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# Humic Substances as Master Regulators of Plant Stress Resilience: From Supramolecular Chemistry to Climate-Smart Agriculture

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# Abstract

Humic substances (HS) are increasingly recognized not merely as soil conditioners but as dynamic biological regulators that orchestrate plant resilience through complex signaling networks. This review synthesizes recent advances in understanding how HS function as master regulators of plant adaptation to abiotic stress. We present a mechanistic framework integrating three interconnected themes: (1) the supramolecular chemistry of HS and its relationship to bioactivity, (2) the early signaling events, encompassing reactive oxygen species (ROS), calcium ($Ca^{2+}$), and phytohormone crosstalk that translate HS perception into systemic stress responses, and (3) the tripartite interaction among plants, HS, and the rhizosphere microbiome. Emerging evidence demonstrates that HS act as chemical priming agents, inducing a state of "eustress" that preconditions plants for enhanced tolerance to subsequent drought, salinity, heat, and heavy metal stress. This preconditioning involves transcriptional reprogramming through transcription factors such as WRKY, NAC, and MYB, leading to enhanced antioxidant defenses, osmotic adjustment, photosynthetic protection, and nutrient homeostasis. We critically evaluate the structure-function relationships of HS, the molecular players in HS signaling, and the synergistic potential of HS with plant growth-promoting microorganisms. Finally, we identify critical knowledge gaps, including the identity of plant HS receptors, the relative contribution of direct versus microbiome-mediated effects, and the performance of HS under multifactorial stress combinations and propose future research directions. By bridging supramolecular chemistry, plant physiology, and microbiome science, this review positions HS as a cornerstone of climate-smart agriculture and provides a roadmap for developing next-generation, mechanism-based HS biostimulants.


# 1. Introduction

Global food production faces unprecedented challenges from climate change, with extreme weather events like droughts, floods, heatwaves, and soil salinization, increasing in frequency and intensity. Abiotic stresses now account for 50–80% of yield losses in major crops, losses projected to intensify as climate change accelerates (Rivero et al., 2022; Canellas et al., 2024). By 2050, global food production must increase by 60–70% to feed a projected population of 9.7 billion, yet arable land is diminishing due to soil degradation, salinization, and urbanization (Johnson et al., 2024). This confluence of challenges demands a paradigm shift from conventional high-input agriculture toward sustainable intensification strategies that maintain productivity while reducing environmental footprint.

Conventional approaches to stress management, breeding for stress-tolerant varieties, genetic engineering, and intensive agrochemical application have achieved significant gains but face inherent limitations. Breeding programs are time-consuming, often requiring 10–15 years to develop improved varieties, and frequently fail to deliver tolerance to multiple, simultaneously occurring stresses (Deolu-Ajayi et al., 2022). The Green Revolution's reliance on synthetic fertilizers and pesticides has resulted in soil degradation, water contamination, biodiversity loss, and greenhouse gas emissions, undermining the long-term sustainability of agricultural systems (Miglani et al., 2025). There is, therefore, an urgent need

for eco-friendly, cost-effective alternatives that enhance plant resilience while reducing dependence on chemical inputs.

Plant biostimulants have emerged as a promising solution to this challenge. Defined by the European Union's Fertilizing Products Regulation (EU 2019/1009) as products that stimulate plant nutrition processes independently of their nutrient content to improve nutrient use efficiency, abiotic stress tolerance, quality traits, and soil nutrient availability (Atero-Calvo et al., 2024). Biostimulants like humic substances, seaweed extracts, and beneficial microorganisms naturally enhance plant growth, nutrient efficiency, and stress tolerance. The global market for these sustainable agricultural inputs is expanding rapidly, with humic-based products alone valued at nearly $1 billion and the broader biostimulants industry scaling past $3.4 billion globally and is projected to grow from USD 3.4 billion in 2026 to USD 5.9 billion by 2033 (Grand View Research, 2026).

Humic substances are the most abundant organic components of soils, comprising up to 80% of soil organic matter and playing central roles in nutrient cycling, soil structure, and ecosystem functioning (Canellas and Olivares, 2014; Nardi et al., 2021). Historically, HS were conceptualized as large, stable macromolecular polymers with well-defined structures, a view that persisted for decades and framed HS primarily as inert soil conditioners rather than biologically active agents. This paradigm has been fundamentally challenged and revised over the past two decades through the pioneering work of Piccolo and colleagues, who demonstrated that HS are not covalent macromolecules but rather supramolecular associations of relatively small, heterogeneous molecules held together by weak intermolecular forces, hydrophobic interactions, hydrogen bonds, and van der Waals forces (Piccolo and Drosos, 2026). This supramolecular model explains the remarkable conformational flexibility of HS and their sensitivity to environmental conditions such as pH, ionic strength, and the presence of root exudates. The "humeomics" approach, developed by Nebbioso and Piccolo (2011and 2012), provides a sequential chemical fractionation method to dissect the humic supramolecular structure and identify individual molecular components, revealing that HS comprises thousands of distinct molecules, including fatty acids, lignin-derived phenols, carbohydrates, peptides, and auxin-like compounds. This paradigm shift has profound implications for understanding HS bioactivity: rather than a single molecule with a defined receptor, HS represent a complex chemical system whose bioactivity emerges from the collective properties of its constituents and their dynamic interactions with plant roots and soil microbiota (Canellas et al., 2024; Yakhin et al., 2017). The weak forces stabilizing the supramolecular structure allow for the controlled release of bioactive molecules upon contact with root exudates, creating a sophisticated communication system between soil organic matter and the plant.

The recognition of HS as dynamic, bioactive assemblies has opened new avenues for understanding their effects on plants. A growing body of evidence supports the view that HS act as chemical priming agents, inducing a state of heightened responsiveness that prepares plants for subsequent stress encounters (Canellas et al., 2020; Savvides et al., 2016). This priming effect operates through the induction of mild, transient stress, a phenomenon termed "eustress"—that activates plant defense mechanisms without causing significant damage. The concept of hormesis, defined as a biphasic dose-

response phenomenon in which low doses of a stressor stimulate adaptive responses (Calabrese and Blain, 2009), provides a useful framework for understanding HS action. Canellas et al. (2020) demonstrated that maize seedlings treated with an optimal concentration of HS exhibited increased catalase activity, proline accumulation, and transcriptional upregulation of stress-responsive genes, including transcription factors and genes involved in calcium signaling, hormone signaling, and stress perception. Importantly, these responses were induced in the absence of any abiotic stressor, indicating that HS themselves act as mild elicitors that prime the plant for subsequent stress tolerance. When these primed seedlings were subsequently exposed to salinity, drought, chromium toxicity, or high HS concentrations, they showed significantly less growth inhibition than unprimed controls. This priming effect represents a fundamental shift in how we conceptualize HS bioactivity: HS are not simply nutrient providers or growth promoters but active modulators of plant stress signaling networks. The pleiotropic effects of HS enhancing tolerance to drought, salinity, heat, cold, and heavy metals—can be understood as manifestations of a common priming mechanism that engages core stress signaling pathways (Amin et al., 2026; Sani and Yong, 2021).

Despite extensive research on HS effects, several critical knowledge gaps remain. First, the relationship between HS supramolecular structure and bioactivity is incompletely understood; while the humeomics approach has revealed the molecular complexity of HS, the specific structural features that confer biological activity remain debated. Second, the early signaling events triggered by HS perception, the identity of receptors, the sequence of second messenger activation, and the integration with phytohormone networks are only beginning to be elucidated. Third, the role of the plant microbiome in mediating HS effects is increasingly recognized but poorly characterized. Fourth, the translation of mechanistic understanding into practical applications for climate-smart agriculture remains a significant challenge. This review addresses these gaps by providing a comprehensive, mechanistic framework for understanding HS action in plant stress tolerance, integrating recent advances in supramolecular chemistry, plant signaling, and microbiome science to present a unified model of HS bioactivity, critically evaluating the evidence for HS as chemical priming agents, examining the molecular and physiological mechanisms underlying HS-mediated stress tolerance, and exploring the potential for developing next-generation HS biostimulants through structural modification and synergistic formulations. Finally, it identifies priority research directions for advancing the field and maximizing the contribution of HS to sustainable, climate-resilient agriculture.

## 2. The Molecular Architecture of Humic Substances: A Structure-Function Perspective

The modern understanding of HS has shifted from the traditional concept of stable, high-molecular-weight polymers to the supramolecular model, which describes HS as dynamic assemblies of diverse, relatively small molecules associated through weak non-covalent interactions (Piccolo, 2001and 2002). This structural paradigm explains the functional flexibility of HS in soils and plant interactions. The supramolecular organization originates from the amphiphilic nature of HS components, which contain hydrophobic domains (aliphatic chains and aromatic structures) and hydrophilic functional groups

(carboxyl, hydroxyl, and phenolic groups). In aqueous environments, these molecules self-assemble through hydrophobic interactions, minimizing exposure of nonpolar regions to water and increasing system entropy through the release of ordered water molecules (Piccolo et al., 2018). Evidence supporting this model includes the disruption of HS aggregates by organic acids, concentration-, pH-, and ionic-strength-dependent changes in molecular size, and the identification of thousands of individual molecular components within HS using advanced analytical approaches (Piccolo et al., 1999; Baigorri et al., 2007; Nebbioso and Piccolo, 2011and 2012).

The humeomics approach, developed by Nebbioso and Piccolo (2011and 2012), has significantly advanced HS structural characterization by sequentially releasing molecular fractions through organic solvent extraction, transesterification, alkaline hydrolysis, and acid hydrolysis, followed by chromatographic and mass spectrometric analyses. This approach revealed that HS consists of highly diverse chemical constituents, including fatty acids, alkanes, sterols, lignin-derived phenols, carbohydrates, and nitrogen-containing compounds. Structure–function studies indicate that HS bioactivity is strongly associated with their chemical composition and molecular organization. Multivariate analyses demonstrated that the hydrophobic/hydrophilic carbon ratio, determined through $^{13}C$ nuclear magnetic resonance spectroscopy, is closely correlated with root growth stimulation and plasma membrane $H^+$-ATPase activation (Canellas et al., 2012). Additionally, methoxyl, aryl, and carboxylic acid groups are positively associated with biological activity, whereas carbohydrate-rich structures show weaker or negative relationships (Aguiar et al., 2013). These findings highlight the importance of hydrophobic domains enriched in aromatic and aliphatic structures for HS-mediated plant responses.

The biological functions of HS arise from their diverse functional groups. Carboxyl and phenolic groups provide negative charges that facilitate metal complexation, improving the availability of essential micronutrients such as Fe, Zn, Cu, and Mn (Yang et al., 2021). Quinone groups contribute to HS redox activity by mediating electron transfer reactions, enabling antioxidant or pro-oxidant behavior depending on environmental conditions (Lamar, 2020). Meanwhile, aliphatic and aromatic regions contribute to amphiphilicity and allow HS to interact with hydrophobic signaling molecules, including plant hormones. These hydrophobic domains can protect and gradually release bioactive compounds, supporting sustained biological stimulation (Piccolo et al., 2018).

The origin and extraction method of HS strongly influence their chemical composition and bioactivity. Vermicompost-derived HS generally exhibit the highest biological activity due to microbial transformation processes that generate diverse bioactive molecules and favorable supramolecular structures (Canellas and Olivares, 2014). Peat-derived HS typically contain more carbohydrate-derived structures and display moderate activity, whereas leonardite- and lignite-derived HS often show reduced bioactivity because of their condensed and recalcitrant molecular structures (Rose et al., 2014; Canellas et al., 2015 ). This variability presents challenges for standardization but enables the development of crop- and stress-specific HS formulations.

A critical aspect of HS functionality is their dynamic interaction with plant root exudates. Plants release 10–40% of photosynthetically fixed carbon into the rhizosphere as sugars, organic acids, amino acids, and secondary metabolites, which can disrupt HS supramolecular assemblies and release bioactive molecules (Piccolo, 2002; Nguyen, 2003 ). Root exudates, particularly organic acids such as citrate, malate, and oxalate, facilitate the liberation of HS-associated molecules that interact with plant receptors (Canellas et al., 2008). Evidence of a positive feedback mechanism shows that HS stimulate root exudation, while increased exudation enhances HS bioactivity (Canellas et al., 2019). Furthermore, auxin-like activity is associated with weakly bound hydrophobic fractions but disappears when strong ester and ether bonds must be cleaved, demonstrating that the spatial arrangement of molecules within the HS supramolecular structure is essential for biological function (Canellas et al., 2011). Thus, HS function as dynamic molecular systems whose structure, composition, and interaction with plants collectively regulate their biostimulant activity.

## 3. The Signaling Cascade: How Plants Perceive and Respond to Humic Substances

# 3.1 Perception and Early Signaling Events (ROS, $Ca^{2+}$, pH)

The initial events in HS perception remain incompletely characterized, but accumulating evidence points to a rapid, multi-faceted signaling response involving reactive oxygen species (ROS), calcium ($Ca^{2+}$), and pH changes that are triggered within minutes to hours of HS application. ROS are among the earliest signals elicited by HS, with studies demonstrating that treatment of maize roots with HS induces a rapid increase in $H_2O_2$ production accompanied by enhanced catalase activity (Cordeiro et al., 2011) (Fig. 1). Vermicompost-derived HS similarly modulates ROS accumulation and metabolism in rice, increasing both superoxide and $H_2O_2$ levels in specific root zones while simultaneously enhancing antioxidant enzyme activities (Garcia et al., 2016). The HS-induced ROS burst appears to be mediated by plasma membrane-localized NADPH oxidases (Rboh), with emerging evidence supporting the central role of the Rboh/ROS/auxin/$H^+$-ATPase module in root growth regulation by HS (De Souza et al., 2025; Zandonadi et al., 2025). Calcium signaling constitutes another early response, with vibrating probe studies demonstrating that HS application induces a clear peak of $Ca^{2+}$ influx in root zones where $H^+$ efflux and anion exudation occur, accompanied by increased expression of genes encoding voltage-gated $Ca^{2+}$ channels and calcium-dependent protein kinases (Ramos et al., 2015). Cytosolic pH changes represent a third early signal, with HS application triggering rapid, transient cytoplasmic acidification that may activate downstream responses (Baia et al., 2020; Felle, 2001). The integration of ROS, $Ca^{2+}$, and pH signals creates a complex early signaling network that amplifies the initial HS perception event, with ROS stimulating $Ca^{2+}$ release from intracellular stores and $Ca^{2+}$ activating NADPH oxidases in a positive feedback loop (Waszczak et al., 2018). Together, these signals activate downstream kinases and transcription factors that orchestrate the plant's adaptive response.

# 3.2 Hormonal Crosstalk: The Regulatory Hub

HS influence multiple phytohormone pathways, acting as master regulators that modulate hormonal balance and crosstalk (Fig. 1). Auxin (IAA) is the most extensively studied, with HS manifesting auxin-like activity through root elongation, lateral root formation, and root hair development (Canellas et al., 2002; Zandonadi et al., 2007). Transcriptomic analyses reveal that HS upregulate auxin-responsive genes, including SAUR and Aux/IAA families (Cha et al., 2021; Canellas et al., 2020). These effects are mediated through multiple mechanisms: presence of IAA and auxin-like molecules within HS supramolecular structure (Muscolo et al., 1998); HS-induced NO production modulating auxin signaling via *S*-nitrosylation of TIR1 receptor (Terrile et al., 2012); and HS-induced changes in auxin transport and distribution (Azevedo et al., 2019). Abscisic acid (ABA) is a key player in HS-mediated stress tolerance, with HS increasing ABA concentrations and ABA signaling required for HS-induced root hydraulic conductivity and shoot growth (Olaetxea et al., 2015 and 2019). The HS-ABA connection operates through NO and IAA pathways, with HS triggering NO that promotes IAA accumulation and activates ABA biosynthesis (Mora et al., 2014). Jasmonic acid (JA) signaling is activated through transient mild-stress mechanisms (De Hita et al., 2020), with JA-dependent regulation enhancing protective responses (Silva et al., 2023). Ethylene and salicylic acid pathways are also modulated, with HS upregulating ethylene-responsive transcription factors and pathogenesis-related proteins (Cha et al., 2021; Silva et al., 2023). The coordinated activation of multiple hormone pathways suggests HS act as broad-spectrum elicitors engaging the entire stress signaling network, shifting hormonal balance toward a state of preparedness that maintains growth while activating stress defenses (Table 1).

Table 1
Molecular Players in the HS Signaling Pathway

| Component | Examples | Functional Role | References |
|---|---|---|---|
| **ROS** | $H_2O_2$, $O_2^-$ | Early signaling; activates stress responses; modulates root growth | Cordeiro et al. (2011); Garcia et al. (2016); De Souza et al. (2025) |
| **$Ca^{2+}$** | Cytosolic $Ca^{2+}$ | Second messenger; activates CDPKs; regulates ion channels | Ramos et al. (2015); Lee and Seo (2021) |
| **pH** | Cytosolic acidification | Signal transduction; modulates ion transport | Felle (2001); Baia et al. (2020) |
| **NADPH oxidases** | Rboh | Generates ROS; mediates HS-induced root growth | Zandonadi et al. (2025); De Souza et al. (2025) |
| **CDPKs** | Calcium-dependent protein kinases | Phosphorylates downstream targets; transduces $Ca^{2+}$ signals | Ramos et al. (2015) |
| **MAPKs** | Mitogen-activated protein kinases | Signal amplification; stress response regulation | Canellas et al. (2020) |
| **Auxin (IAA)** | SAUR, Aux/IAA genes | Root growth; lateral root formation; stress responses | Canellas et al. (2002); Zandonadi et al. (2007) |
| **ABA** | ABA-responsive genes | Stomatal regulation; osmotic adjustment; stress tolerance | Olaetxea et al. (2015, 2019); Mora et al. (2014) |
| **JA** | JA-Ile; JA-responsive genes | Defense responses; stress signaling | De Hita et al. (2020); Silva et al. (2023) |
| **Ethylene** | ERF transcription factors | Stress responses; growth regulation | Cha et al. (2021) |
| **Transcription Factors** | WRKY, NAC, MYB, bZIP | Regulate stress-responsive gene expression | Canellas et al. (2020); Kimotho et al. (2019) |
| **Heat Shock Proteins** | HSP101, HSP81.1, HSP17.6 | Protein protection; thermotolerance | Cha et al. (2020, 2021) |
| **Antioxidant Enzymes** | SOD, CAT, APX, POD | ROS scavenging; oxidative stress protection | Garcia et al. (2016); Cordeiro et al. (2011) |

# 4. Physiological and Metabolic Rewiring: The Manifestations of Priming

# 4.1 Fortifying the Frontline: Antioxidant Defense and ROS Management

The enhanced antioxidant capacity induced by HS is a cornerstone of their stress-protective effects, with both enzymatic and non-enzymatic systems upregulated to manage oxidative stress. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) activities are consistently enhanced in HS-treated plants under various stress conditions (Kaya et al., 2018; Abbas et al., 2022). The ascorbate-glutathione (AsA-GSH) cycle represents a key target of HS action, with HS treatment enhancing APX, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase activities, thereby creating a more robust system for $H_2O_2$ detoxification and redox homeostasis maintenance (Ozfidan-Konakci et al., 2018; Hasanuzzaman et al., 2021). Non-enzymatic antioxidants including ascorbate, glutathione, and phenolic compounds (flavonoids and phenolic acids) accumulate in response to HS treatment, contributing to antioxidant capacity and providing precursors for lignin biosynthesis and defense compounds (Silva et al., 2023; Abu-Ria et al., 2023). The activation of antioxidant systems by HS represents a form of "pre-adaptation" that prepares plants for oxidative stress by upregulating antioxidant enzymes and metabolites in the absence of stress, creating a state of readiness for more rapid and effective responses when stress is encountered (Canellas et al., 2020). This priming effect is reflected in the significantly lower levels of lipid peroxidation (malondialdehyde) and reduced electrolyte leakage consistently observed in HS-treated plants under stress conditions (Yildirim et al., 2021; Abbas et al., 2022; Abu-Ria et al., 2024).

## 4.2 Maintaining the Balance: Osmotic Adjustment and Water Relations

Osmotic adjustment is critical for plant survival under drought and salinity stress, with HS promoting the accumulation of compatible solutes small, nontoxic molecules that lower cellular osmotic potential and protect macromolecules from stress-induced damage. Proline is the most widely studied osmoprotectant in HS action, with multiple studies reporting increased proline accumulation under drought and salinity stress (Kaya et al., 2018; Hatami et al., 2018; Yildirim et al., 2021). Proline functions as an osmolyte, molecular chaperone, and ROS scavenger, contributing to multiple stress tolerance mechanisms. The HS-induced proline increase is often associated with enhanced activity of pyrroline-5-carboxylate synthetase (P5CS), the rate-limiting enzyme in proline biosynthesis (Meng et al., 2023). However, in some cases, HS treatment reduces proline accumulation under stress, which may reflect lower stress levels due to protective effects (Abu-Ria et al., 2024). Soluble sugars including glucose, fructose, sucrose, and trehalose accumulate in HS-treated plants under stress, contributing to osmotic adjustment while serving as energy sources and signaling molecules (Chen et al., 2022; Shen et al., 2020). Trehalose has been identified as a key metabolite whose accumulation is enhanced by HS under drought stress, acting as an osmoprotectant and participating in stress signaling and carbon metabolism regulation (Chen et al., 2022).

Water relations are improved by HS through multiple mechanisms. HS-treated millet seedlings maintained higher relative water content (RWC) under drought stress, reflecting improved water uptake and retention (Shen et al., 2020). HS regulate root hydraulic conductivity through ABA-dependent modulation of aquaporin gene expression, with aquaporin regulation contributing to improved water

uptake and transport (Olaetxea et al., 2015). The combined effects of osmolyte accumulation and improved water relations result in enhanced water use efficiency (WUE) in HS-treated plants, with HS-treated maize showing higher WUE under drought stress associated with improved photosynthetic performance and reduced transpiration rates (Chen et al., 2022). These integrated responses demonstrate that HS-mediated osmotic adjustment operates through complementary mechanisms involving compatible solute accumulation, aquaporin regulation, and stomatal control, collectively enabling plants to maintain cellular hydration and metabolic function under water-limited conditions.

## 4.3 Protecting the Engine: Photosynthesis and Carbon Assimilation

Photosynthesis is a primary target of abiotic stress, and HS protect the photosynthetic apparatus through multiple mechanisms that collectively preserve carbon assimilation capacity under adverse conditions. Chlorophyll content is consistently higher in HS-treated plants under stress, with SPAD values typically elevated, reflecting reduced chlorophyll degradation through HS-induced inhibition of chlorophyllase activity and protection of chloroplast membranes from oxidative damage (Shen et al., 2020; Chen et al., 2022; Li et al., 2023). Photosystem II (PSII) efficiency, measured as Fv/Fm and other chlorophyll fluorescence parameters, is protected by HS treatment, with HS-treated rapeseed and millet maintaining higher PSII activity, enhanced electron transport rates, and reduced non-photochemical quenching under drought stress (Lotfi et al., 2018; Shen et al., 2020). These parameters indicate that HS protect the photosynthetic apparatus from stress-induced damage, maintaining the efficiency of light capture and energy conversion. Rubisco activity is enhanced by HS treatment under stress, with HS-treated maize showing higher Rubisco activity associated with increased $CO_2$ assimilation rates, reflecting both direct effects on enzyme activity and indirect effects through upregulation of Rubisco small subunit genes (Chen et al., 2022). Stomatal conductance is often maintained at higher levels in HS-treated plants under stress, allowing for continued $CO_2$ uptake, with this effect mediated by HS-induced changes in ABA signaling that regulate stomatal aperture (Shen et al., 2020; Chen et al., 2022). The maintenance of stomatal conductance, combined with protected photosynthetic machinery, results in higher net photosynthetic rates in HS-treated plants under stress. The protection of photosynthesis by HS has significant implications for plant productivity, as maintaining carbon assimilation under stress enables continued growth and biomass accumulation, leading to improved yield under adverse conditions.

## 4.4 Nutri-Priming: Enhancing Nutrient Acquisition and Homeostasis

HS enhance nutrient acquisition through multiple mechanisms, including improved root architecture, increased nutrient transporter expression, and enhanced nutrient bioavailability. These effects contribute to improved plant nutrition under both optimal and stress conditions. Root architecture is profoundly modified by HS, with increased root length, surface area, and branching (Nabi et al., 2025; Maffia et al., 2025). These changes increase the soil volume explored by roots and enhance nutrient and water uptake

capacity. The auxin-like activity of HS is a key driver of these root architectural changes, which are mediated through NO and auxin signaling pathways.

Nutrient transporter expression is upregulated by HS. Quaggiotti et al. (2004) showed that HS treatment upregulated the expression of nitrate transporters (NRT2.1) in maize roots (Balusamy et al., 2026). Jannin et al. (2012) reported that HS upregulated nitrate transporters (NRT1.1, NRT2.1) and sulfate transporters (SULTR1.1, SULTR1.2) in *Brassica napus*. Zanin et al. (2018) found that HS upregulated phosphate transporters (PT2) in tomato. These effects are mediated through HS-induced activation of $H^+$-ATPase, which creates the electrochemical gradient required for nutrient uptake.

Nutrient bioavailability is enhanced by HS through chelation and complexation. HS form stable complexes with micronutrients such as Fe, Zn, Cu, and Mn, preventing their precipitation and maintaining them in bioavailable forms (Chen et al., 2004; Zanin et al., 2019). This is particularly important under alkaline soil conditions where micronutrient availability is limited. HS also enhance phosphorus availability by competing with phosphate for adsorption sites on soil particles and by forming soluble metal-phosphate-HS complexes (Yang et al., 2021). Ionic homeostasis under salinity stress is improved by HS. (Abbas et al. 2022) showed that HS treatment reduced $Na^+$ accumulation and increased $K^+$ accumulation in wheat under salinity stress, resulting in improved $K^+/Na^+$ ratios. This effect is mediated through HS-induced upregulation of high-affinity $K^+$ transporters (HKT1) and salt overly sensitive (SOS) pathway components (Khaleda et al., 2017; Souza et al., 2021). The maintenance of ionic balance is critical for salt tolerance, as excessive $Na^+$ accumulation disrupts cellular metabolism and causes toxicity.

The combined effects of improved root architecture, enhanced transporter expression, and increased nutrient bioavailability result in improved nutrient use efficiency (NUE) in HS-treated plants. This has significant implications for sustainable agriculture, as improved NUE reduces the need for fertilizer applications and associated environmental impacts.

# 4.5 Secondary Metabolism: A Defense Arsenal

Humic substances induce the phenylpropanoid pathway, leading to increased synthesis of phenolic compounds, flavonoids, and lignin that play diverse roles in plant defense against biotic and abiotic stresses. Phenylalanine ammonia-lyase (PAL), the key enzyme catalyzing phenylalanine conversion to trans-cinnamic acid, is upregulated by HS treatment in maize and citrus, resulting in increased total phenolic content (Schiavon et al., 2010; Silva et al., 2023). Phenolic compounds, including flavonoids, phenolic acids, and lignin precursors accumulate in HS-treated plants, contributing to antioxidant capacity against oxidative stress while serving as precursors for lignin biosynthesis that strengthens cell walls and provides physical barriers against pathogen invasion (Schiavon et al., 2010; Verrillo et al., 2021). Lignin deposition is enhanced by HS treatment through upregulation of laccase and peroxidase genes involved in lignin polymerization, with increased lignification reducing water loss under drought and providing protection against pathogen attack (Arslan et al., 2021). Pathogenesis-related (PR) proteins are induced by HS treatment, with PR-1, PR-2 (β-1,3-glucanase), and PR-3 (chitinase) expression

upregulated in citrus leaves (Silva et al., 2023). These PR proteins are integral components of plant defense against pathogens and are also involved in abiotic stress tolerance. The activation of secondary metabolism by HS represents a form of "defense priming" that prepares plants for both biotic and abiotic stresses by upregulating the phenylpropanoid pathway and PR protein expression in the absence of stress, creating a state of readiness that enables more rapid and effective defense responses when stress is encountered.

## 5. The Microbiome Dimension: A Tripartite Interaction

# 5.1 Humic substances as Microbial Modulators: The Rhizosphere Effect

Humic substances influence rhizosphere microbial communities, creating feedback loops that amplify their beneficial effects on plants. Microbial community composition is significantly altered by HS treatment, with studies demonstrating changes in bacterial community structure in the maize rhizosphere dependent on HS molecular size (Yuan et al., 2026). Humic substances enrich the rice microbiome with bacterial taxa associated with plant defense and growth promotion, including *Pseudomonas* and *Chitinophaga* (da Silva et al., 2021), while increasing beneficial bacterial genera such as Firmicutes, Basidiomycota, and Mortierellomycota in continuous cropping systems (Li et al., 2019). Microbial activity is stimulated as HS serve as carbon and energy sources for soil microorganisms, promoting growth and metabolic activity (Pukalchik et al., 2019). The redox-active functional groups in HS serve as electron donors for bacterial respiration, supporting microbial metabolism (Zhao et al., 2026), while enhancing enzyme activities involved in nutrient cycling, including urease, phosphatase, and dehydrogenase (Pukalchik et al., 2019). Plant growth-promoting rhizobacteria (PGPR) are enriched by HS treatment, with combinations often producing synergistic effects on plant growth and stress tolerance (da Silva et al., 2021). Humic substances enhance PGPR survival and colonization by providing nutrients and physical protection (Beheshti et al., 2026), while PGPR enhance the release of bioactive molecules from HS through organic acid and enzyme production, creating mutualistic positive feedback loops. The multifaceted mechanisms by which HS modulate the microbiome include providing physical habitat, protecting microorganisms from predation and environmental stress, serving as a carbon source supporting microbial growth, chelating metals to reduce toxicity, and acting as signaling molecules influencing microbial gene expression and behavior.

# 5.2 The Synergy with PGPR: A Two-Pronged Approach

The combination of HS with PGPR offers a powerful strategy for enhancing plant growth and stress tolerance through complementary mechanisms and positive interactions. Enhanced root colonization by PGPR is promoted by HS, with studies demonstrating that HS increase colonization of maize roots by *Herbaspirillum seropedicae* through HS-induced changes in root morphology and exudation that create favorable conditions for bacterial attachment and proliferation (Canellas and Olivares, 2017). Improved nutrient acquisition results from combined effects, with HS enhancing nutrient bioavailability through

chelation and complexation while PGPR increase nutrient availability through nitrogen fixation, phosphate solubilization, and siderophore production, leading to greater nutrient uptake and improved plant nutrition (da Silva et al., 2021). Enhanced stress tolerance is achieved through complementary mechanisms, with HS priming plant stress response systems while PGPR produce stress-protective compounds such as ACC deaminase that reduces ethylene levels under stress, with the combination also enhancing antioxidant enzyme activities and osmolyte accumulation to provide multiple protection layers (da Silva et al., 2021). Biocontrol activity is enhanced as HS induce systemic resistance while PGPR produce antimicrobial compounds and compete with pathogens for nutrients and space, resulting in improved disease resistance and reduced pathogen pressure (Silva et al., 2023). The synergy between HS and PGPR has been demonstrated across multiple crop species, with combinations increasing tomato production by 44–80% compared to controls and enhancing maize growth and nitrogen use efficiency (Canellas et al., 2013; Olivares et al., 2015 ). These findings highlight the substantial potential of integrated HS-PGPR formulations for sustainable agriculture, offering a dual-pronged approach that simultaneously enhances plant nutrition, stress tolerance, and disease resistance while reducing dependence on synthetic inputs.

## 5.3 The "DefenseBiome": Recruiting Microbial Allies

The concept of the "DefenseBiome" extends the priming effect from the plant to its associated microbiome, with HS acting as "microbiome engineers" that shape plant-associated microbial communities to enhance stress tolerance and disease resistance. Endophytic microbiome composition is significantly influenced by HS treatment, with HS application enriching the rice endophytic microbiome with bacterial taxa associated with defense and stress tolerance, reflecting HS-induced changes in root exudation that selectively attract beneficial microorganisms (da Silva et al., 2021). Disease suppression is enhanced through microbiome-mediated mechanisms, with HS treatment of citrus inducing systemic resistance and enhancing defense-related enzyme activity while reducing disease symptoms, effects associated with rhizosphere microbiome changes that suggest HS act through both direct and microbiome-mediated mechanisms (Silva et al., 2023). Stress tolerance may be enhanced by HS-induced recruitment of stress-tolerant microorganisms, with enrichment of microorganisms possessing stress-protective traits such as osmoprotectant production and antioxidant activity providing an additional protection layer beyond direct physiological effects. While the mechanisms by which HS recruit beneficial microorganisms are not fully understood, the available evidence suggests HS can act as "microbiome engineers" that shape the plant-associated microbial community in ways that enhance stress tolerance and disease resistance. The emerging DefenseBiome concept represents a paradigm shift in understanding HS action, highlighting that the beneficial effects of HS on plant stress tolerance may be partially mediated through their influence on the plant microbiome, creating opportunities for developing integrated HS-microbiome management strategies for sustainable agriculture.

# 6. From Lab to Field: Translating Mechanisms into Applications

# 6.1 Navigating the Complexity: The Influence of Dose, Source, and Mode of Application

The effectiveness of HS is highly context-dependent, influenced by dose, source, molecular weight, application method, and environmental conditions. Understanding these factors is essential for optimizing HS applications and ensuring consistent, predictable benefits. Dose-response relationships for HS typically follow a hormetic (bell-shaped) curve, with optimal effects at intermediate concentrations and inhibitory effects at high concentrations (Canellas et al., 2020; Rose et al., 2014). The optimal concentration varies depending on the HS source, plant species, and growth conditions, but is typically in the range of 10–100 mg $CL^{-1}$ for liquid applications. The hormetic response reflects the dual role of HS as both signaling molecules and mild stressors; at appropriate concentrations, HS activate beneficial stress responses, while at high concentrations, they cause excessive stress and growth inhibition. HS source significantly influences bioactivity, with vermicompost-derived HS generally exhibiting greater effects than HS from peat, leonardite, or coal (Canellas and Olivares, 2014; Rose et al., 2014). The differences in bioactivity reflect variations in chemical composition, functional group distribution, and supramolecular organization. The source of HS should be considered when selecting products for specific applications. Molecular weight influences HS bioactivity, with low molecular weight (LMW) fractions generally being more bioactive than high molecular weight (HMW) fractions (Canellas et al., 2010; Nardi et al., 2021). The LMW HS can enter plant cells and directly trigger signaling cascades, while HMW HS interact with cell surface receptors. However, the distinction between LMW and HMW HS is somewhat artificial, as the supramolecular structure of HS allows for the release of LMW components upon interaction with root exudates. Application method affects HS efficacy. Soil application allows for direct interaction with roots and the rhizosphere microbiome, while foliar application enables rapid uptake and systemic effects. Seed treatment provides early protection during germination and seedling establishment. The optimal application method depends on the target crop, the stress of interest, and practical considerations such as equipment availability and labor costs. Timing of application is critical for maximizing HS benefits. Early application during seedling establishment is generally most effective, as it allows for the establishment of the primed state before stress occurs (Qin and Leskovar, 2020). Multiple applications throughout the growing season may provide sustained benefits, particularly under prolonged stress conditions.

# 6.2 Engineering the Humic Molecule: Modification and Formulation

Humic substances can be modified through chemical, physical, and biological approaches to enhance functionality and tailor applications, representing a frontier in HS research and product development. Chemical modification enhances HS bioactivity by introducing new functional groups or altering existing ones. Oxidation with hydrogen peroxide, ozone, or potassium permanganate increases carboxyl and phenolic group content, enhancing metal chelation and antioxidant capacity (Sarlaki et al., 2024). Nitrogen functionalization through introduction of nitro, nitroso, amide, or amine groups enhances nutrient interactions and enables slow-release nitrogen fertilizer development (Sarlaki et al., 2023).

Sulfonation introduces sulfonic acid groups, increasing solubility and cation exchange capacity. Physical modification alters HS molecular size and conformation, with ultrasonication reducing molecular size and increasing solubility to potentially enhance bioactivity (Al-Akbari et al., 2024). Nanotechnology approaches, including nano-HS production, can improve delivery efficiency and bioavailability, though environmental and health implications require careful consideration. Biological modification through microbial or enzymatic treatment can alter HS structure and enhance bioactivity, with microbial incubation introducing new functional groups and increasing bioactive molecule diversity (Kluczek-Turpeinen et al., 2005). Enzymatic treatment with laccases or peroxidases can polymerize phenolic compounds, altering supramolecular structure and potentially enhancing bioactivity (Aranaz et al., 2023). Formulation with other biostimulants or adjuvants can enhance HS efficacy, with co-formulation with PGPR, seaweed extracts, or protein hydrolysates producing synergistic effects (da Silva et al., 2021; Atero-Calvo et al., 2024). Surfactants or penetrants can improve foliar uptake and translocation, while encapsulation in biodegradable carriers can provide controlled release and protect HS from degradation. These modification approaches offer substantial opportunities for developing next-generation HS products with enhanced functionality, targeted applications, and improved efficacy, though systematic comparisons across modification strategies and rigorous environmental safety assessments remain essential for translating these advances into practical agricultural applications.

## 6.3 Integrated Stress Management: The Power of Combination

HS are most effective when integrated into comprehensive stress management strategies that combine multiple complementary approaches, recognizing that stress tolerance is a complex trait influenced by genetics, environment, and management. Combination with other biostimulants produces synergistic effects, with HS combined with seaweed extracts, protein hydrolysates, or silicon-based biostimulants enhancing stress tolerance beyond individual components through complementary mechanisms providing multiple protection layers against stress (Atero-Calvo et al., 2024; Abbas et al., 2022). Combination with PGPR is particularly promising, with synergistic effects reflecting complementary modes of action and positive interactions, making co-formulation of HS and PGPR an active area of product development with several commercial products now available (da Silva et al., 2021). Integration with broader management practices enhances HS efficacy, as crop rotation, cover cropping, conservation tillage, and organic amendments all contribute to soil health and enhance HS benefits, while integration with precision agriculture tools such as variable-rate application and sensor-based monitoring can optimize HS use and maximize returns. Tailoring to specific stress scenarios is critical for maximizing HS benefits, as different HS sources and formulations may be more effective against specific stresses, with high antioxidant capacity HS particularly effective against oxidative stress and high metal chelation capacity HS more effective for heavy metal stress management. The integrated approach to HS application represents a paradigm shift from single-input solutions to holistic management strategies that leverage the complementary benefits of multiple approaches, offering substantial potential for sustainable agriculture by simultaneously enhancing plant resilience, improving resource use efficiency,

and reducing dependence on synthetic inputs while adapting to specific crop, stress, and environmental contexts.

## 7. Applications of Humic Substances in Enhancing Plant Abiotic Stress Tolerance

Humic substances have emerged as versatile biostimulants capable of enhancing plant tolerance across a wide spectrum of abiotic stresses, including drought, salinity, heat, cold, heavy metal toxicity, and nutrient limitation (Fig. 2 and Table 2). The mechanisms underlying these protective effects are multifaceted, involving coordinated physiological, biochemical, and molecular reprogramming that collectively prepare plants for stress encounters—a phenomenon increasingly recognized as "chemical priming" or "eustress" induction (Ibraeva et al., 2026). The efficacy of HS is highly dependent on factors such as source, concentration, application method, and plant species, with recent studies highlighting significant genotype-specific responses (Jaros-Tsoj et al., 2026).

# 7.1. Drought Stress

Drought stress severely limits crop production, reducing growth parameters and triggering oxidative damage. Humic substances application has demonstrated consistent protective effects across multiple species, primarily by enhancing the plant's antioxidant defense and osmotic adjustment systems. In faba bean, foliar-applied AsAand soil-applied humic acid (HA) significantly improved drought tolerance across three cultivars by enhancing physiological performance and mitigating oxidative damage under moderate (300 mm) and severe (200 mm) drought conditions (Al-Demrdash et al., 2025). Drought stress significantly reduced chlorophyll content (up to − 57.5%), RWC (− 37.9%), and yield traits such as plant height (− 9.6%) and seed yield (− 20.8%), while increasing oxidative stress markers like MDA (+ 192.8%) and hydrogen peroxide ($H_2O_2$, + 105.0%). Ascorbic acid and HA alleviated these effects, improving chlorophyll retention (up to + 33.7%), water status (+ 17.0%), and reducing MDA (− 19.1%) and electrolyte leakage (− 11.5%). Enhanced accumulation of proline (+ 27.4%) and soluble sugars (+ 18.0%) contributed to improved osmotic balance, while antioxidant enzyme activities (SOD, CAT, POD, APX, and GR) were also upregulated, particularly with AsA. The study identified Nubaria-5 as the most drought-resilient cultivar, exhibiting superior pigment stability, antioxidant activity, and yield preservation across stress conditions (Al-Demrdash et al., 2025).

*In vitro* studies on soybean have demonstrated that HA with molecular weights greater than 30 kDa and unfractionated HA (Mix) provide the greatest protective and stimulatory effects under drought stress induced by mannitol (Kulpa et al., 2025). Drought stress reduced plant height by 24%, root length by 55%, number of leaves by 36%, root weight by 50%, and above-ground biomass by 43%. However, HA application led to average increases of 20–40% in analyzed biometric parameters, with the HA Mix showing an exceptional 87% increase in leaf number under drought conditions (Kulpa et al., 2025). These protective effects were associated with enhanced stomatal density, with HA treatments increasing this parameter by up to 234% compared to stress-only treatments.

The mechanisms through which HS enhance drought tolerance are multifaceted. Fulvic acid (FA) acts as a mobile electron shuttle in redox-active soils, mediating extracellular electron transfer between metal-reducing microorganisms and mineral electron acceptors, thereby influencing metal mobilization and nutrient availability (Ahmed et al., 2026). FA's higher carboxyl functional group density and oxidation state confer superior electron-accepting and electron-donating properties, enabling rapid diffusion between microbial cells and mineral surfaces and sustaining efficient redox cycling under fluctuating moisture conditions (Ahmed et al., 2026). Furthermore, HS application stimulates plasma membrane $H^+$-ATPase activity, leading to enhanced proton extrusion and localized rhizosphere acidification under nutrient-deficient conditions, which strengthens the transmembrane electrochemical gradient required for secondary active transport (Ahmed et al., 2026).

# 7.2. Salinity Stress

Salt stress induces ionic imbalance, osmotic stress, and oxidative damage. Humic substances mitigate these effects through coordinated regulation of ion homeostasis, osmolyte accumulation, and antioxidant defense. In soybean *in vitro* cultures, salt stress (100 mmol·dm$^{-3}$ NaCl) significantly reduced plant height by 80%, root length by 65%, number of leaves by 79%, root weight by 51%, and above-ground biomass by 85% (Kulpa et al., 2025). However, HA treatments, particularly the > 30 kDa fraction and unfractionated HA Mix, demonstrated significant protective effects, increasing plant height by 58–156%, number of leaves by 119–165%, and fresh mass of above-ground parts by 44–122% compared to salt-stressed plants. Root-related parameters showed more modest improvements, with root mass and length increasing by 16–39% and 14–30%, respectively (Kulpa et al., 2025).

The protective mechanisms of HS under salinity involve modulation of ion uptake. Salt stress increased sodium (Na) content in soybean seedlings by approximately 350%, while reducing potassium (K), magnesium (Mg), and calcium (Ca) content by 19.3%, 27.5%, and 28.9%, respectively (Kulpa et al., 2025). Humic acid fractions, particularly the HA Mix, significantly reduced Na uptake by more than 30% while maintaining K content at or above control levels. The unfractionated HA Mix showed the broadest protective effect, restoring Na and Mg to control levels under drought conditions and significantly increasing Ca content. For micronutrients, the > 30 kDa fraction demonstrated a pronounced regulatory effect, mitigating the negative impact of salinity on Zn and strongly increasing Fe uptake, consistent with the proposed chelating and Fe-mobilizing role of high-molecular-weight humic compounds (Kulpa et al., 2025).

Stomatal regulation represents another critical mechanism of HS-mediated salinity tolerance. Salt stress significantly reduced stomatal length by 16% and stomatal density by 40% in soybean (Kulpa et al., 2025). However, HA application, particularly the > 30 kDa fraction, increased stomatal length by 15% and stomatal density by 144% compared to stress-only treatments, and by 47% compared to control plants. These improvements in stomatal parameters contribute to enhanced photosynthetic efficiency and water-use efficiency under saline conditions (Kulpa et al., 2025).

The structural and chemical properties of HS are fundamental to their protective functions. Humic substances contain multiple functional groups, including carboxyl, phenolic, hydroxyl, carbonyl, and quinone moieties, which enable them to bind various components and form stable complexes with metal ions (Ibraeva et al., 2026). The carboxylic and phenolic hydroxyl groups in HS form metal ion complexes that transform functional properties into less toxic and less mobile forms, improving soil fertility through nutrient release. Additionally, HS exhibit ion-exchange properties, with negatively charged surfaces enabling cation exchange that improves soil fertility and maintains nutrient availability under saline conditions (Ibraeva et al., 2026).

## 7.3. Heat Stress

Rising global temperatures pose increasing threats to crop production. Humic substances enhance heat stress tolerance through multiple protective mechanisms, including the transcriptional activation of heat-shock proteins (HSPs). In *Arabidopsis*, HA was found to enhance heat stress tolerance via transcriptional activation of several HSP coding genes, including *HSP101*, *HSP81.1*, *HSP26.5*, *HSP23.6*, and *HSP17.6A* (Ibraeva et al., 2026). This molecular response is crucial for preventing protein denaturation under high temperatures. The protective mechanisms include: (i) upregulation of heat shock proteins that prevent protein denaturation; (ii) enhanced antioxidant defense to scavenge heat-induced ROS; (iii) maintenance of membrane integrity and photosynthetic efficiency; and (iv) accumulation of compatible solutes that stabilize proteins and membranes under high temperatures.

Research has demonstrated that HS facilitate plant adaptation to heat stress by stimulating various physiological responses, including the generation of ROS, which play a crucial role in signaling pathways that help plants cope with stress (Ibraeva et al., 2026). The quinone and semiquinone structures in HS function as pro-oxidant sites, attracting electrons and causing the development of temporary ROS, establishing redox signaling in plant roots. Conversely, phenolic hydroxyl groups serve as antioxidant sites, donating electrons to radicals and effectively neutralizing them while preserving redox balance throughout cells. This dual pro-oxidant and antioxidant activity enables HS to establish an optimal balance between stress signaling and protection (Ibraeva et al., 2026).

## 7.4. Cold Stress

Low-temperature stress disrupts membrane structure, induces oxidative damage, and inhibits photosynthesis. Humic substances improve cold tolerance through multiple mechanisms, including regulation of nitrogen metabolism, proline synthesis, and activation of C-repeat binding factor (CBF) pathways. In melon seedlings, HA alleviated low-temperature stress by regulating nitrogen metabolism and proline synthesis, significantly increasing the expression of genes related to these pathways and enhancing antioxidant enzyme activities (Zhu et al., 2024).

In oat, different application methods of HA and zinc differentially regulate osmotic balance and the CBF pathways under cold stress. Soil application of HA + Zn provided the greatest protection, as evidenced by improved membrane durability index, RWC, and proline levels, and a stronger induction of *CBF* genes (Zhu et al., 2024). These findings indicate that HS reprogram plant metabolism and modulate genes

involved in ROS scavenging, phytohormone metabolism, and signaling pathways, with soil application often proving more effective than foliar application for cold stress mitigation.

The supramolecular nature of HS is critical to understanding their biological activity. Contemporary evidence suggests that humic compounds can be defined as groups of different kinds of small organic molecules that remain together through weak, noncovalent interactions such as hydrogen bonding, metal bridging, and hydrophobic forces (Ibraeva et al., 2026). This perspective considers HA and fulvic acids (FA) as operationally defined fractions whose characteristics vary according to their extraction methods rather than legitimate polymers with stable molecular structures. The supramolecular view more accurately represents HS in natural soil systems, explaining their variable biological activity and the importance of extraction methods in determining their efficacy (Ibraeva et al., 2026).

# 7.5. Heavy Metal Toxicity

Heavy metal contamination poses significant threats to crop production and food safety. Humic substances mitigate heavy metal toxicity through two primary mechanisms: (i) immobilization of metals in soil through complexation and chelation, reducing bioavailability; and (ii) activation of plant antioxidant defenses to counteract metal-induced oxidative stress. High-molecular-weight humic acid tends to form stable macromolecular complexes with heavy metals like cadmium (Cd) and lead (Pb), effectively immobilizing them and reducing their bioavailability (Jaros-Tsoj et al., 2026; Li et al., 2022). The functional groups of HS (carboxyl, phenolic, hydroxyl) are key to forming stable complexes with heavy metals, preventing their uptake and translocation.

A field study on metal-contaminated soil (Zn: 8057 mg $kg^{-1}$, Pb: 2940 mg $kg^{-1}$, Cd: 51.56 mg $kg^{-1}$) investigated the effects of HS, alone or combined with mycorrhizal inoculation, on oxidative stress and antioxidative responses in *Cannabis sativa*, *Sorghum sudanense × bicolor*, and *Miscanthus × giganteus* (Jaros-Tsoj et al., 2026). The results demonstrated strong species-specific responses. In hemp, biostimulants increased Cd and Pb concentrations in leaves, which were associated with increased SOD, APX, and GR activities (by 33–267%), without affecting growth. In sorghum, biostimulants increased proline and sugars (by up to 55% and 80%, respectively), accompanied by reduced oxidative stress indicators and improved biomass (by 26%). In miscanthus, increased lipid peroxidation (by 37–60%) occurred alongside enhanced GR and APX activities, indicating distinct physiological strategies of stress adaptation (Jaros-Tsoj et al., 2026).

The mechanisms of HS-mediated heavy metal tolerance involve both soil-level and plant-level processes. In soil, HS form soluble metal-organic complexes that buffer ion activity, limit precipitation, and sustain nutrient diffusion toward the root surface (Ahmed et al., 2026). Under acidic conditions (pH ≤ 3), protonation of FA functional groups reduces electrostatic binding capacity, resulting in comparatively weak chelation. However, at near-neutral pH (≈ 6–7), progressive deprotonation of carboxyl and phenolic groups enables stable bidentate and bridging coordination with essential micronutrients and toxic metals (Ahmed et al., 2026; Li et al., 2022). This pH-dependent chelation is fundamental to HS-mediated metal detoxification, as it regulates metal mobility and bioavailability in the rhizosphere.

At the plant level, HS application enhances the activity of antioxidant enzymes that detoxify ROS and support osmotic balance, hormone signaling, and cellular stability (Ahmed et al., 2026). This enzymatic action is complemented by elevated non-enzymatic antioxidants such as glutathione and ascorbate, which reinforce redox homeostasis. Humic substances also facilitate osmotic balance by increasing the accumulation of compatible solutes, including proline, soluble sugars, and glycine betaine, which stabilize cellular proteins, membranes, and turgor under metal-induced osmotic stress (Ahmed et al., 2026). The hormonal regulation mediated by HS, including modulation of ABA, SA, and JA levels, coordinates stomatal behavior, defense gene expression, and stress-responsive signaling, contributing to enhanced metal tolerance.

## 7.6. Nutrient Stress

Humic substances enhance nutrient use efficiency under both optimal and limiting conditions, playing a significant role in nitrogen metabolism, particularly under stress. The mechanisms through which HS improve nutrient acquisition include: (i) stimulation of plasma membrane $H^+$-ATPase activity, leading to enhanced proton extrusion and rhizosphere acidification; (ii) strengthening of the transmembrane electrochemical gradient for secondary active transport; (iii) chelation of nutrients to maintain solubility and prevent precipitation; and (iv) modulation of nutrient transporter expression and uptake efficiency (Ahmed et al., 2026).

In melon seedlings under low-temperature stress, HA promoted the degradation of nitrate nitrogen ($NO_3^-$-N), the synthesis of ammonium nitrogen ($NH_4^+$-N), free amino acids, proline, and soluble protein (Kulpa et al., 2025). This was associated with increased activity of key nitrogen metabolism enzymes (NR, GS, GOGAT, GDH) and proline-related enzymes (P5CS, OAT). This regulatory effect on nitrogen assimilation helps plants maintain metabolic function and build stress resilience, highlighting the critical role of HS in optimizing nitrogen use efficiency under challenging conditions.

The molecular mechanisms underlying HS-mediated nutrient uptake involve modulation of transporter gene expression. FA application under low-phosphorus conditions increased phosphorus uptake in rice and was accompanied by upregulation of phosphate transporter genes (*OsPT1*, *OsPT2*, *OsPT4*, *OsPT8*) together with plasma membrane $H^+$-ATPase genes (Ahmed et al., 2026). For nitrogen acquisition, purified FA enhanced nitrate influx in apple rootstock seedlings and increased transcript abundance of nitrate transporter genes (*MdNRT1.1*, *MdNRT1.2*, *MdNRT2.1*), indicating improved coupling between membrane bioenergetics and transporter-mediated uptake. These findings demonstrate that HS reinforce canonical nutrient-deficiency programs rather than bypassing them, acting through bioenergetic and signaling modulation rather than direct genetic regulation (Ahmed et al., 2026).

## 7.7. Species-Specific Responses and Integrated Mechanisms

The efficacy of HS in enhancing abiotic stress tolerance is strongly species-specific, reflecting differences in intrinsic stress tolerance strategies, metabolic regulation, and interactions with the soil

microbiome (Jaros-Tsoj et al., 2026). In field studies, sorghum exhibited the most pronounced positive responses to HS application, including improved growth, decreased lipid peroxidation, and increased sugar and proline concentrations, despite elevated leaf metal concentrations. This response was associated with a strong non-enzymatic antioxidant system that may compensate for the lack of induction of antioxidant enzymes. In contrast, hemp responded primarily through activation of enzymatic antioxidant defenses, with increased SOD, APX, and GOPX activities, representing a potentially more costly stress response strategy. Miscanthus maintained biomass despite oxidative signals and relatively limited physiological responses, indicating a stable tolerance-based strategy rather than a predominantly stress-responsive profile (Jaros-Tsoj et al., 2026).

Principal component analysis (PCA) confirmed strong species-specific responses to biostimulant application, with species-specific traits being the primary drivers of variability in the dataset (Jaros-Tsoj et al., 2026). In hemp, biostimulant application, particularly HS combined with mycorrhiza (HS + M), was correlated with increased activities of APX, SOD, GOPX, and GR, together with higher proline concentrations and elevated metal accumulations in leaf tissues. In sorghum, HS treatment was positively correlated with plant height, proline and sugar concentrations, as well as Cd, Pb, and Zn concentrations, while being negatively correlated with TBARS concentrations. In miscanthus, a different pattern was observed, with HS + M treatment characterized by positive correlations with TBARS concentration, APX activity, and plant height, and negative correlations with CAT and SOD activities (Jaros-Tsoj et al., 2026).

These species-specific responses highlight that oxidative stress-related parameters are not universal biomarkers of stress tolerance but rather reflect distinct physiological strategies for coping with environmental challenges. The lack of consistent synergistic effects when HS were combined with mycorrhizal inoculation further emphasizes the complex nature of plant-biostimulant interactions and the importance of considering species-specific strategies when selecting crops for cultivation on marginal lands (Jaros-Tsoj et al., 2026).

**Molecular Mechanisms and Signaling Pathways**

The molecular mechanisms underlying HS-mediated stress tolerance involve complex transcriptional networks and signaling pathways. Humic substances influence plant physiology via regulation of the expression of numerous genes, most of which are involved in the cell cycle, meristem organization, and cytoskeleton dynamics (Ibraeva et al., 2026). The biological activity of HS stems from their multiple functional groups, colloidal behavior, and diverse chemical composition, which enable participation in chemical redox reactions, formation of organometallic complexes, influence on osmotic pressure, enzyme-substrate interactions, and detoxification of xenobiotics (Ibraeva et al., 2026).

Calcium ($Ca^{2+}$) signaling represents a potential early signaling layer in plant responses to HS treatment. Studies using humic substance fractions, including fulvic-rich extracts, have reported rapid alterations in $Ca^{2+}$ fluxes in root tissues accompanied by increased activity of calcium-dependent protein kinases (CDPKs/CPKs) and modulation of $Ca^{2+}$-related gene expression during early growth responses (Ahmed

et al., 2026). Elevated cytosolic $Ca^{2+}$ is decoded by CDPKs through EF-hand motifs, triggering phosphorylation of downstream targets involved in ion transport regulation, redox balance, and stress-responsive gene expression. Because $H^+$-ATPase activation, reactive oxygen species dynamics, and hormonal signaling networks can all intersect with $Ca^{2+}$-dependent pathways, HS-associated responses may involve coordinated integration of these signaling modules (Ahmed et al., 2026).

The entry of HS into plants leads to wall hydrophilization of conducting tissues, which enables pinocytosis and affects bioelectric responses, osmotic pressure, and ion transport, thus speeding up nutrient distribution throughout the plant (Ibraeva et al., 2026). The improved nutrient transport system allows plants to perform photosynthesis at elevated levels and take in minerals better, leading to faster plant development and growth. The biostimulatory activity of HS is most pronounced in fractions with relatively low molecular weight, as low-molecular-weight HS penetrates plant cells more readily and moves through the symplastic pathway to directly influence plant metabolism, whereas high-molecular-weight HS moves through the apoplast and affects processes related to plant cell wall functioning (Ibraeva et al., 2026).

Table 2
Representative Studies on Humic Substance-Mediated Abiotic Stress Tolerance in Crop Plants

| Type of HS | Target Plant | Application Method & Dose | Primary Mechanisms | Key Results | References |
|---|---|---|---|---|---|
| **Salinity Stress** | | | | | |
| Humic acid | Maize (*Zea mays*) | HA at 50 mg/L, single application | Enhanced seed germination and seedling growth | Increased germination and growth under saline conditions | Yang et al. (2023) |
| Humic acid | Quinoa (*Chenopodium quinoa*) | HA at 1% (v/v), weekly for 60 days (~ 1 L/m²) | Improved growth parameters | Enhanced plant height, fresh weight, and dry matter | Rekaby et al. (2023) |
| Humic acid | Rice (*Oryza sativa*) | HA at 100 mg/L, at transplanting for 30 days | Antioxidant enzyme activation; root development | Improved antioxidant enzyme activity and root growth | Abu-Ria et al. (2023) |
| Humic acid | Wheat (*Triticum aestivum*) | HA at 200 mg/kg soil, single application for 45 days | Enhanced nutrient uptake and productivity | Increased grain yield and overall productivity | Huang (2022) |
| Humic acid | Papaya (*Carica papaya*) | HA at 3.5 mL/L, biweekly foliar spray for 8 weeks | Photosynthesis enhancement; stress alleviation | Alleviated salt stress, promoted growth, enhanced photosynthesis | Silva et al. (2025) |
| Humic substances | Papaya (*Carica papaya*) | HS at 20 g/kg, soil mixed before planting for 60 days | Improved photosynthetic efficiency | Enhanced $CO_2$ assimilation, WUE, and chlorophyll under saline conditions | Dias et al. (2020) |
| Humic acid (fractions) | Soybean | HA > 30 kDa and Mix | Reduced $Na^+$ uptake; maintained $K^+$ homeostasis; enhanced stomatal traits | 58–156% increase in plant height; 144% increase in stomatal density; >30% $Na^+$ reduction (Mix) | Kulpa et al. (2026) |
| Humic substances | Various crops | Radicular/foliar application | Reduced $Na^+$ translocation; enhanced $K^+$ accumulation; improved | Decreased $Na^+/K^+$ ratio; improved Rubisco efficiency and | Ahmed et al. (2026) |

| Type of HS | Target Plant | Application Method & Dose | Primary Mechanisms | Key Results | References |
|---|---|---|---|---|---|
| **Salinity Stress** | | | | | |
| | | | photosynthetic efficiency | PSII performance | |
| **Drought Stress** | | | | | |
| Humic acid | Maize (*Zea mays*) | HA at 45 kg/ha, soil at sowing for 90 days | Improved nutrient availability; Rubisco activation; osmolyte accumulation | Improved nutrient availability, WUE, Rubisco activity, sugars, and osmolyte content | Chen et al. (2022) |
| Humic acid | Soybean (*Glycine max*) | HA at 5 mg/dm³, at sowing for 45 days | Antioxidant enzyme activation; root elongation | Enhanced antioxidant enzyme activity, biomass, and root length | Matuszak-Slamani et al. (2022) |
| Humic acid | Pepper (*Capsicum annuum*) | HA at 4.5 L/ha, weekly irrigation for 75 days | Enhanced shoot development | Increased shoot biomass and growth under drought | Qin & Leskovar (2020) |
| Humic acid (soil) | Faba bean | HA (soil application) | Enhanced antioxidant enzymes; proline and soluble sugar accumulation; improved water relations | Increased chlorophyll (up to + 33.7%), RWC (+ 17.0%), reduced MDA (− 19.1%) | Al-Demrdash et al. (2025) |
| Ascorbic acid + Humic acid | Faba bean | Foliar + soil application | Upregulation of SOD, CAT, POX, APX, GR; osmoprotectant accumulation | Superior drought resilience; improved yield traits and WUE | Al-Demrdash et al. (2025) |
| Humic acid (fractions) | Soybean | HA > 30 kDa and Mix | Increased stomatal density; enhanced biometric parameters; improved nutrient balance | 20–40% increase in biometric parameters; 87% increase in leaf number (Mix); restored Ca, Na, Mg to control levels | Kulpa et al. (2026) |
| **Extreme Temperature Stress** | | | | | |

| Type of HS | Target Plant | Application Method & Dose | Primary Mechanisms | Key Results | References |
|---|---|---|---|---|---|
| **Salinity Stress** | | | | | |
| Humic acid | Tomato (*Solanum lycopersicum*) | HA at 500 mg/L, foliar spray before heat exposure | Enhanced antioxidant activity; heat-responsive gene expression | Improved growth, fluorescence, antioxidant activity, and gene expression | Turan et al. (2021) |
| Humic acid | *Arabidopsis thaliana* | Commercial HA at 860 mg/L, single spray | Transcriptional activation of Heat-Shock Proteins (HSPs) | Upregulated HSP101, HSP81.1, HSP26.5, HSP23.6, HSP17.6A; enhanced heat tolerance | Cha et al. (2020); Ibraeva et al. (2026) |
| Humic acid | Coriander (*Coriandrum sativum*) | HA at 50 mg/L, during irrigation for 30 days | Enhanced antioxidants; secondary metabolite production | Promoted growth, enhanced antioxidants, and secondary metabolites | Hassanein et al. (2021) |
| Humic acid | Melon | HA application | Regulation of nitrogen metabolism and proline synthesis | Enhanced antioxidant enzymes; promoted $NO_3^-$-N degradation and $NH_4^+$-N synthesis | Zhu et al. (2024) |
| Humic acid + Zinc | Oat | Soil application | Regulation of osmotic balance; activation of CBF pathways | Improved membrane durability, RWC, and proline; stronger induction of CBF genes | Kutlu et al. (2026) |
| Humic acids (CHA, FA, BHA) | Zucchini | Various HA types | Increased antioxidative enzymes; soluble sugars; proline; TF regulation (NAC, WRKY) | Improved low-temperature tolerance; BHA most effective | Li et al. (2023) |
| **Heavy Metal Stress** | | | | | |
| Humic acid | Strawberry (*Fragaria ×* | HA at 5 mM, foliar for 45 | Improved membrane | Reduced Cd toxicity via | Dogan et al. (2022) |

| Type of HS | Target Plant | Application Method & Dose | Primary Mechanisms | Key Results | References |
|---|---|---|---|---|---|
| **Salinity Stress** | | | | | |
| | *ananassa*) | days | stability; increased proline | improved membrane stability and increased proline | |
| Humic acid | Rapeseed (*Brassica napus*) | HA at 2000 mg/kg, soil for 60 days | Reduced metal accumulation; enhanced antioxidant activity | Increased growth, reduced metal accumulation and oxidative stress | Boysan Canal et al. (2022) |
| Humic substances | Garden cress (*Lepidium sativum*) | HS at 7000 mg/L, hydroponic for 20 days | Reduced Cd uptake; enhanced growth | Increased biomass and root diameter; reduced Cd uptake by up to 95% | Yildirim et al. (2021) |
| Humic substances | Hemp, Sorghum, Miscanthus | HS application | Species-specific enzymatic vs. non-enzymatic antioxidant strategies | Sorghum: +26% biomass, + 80% sugars; Hemp: +267% APX; Miscanthus: mixed responses | Jaros-Tsoj et al. (2026) |
| Fulvic acid | Various crops | FA application | pH-dependent chelation; metal complexation; redox modulation | Maintains micronutrient availability; buffers metal toxicity; electron shuttle function | Ahmed et al. (2026) |
| **Nutrient Stress / Multiple Stress** | | | | | |
| Humic acid | Various crops | HA application | $H^+$-ATPase activation; transporter gene upregulation; nitrogen assimilation | Enhanced P and N uptake; upregulated OsPT and MdNRT genes; improved N metabolism | Ahmed et al. (2026) |

# 8. Future Perspectives and Unanswered Questions

# 8.1 Deconstructing the "Black Box": The Need for Mechanistic Clarity

Despite significant advances, the molecular mechanisms of HS perception and action remain incompletely understood. Identifying specific plant receptors and primary molecular targets of HS is a critical priority. While HS trigger rapid signaling responses, the receptors that perceive these complex mixtures have not been identified. Potential candidates include receptor-like kinases (RLKs), receptor-like proteins (RLPs), and pattern recognition receptors (PRRs). Similarly, primary molecular targets including plasma membrane $H^+$-ATPase, ion channels, and components of ROS and $Ca^{2+}$ signaling pathways remain uncharacterized. Furthermore, while correlations between HS chemical properties and bioactivity exist, the specific structural features conferring biological activity remain unclear. Advanced analytical approaches, high-resolution mass spectrometry, NMR spectroscopy, and computational chemistry, integrated with biological assays could address these knowledge gaps. Multi-omics approaches offer potential for systems-level understanding. Transcriptomics has revealed that HS regulate hundreds to thousands of genes, though temporal dynamics and regulatory networks remain incompletely understood. Proteomics can identify changes in protein abundance and post-translational modifications, as demonstrated by Roomi et al. (2018) in Arabidopsis roots, revealing proteins involved in metabolism, ROS homeostasis, and stress responses. Metabolomics provides insights into HS-induced metabolic changes, with studies in sugarcane revealing alterations in carbohydrate, amino acid, and secondary metabolism (Aguiar et al., 2018). Metagenomics can characterize HS-induced changes in rhizosphere microbiomes, though functional significance requires further investigation. The integration of multi-omics data through systems biology approaches could provide comprehensive understanding of HS action, from molecular interactions to physiological responses, guiding development of more effective HS-based biostimulants and enabling prediction of HS effects under diverse conditions (Fig. 3).

# 8.2 The Omics Revolution: A Systems-Level Understanding

Multi-omics approaches offer the potential to develop a systems-level understanding of HS action, integrating transcriptomics, proteomics, metabolomics, and metagenomics data. Transcriptomics provides insights into the gene expression changes induced by HS. RNA-seq and microarray analyses have revealed that HS regulate hundreds to thousands of genes, but the temporal dynamics and regulatory networks controlling these changes are not fully understood. Time-series transcriptomics could reveal the sequence of events following HS perception and identify early response genes that initiate the signaling cascade. Proteomics can identify changes in protein abundance and post-translational modifications induced by HS. Roomi et al. (2018) used label-free proteomics to identify proteins differentially abundant in HS-treated Arabidopsis roots, including proteins involved in metabolism, protein synthesis, ROS homeostasis, and stress responses. Proteomic approaches could also identify the proteins that directly interact with HS components. Metabolomics provides insights into the metabolic changes induced by HS. Aguiar et al. (2018) used metabolomics to identify changes in the metabolic profile of sugarcane leaves treated with HS, including changes in carbohydrate metabolism, amino acid metabolism, and secondary metabolism. Metabolomics can also identify biomarkers of HS

efficacy that could be used for quality control and product development. Metagenomics can characterize the changes in the rhizosphere and endophytic microbiome induced by HS. Studies have shown that HS alter microbial community composition, but the functional significance of these changes is not fully understood. Metagenomics, combined with metatranscriptomics and metabolomics, could reveal the functional changes in the microbiome that contribute to HS effects. The integration of multi-omics data through systems biology approaches could provide a comprehensive understanding of HS action, from molecular interactions to physiological responses. This understanding could guide the development of more effective HS-based biostimulants and enable the prediction of HS effects under different conditions.

## 8.3 The Soil Microbiome as a Conduit for HS Action

The role of the plant microbiome in mediating HS effects represents an emerging research frontier with significant implications for understanding and optimizing HS applications. Key research priorities include disentangling direct versus microbiome-mediated effects through studies using sterile systems or defined microbial consortia to distinguish the relative contributions of each pathway; elucidating microbiome recruitment mechanisms by investigating how HS influence root exudation and other plant traits to selectively attract beneficial microorganisms, potentially through identification of specific exudate compounds and optimization of HS formulations; functionally characterizing HS-induced microbiome changes using metatranscriptomics and metabolomics to reveal how altered microbial community composition translates to plant growth promotion and stress tolerance; and developing microbiome engineering approaches, ranging from combining HS with specific GPR strains to designing synthetic microbial communities (SynComs) that complement HS effects for enhanced agricultural benefits (Nabi et al., 2025).

## 8.4 Synergistic Formulations and Precision Agriculture

The development of next-generation HS formulations integrated with precision agriculture technologies presents strategic opportunities to maximize HS benefits through targeted applications. Research priorities include: (1) Designer HS formulations tailored for specific crops, stress types, and environmental conditions, requiring deeper understanding of structure-function relationships, predictive models linking HS properties to bioactivity, and high-throughput screening approaches to accelerate identification of optimal HS sources; (2) Controlled release formulations using biodegradable carriers such as alginate or chitosan beads to provide sustained HS effects, reduce application frequency, and protect HS from degradation (Aras et al., 2026; Shabir et al., 2026); (3) Precision agriculture technologies including sensor-based monitoring of plant stress status, soil conditions, and weather forecasts to guide optimal application timing and rates, alongside variable-rate application technologies enabling site-specific HS application that accounts for spatial variability in soil conditions and crop stress; and (4) Decision support systems integrating HS application with crop type, stress history, soil conditions, and economic factors to provide tailored recommendations that help farmers optimize HS use and maximize returns (Nabi et al., 2025).

## 8.5 A Grand Challenge: Understanding Multifactorial Stress

A critical research priority is understanding how HS influence plant tolerance to multifactorial stress combinations, as field-grown plants typically experience multiple concurrent stresses rather than single stressors. This requires stress combination experiments that characterize HS effects under realistic scenarios, examining various stress combinations (drought, salinity, heat, and others) along with stress timing and duration—to capture the complex dynamics of plant responses. The mechanisms underlying HS-induced multi-stress tolerance need elucidation, distinguishing between common pathways such as antioxidant defense and osmotic adjustment versus stress-specific mechanisms, which would enable development of HS formulations optimized for particular stress combinations. Field validation under multifactorial stress is essential to complement controlled environment studies, with field experiments accounting for natural stress variability and stress factor interactions. This challenge is particularly relevant under climate change, which is increasing the frequency and severity of stress combinations, positioning HS-based biostimulants as promising tools for climate-smart agriculture that can enhance tolerance to multiple stresses simultaneously (Nabi et al., 2025).

## 9. Conclusion

Humic substances have emerged as master regulators of plant stress resilience, orchestrating complex responses that enhance tolerance to drought, salinity, heat, cold, and heavy metal stress through mechanisms involving chemical priming, hormonal crosstalk, antioxidant defense, and microbiome modulation. The supramolecular structure of HS, characterized by dynamic assemblies of small molecules, provides the foundation for understanding their bioactivity and structure-function relationships. Despite significant advances, critical knowledge gaps remain, including the identification of HS receptors, the relative contribution of direct versus microbiome-mediated effects, and the performance of HS under multifactorial stress combinations. Future research must integrate cutting-edge molecular tools, systems biology, and field validation to develop next-generation HS-based biostimulants. Addressing these priorities—receptor identification, microbiome recruitment mechanisms, predictive structure-function models, multifactorial stress characterization, and field efficacy validation will enable the development of targeted, sustainable HS products that enhance crop resilience, reduce chemical inputs, and support climate-smart agriculture, ultimately contributing to food security and environmental sustainability (Nabi et al., 2025).

## Declarations

# Conflicts of Interest / Competing Interest

♣ **s**: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

# Ethics Approval & Consent to Participate:

♣ Not applicable, as this review study did not involve human participants, animal experiments, or tissue samples.

**Consent for Publication:**

♣ Not applicable.

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**Mehedi Amin**: Conceptualization, Methodology, Investigation, Literature search, Writing – original draft, Visualization, Writing – review & editing. **Md Motasim Billah Sagor**: Participated writing – original draft, Visualization. **Muhammad Abdul Mannan**: Literature search, Writing – review and editing. **Sayed Mohammad Mohsin**: Writing – review & editing, Supervision. **Abu Noman Faruq Ahmmed**: Writing – review & editing, Supervision. All authors read and approved the final manuscript.

# Data Availability Statement:

Data sharing is not applicable to this article as no new primary datasets were generated or analyzed during the current review.

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# Figures

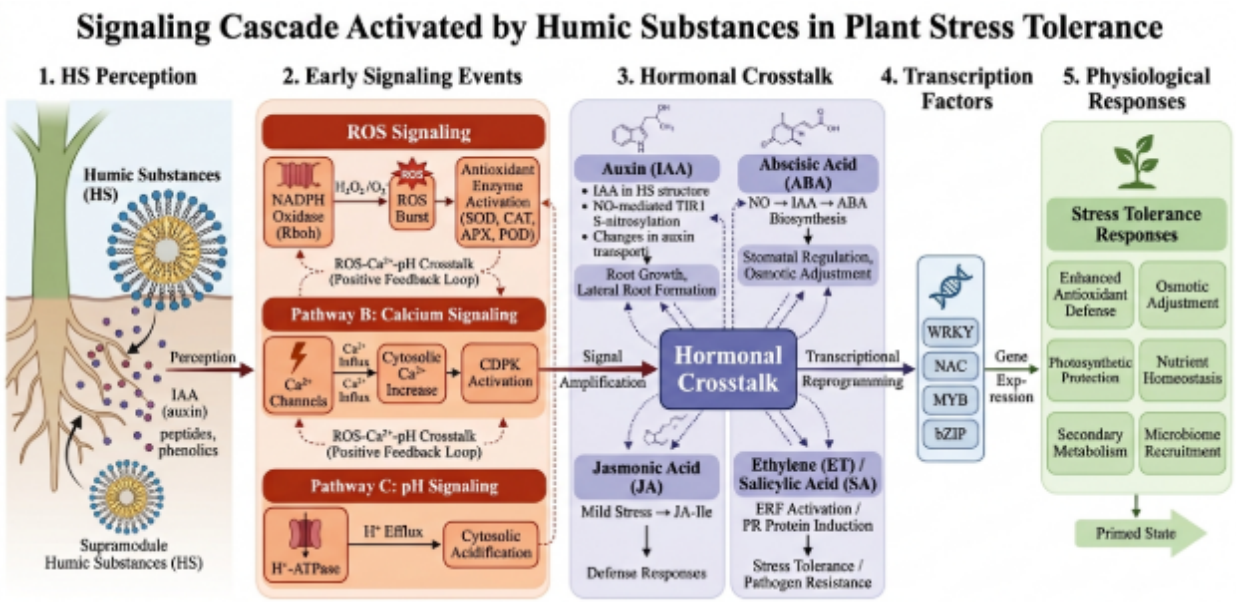



**Figure 1**

Schematic representation of the humic substances (HS)-induced signaling network in plants. HS activate membrane acidification, ROS/NO bursts, and phytohormone cross-talk (IAA, JA, ET, SA, ABA), leading to enhanced nutrient assimilation, secondary metabolism, and activation of defense and stress-responsive genes via key transcription factors (MYB, bZIP, NAC) and kinases (CDPK, MAPK), ultimately conferring stress tolerance and pathogen resistance.

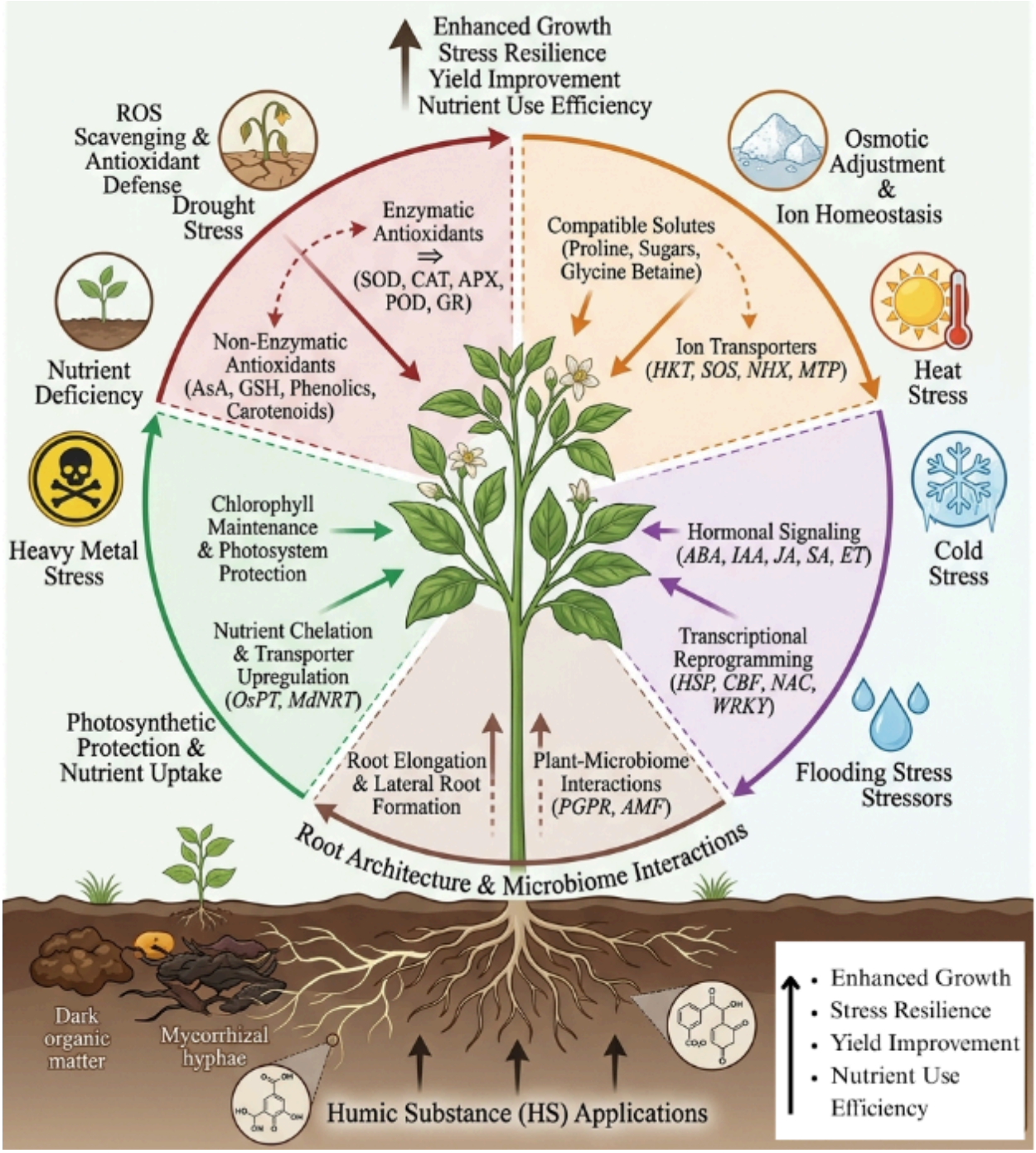



**Figure 2**

Humic Substances: Multifaceted Mechanisms for Enhancing Plant Abiotic Stress Tolerance

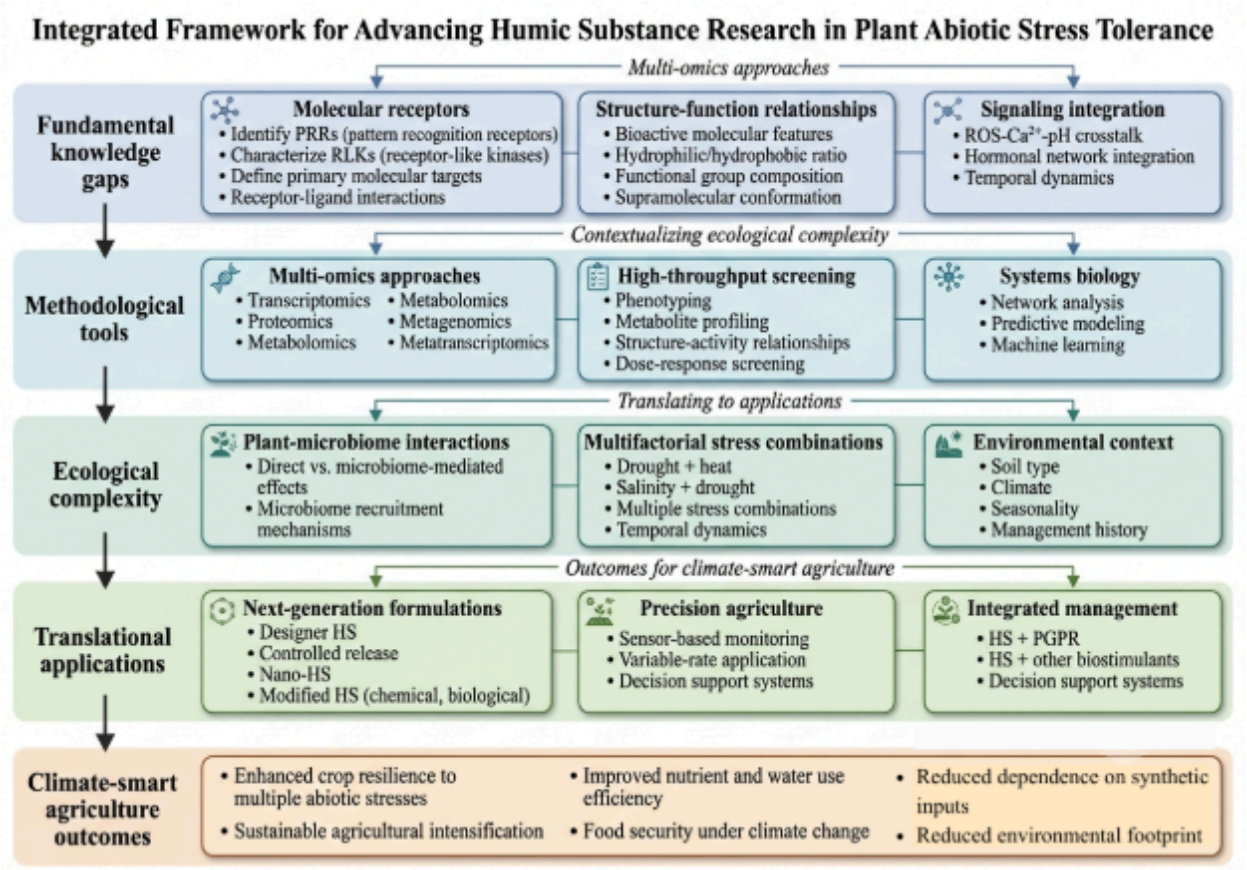



**Figure 3**

Integrated research framework for advancing humic substance (HS) applications in agriculture. The framework outlines a multidisciplinary pathway from resolving fundamental knowledge gaps in molecular recognition and signaling, through multi-omics and systems biology, to contextualizing these mechanisms within complex ecological environments. This foundational understanding is then translated into next-generation formulations and precision management strategies, ultimately aiming to deliver outcomes for climate-smart agriculture.