and plant resistance markers in wheat seedlings inoculated with this strain conidia. *International Journal of Molecular Sciences*, *20*(19), 4923.

Jiang, J., Su, M., Chen, Y., Gao, N., Jiao, C., Sun, Z., ... & Wang, C. (2013). Correlation of drought resistance in grass pea (Lathyrus sativus) with reactive oxygen species scavenging and osmotic adjustment. *Biologia*, *68*(2), 231-240.

Kant, S., Deepika, & Roy, S. (2025). Integrative multi-omics and artificial intelligence: a new paradigm for systems biology. *Omics: a journal of integrative biology*, *29*(12), 576-587.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Kaur, R., Kaur, C., Kaur, G., Kaur, J., Rath, S. K., & Dwibedi, V. (2025). From microscopy to omics: a comprehensive review of tools and techniques in studying endophytic adaptation under abiotic and biotic stress. *Journal of Plant Growth Regulation*, *44*(12), 6766-6784.

Khan, M. N., Zhang, J., Luo, T., Liu, J., Rizwan, M., Fahad, S., ... & Hu, L. (2019). Seed priming with melatonin coping drought stress in rapeseed by regulating reactive oxygen species detoxification: Antioxidant defense system, osmotic adjustment, stomatal traits and chloroplast ultrastructure perseveration. *Industrial Crops and Products*, *140*, 111597.

Kim, M., & Tagkopoulos, I. (2018). Data integration and predictive modeling methods for multi-omics datasets. *Molecular omics*, *14*(1), 8-25.

Kumar, D., Sattiraju, K. S., & Reddy, M. S. (2026). A systems approach to endophyte-mediated plant holobiont and microbiome dynamics. *Plants*, *15*(5), 802.

Kumar, K. (2026). Frameworks for robust information extraction from multi-omics data integration to network-based biological insight. *Gene Reports*, 102468.

Kumar, V., & Nautiyal, C. S. (2026). From hidden allies to precision symbionts: unleashing endophytes for sustainable agroecosystems. *World Journal of Microbiology and Biotechnology*, *42*(4), 172.

Kuo, W. H. J., Chung, C. L., Juang, K. W., Tung, C. W., & Liu, L. Y. D. (2025). Challenges to agriculture production under climate change. In *Agricultural Nutrient Pollution and Climate Change: Challenges and Opportunities* (pp. 29-56). Cham: Springer Nature Switzerland.

Li, S., Chiodi, C., Maucieri, C., Della Lucia, M. C., Zardinoni, G., Ravi, S., ... & Stevanato, P. (2026). Profiling soil–plant–microbial communities: DNA and multi-omics techniques. *Genes*, *17*(3), 303.

Liu, F., & Zhang, X. H. F. (2025). Decoding metastatic microenvironments through single-cell omics reveals new insights into niche dynamics and tumor evolution. *PLoS biology*, *23*(7), e3003299.

Liu, L., & Wang, B. (2021). Protection of halophytes and their uses for cultivation of saline-alkali soil in China. *Biology*, *10*(5), 353.

Liu, X., Lin, S., Li, C., Ren, S., Tang, Y., Tang, L., & Zhang, Z. (2026). Functional characteristics and distribution of microorganisms in saline and alkaline soils and their probiotic effects: a review. *Microbial Cell Factories*, *25*(1), 39.

Lv, Y., & Fan, G. (2026). Network-driven insights into plant immunity: integrating transcriptomic and proteomic approaches in plant–pathogen interactions. *International journal of molecular sciences*, *27*(3), 1242.

Mametja, N. M., Ramadwa, T. E., Managa, M., & Masebe, T. M. (2025). Recent advances and developments in bacterial endophyte identification and application: A 20-year landscape review. *Plants*, *14*(16), 2506.

Mametja, N. M., Ramadwa, T. E., Managa, M., & Masebe, T. M. (2025). Recent advances and developments in bacterial endophyte identification and application: A 20-year landscape review. *Plants*, *14*(16), 2506.

Manna, D., Laishram, R., Arpitha, S. R., Rai, M., & Dahuja, A. (2025). Omics for thriving plant microbiomes: growing food security for a sustainable future. *Microbial Metabolomics: Recent Developments, Challenges and Future Opportunities*, 315-346.

Matondo, J. D., Anatoli, A. I., Nyagesera, N. J., & Kilemile, W. H. (2026). Climate change threatens food security through cascading production and nutritional vulnerabilities: a comprehensive review. *Cogent Food & Agriculture*, *12*(1), 2715259.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Mishra, A. K., Sudalaimuthuasari, N., Hazzouri, K. M., Saeed, E. E., Shah, I., & Amiri, K. M. (2022). Tapping into plant–microbiome interactions through the lens of multi-omics techniques. *Cells*, *11*(20), 3254.

Muluneh, M. G. (2021). Impact of climate change on biodiversity and food security: a global perspective a review article. *Agriculture & Food Security*, *10*(1), 36.

Nguyen, H., Eapen, M. M., Nguyen, Q., & Sharma, A. (2026). Spatial omics for profiling the dynamic tumor microenvironment. *Clinical & Translational Immunology*, *15*(4), e70084.

Oshunsanya, S. O., Nwosu, N. J., & Li, Y. (2019). Abiotic stress in agricultural crops under climatic conditions. In *Sustainable agriculture, forest and environmental management* (pp. 71-100). Singapore: Springer Singapore.

Patra, D. K., Nayak, M., Pradhan, C., & Patra, H. K. (2026). Heavy metals tolerance and toxic effects on plants: A review. *Chemistry and Ecology*, *42*(5), 640-672.

Pattabiram, S., Gangadaran, P., Dhayalan, S., Chatterjee, G., Reyaz, D., Prakash, K., ... & Aruljothi, K. N. (2025). Decoding the tumor microenvironment: Insights and new targets from single-cell sequencing and spatial transcriptomics. *Current Issues in Molecular Biology*, *47*(9), 730.

Polu, P. R. (2026). AI-driven chemometrics for multi-omics data integration: advances, challenges, and future directions. *Critical Reviews in Analytical Chemistry*, 1-36.

Rathod, A., & Verma, N. S. (2023). Impact of abiotic stress on agronomical crops. *Frontiers of agronomy*, *27*.

Richardson, A. E., Barea, J. M., McNeill, A. M., & Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms.

Saleem, M. H., Noreen, S., Ishaq, I., Saleem, A., Khan, K. A., Ercisli, S., ... & Fahad, S. (2025). Omics technologies: unraveling abiotic stress tolerance mechanisms for sustainable crop improvement. *Journal of Plant Growth Regulation*, *44*(7), 4165-4187.

Salem, K. F., & Ibrahim, A. A. (2024). Plant biodiversity in the context of food security under climate change. In *Sustainable utilization and conservation of plant genetic diversity* (pp. 3-30). Singapore: Springer Nature Singapore.

Shishodia, S. K., Thakur, R., Shankar, J., Singh, R., & De LosRios-Escalante, P. (2025). Unlocking endophytic mysteries by leveraging multi-omics technologies. In *Multi-omics Approach to Investigate Endophyte Diversity* (pp. 125-144). Singapore: Springer Nature Singapore.

Singh, J., Mishra, A. K., Madheshiya, P., Tiwari, S., & Singh, A. A. (2026). Individual and combined impacts of potentially toxic elements, and tropospheric ozone on crop physiology, transcriptomic responses, and yield performance: a review. *Physiology and Molecular Biology of Plants*, 1-27.

Su, X., Yao, L., Wang, X., Zhang, Y., Zhang, G., & Li, X. (2025). Mechanisms for cell survival during abiotic stress: focusing on plasma membrane. *Stress Biology*, *5*(1), 1.

Suzuki, N., Miller, G., Salazar, C., Mondal, H. A., Shulaev, E., Cortes, D. F., ... & Mittler, R. (2013). Temporal-spatial interaction between reactive oxygen species and abscisic acid regulates rapid systemic acclimation in plants. *The Plant Cell*, *25*(9), 3553-3569.

Svinin, G., Loo, R. T. J., Soudy, M., Nasta, F., Le Bars, S., & Glaab, E. (2025). Computational Systems Biology Methods for Cross-Disease Comparison of Omics Data. *Wiley Interdisciplinary Reviews: Computational Molecular Science*, *15*(4), e70042.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Wu, R., Veličković, M., & Burnum-Johnson, K. E. (2024). From single cell to spatial multi-omics: unveiling molecular mechanisms in dynamic and heterogeneous systems. *Current Opinion in Biotechnology*, *89*, 103174.