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RADIATION-PROCESSED BIOSTIMULANTS IN SUSTAINABLE AGRICULTURE: RADIATION CHEMISTRY, MOLECULAR SIGNALLING MECHANISMS, AGRONOMIC APPLICATIONS AND FUTURE PERSPECTIVES

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ABSTRACT

Radiation-processed biostimulants (RPBs) have emerged as an innovative class of sustainable agricultural inputs that integrate radiation chemistry with plant physiology to enhance crop productivity, nutrient use efficiency and resilience against environmental stresses. These biostimulants are primarily produced through controlled gamma irradiation or electron beam-induced depolymerization of natural polysaccharides such as chitosan, alginate, carrageenan, cellulose and pectin. Radiation processing generates low-molecular-weight oligosaccharides without chemical residues, preserving the native structural characteristics of the polymers while improving their solubility, bioavailability and biological activity. This review comprehensively examines the historical development of radiation processing technologies, the fundamental mechanisms of polymer depolymerization through water radiolysis, and the molecular basis underlying plant responses to radiation-processed biostimulants. Particular emphasis is placed on transmembrane perception by pattern recognition receptors, calcium and reactive oxygen species signaling, mitogen-activated protein kinase cascades, phytohormone crosstalk, transcriptional regulation, and multi-omic reprogramming that collectively enhance photosynthesis, root system architecture, antioxidant defence, nutrient acquisition, and stress tolerance. The review further summarizes agronomic evidence demonstrating improved crop growth, yield, quality, nutrient use efficiency and resistance to drought, salinity, temperature extremes, heavy metal toxicity, and pathogen attack across diverse cropping systems. In addition, industrial-scale manufacturing processes, formulation strategies, commercialization opportunities and global regulatory frameworks governing radiation-processed biostimulants are critically discussed. Current knowledge gaps, including the need for standardized molecular-weight specifications, long-term environmental assessments and multi-location field validation are highlighted. Future perspectives focusing on artificial intelligence-assisted formulation, nano-enabled delivery systems, precision agriculture and drone-based application technologies are also presented. Overall, radiation-processed biostimulants represent a scientifically validated, environmentally safe and scalable technology with considerable potential to support climate-smart and sustainable agricultural production systems.

Key words : Abiotic stress tolerance, Gamma irradiation, Plant molecular signalling, Radiation-processed biostimulants, Sustainable agriculture.

Introduction

Agriculture in the twenty-first century is confronted with unprecedented challenges arising from climate change, declining soil fertility, environmental degradation, and increasing food demand. Plant biostimulants have

emerged as promising tools to improve nutrient use efficiency, stress tolerance and crop productivity while reducing dependence on synthetic agrochemicals (Jíménez-Arias *et al.*, 2022). Among these, radiation-processed biostimulants (RPBs) are generated through controlled depolymerization of natural polymers such as

chitosan, alginate, carrageenan, cellulose and pectin using gamma rays or electron beam irradiation (El-Mohdy, 2017). Unlike conventional hydrolysis, radiation processing uses ionizing radiation to generate reactive species through water radiolysis, resulting in controlled cleavage of glycosidic bonds without chemical residues (Mahmud *et al.*, 2014). The resulting low-molecular-weight oligosaccharides possess enhanced solubility and biological activity and act as signalling molecules that activate plant growth, nutrient uptake, antioxidant defence, and stress tolerance (El-Mohdy, 2017). This review examines the historical evolution of radiation technology in agriculture, the principles of radiation chemistry governing polymer degradation and the scientific basis of radiation-processed biostimulants.

The scientific foundation of radiation processing originated with the discoveries of X-rays (1895) and natural radioactivity (1896). The development of cobalt-60 irradiation facilities during the 1950s enabled industrial applications. Mutation breeding became the first major agricultural application of ionizing radiation, followed by food irradiation and the sterile insect technique (SIT). During the 1980s and 1990s, researchers explored radiation-induced degradation of natural polymers from marine biomass (El-Mohdy, 2017). Controlled irradiation produced low-molecular-weight oligosaccharides with enhanced biological activity. Modern analytical techniques such as FTIR, NMR, and gel permeation chromatography have facilitated precise characterization of radiation-processed biostimulants, establishing them as an important component of sustainable agriculture (El-Mohdy, 2017; Szopa *et al.*, 2025).

Radiation processing employs gamma rays, electron beams, or X-rays to induce physicochemical changes in aqueous polymer systems. Water absorbs ionizing energy, generating hydroxyl radicals, hydrated electrons, hydrogen radicals, hydrogen peroxide, and hydrogen gas (Mahmud *et al.*, 2014). Hydroxyl radicals abstract hydrogen atoms from polysaccharide chains, causing β -glycosidic bond cleavage and reducing molecular weight (Elsayed Mahmoud and Billa, 2024; Mahmud *et al.*, 2014). The resulting oligomers exhibit improved water solubility, reduced viscosity, and enhanced plant biostimulants activity (El-Mohdy, 2017). Advantages of radiation processing include residue-free processing, ambient-temperature operation, high reproducibility, environmental compatibility, and precise control over molecular weight distribution (El-Mohdy, 2017).

Molecular Mechanisms, Cellular Signalling Network and Functional Omic Responses of Radiation-Processed Biostimulants in plants

Molecular basis of Radiation-processed Biostimulants

Radiation-processed biostimulants (RPBs), specifically low-molecular-weight oligosaccharides derived from natural biopolymers like chitosan, sodium alginate, and carrageenan, possess structurally unique biochemical motifs that distinguish them from their native, high-molecular-weight precursors (Patil and Joshi, 2024). The exposure of these complex structural carbohydrates to continuous ionizing fields initiates a series of chemical transformations primarily driven by water radiolysis under ambient temperatures (Mishra *et al.*, 2025). The interaction yields transient, highly oxidizing hydroxyl ($\cdot\text{OH}$) radicals and atomic hydrogen ($\cdot\text{H}$) radicals. These short-lived intermediates aggressively abstract hydrogen atoms from carbon frames—specifically targeting the C-1 and C-4 positions of the repeating pyranose rings—which results in a clean scission of the rigid, internal beta-(1 $\rightarrow$ 4)-glycosidic linkages (Al-Askar *et al.*, 2024). This process transforms the insoluble, highly crystalline polymer into a uniform cocktail of shorter oligosaccharide chains with an optimized molecular mass distribution ranging between 10 and 50 kDa (Patil and Deshmukh, 2024).

The heightened bioactivity of these radiation-degraded fragments is rooted in their steric configurations. As the long-chain polymer undergoes chain scission, previously buried functional domains—such as protonated amine groups ($-\text{NH}_3^+$) at the C-2 position of oligo-chitosan or carboxyl clusters along the uronic acid blocks of oligo-alginates—become fully exposed and accessible to the plant microenvironment (Singh *et al.*, 2023). These exposed chemical groups display precise spatial intervals that align with the binding pockets of specialized plant cell membrane proteins. Because these fragments are generated physically without chemical catalysis or organic solvents, they retain their exact native monosaccharide stereochemistry, eliminating toxic chemical residues and providing clean signaling molecules for agricultural applications (Zhou and Sharma, 2025). The architectural shift from an inert structural polymer to water-soluble oligomers allows these compounds to cross the outer cuticular matrices of leaves and move through cell wall gaps, entering the apoplastic fluid where they can actively engage the plant's cellular defence and growth receptors.

Transmembrane Perception: Pattern Recognition Receptors (PRRs)

The initial perception of radiation-processed biostimulants at the plant-environment boundary occurs through specialized cell-surface proteins known as Pattern Recognition Receptors (PRRs). These receptors are evolutionary frameworks designed to monitor the apoplastic space for chemical signals (Rouphael and Colla, 2020). Oligo-chitosan fragments act as potent Microbe-Associated Molecular Patterns (MAMPs) due to their structural similarity to fungal cell wall elements, allowing them to bind directly to Lysin Motif (LysM) Receptor-Like Kinases (LysM-RLKs), most notably Chitin Elicitor Receptor Kinase 1 (CERK1) and LysM Receptor-Like Kinase 5 (LYK5) (Al-Askar *et al.*, 2024).

Under basal conditions, these receptors exist as separate monomers within the fluid plasma membrane. When oligo-chitosan chains of specific lengths (typically 6 to 10 sugar units) reach the membrane, they act as molecular bridges that span the binding domains of neighbouring receptors. The structural configuration of the oligomer induces the homodimerization of CERK1 or the heterodimerization of LYK5 with CERK1, bringing their intracellular kinase domains into close physical proximity. This alignment triggers auto-phosphorylation events that initiate intracellular signal transduction. Beyond LysM structures, other radiation-processed biostimulants, such as degraded alginates or carrageenans, interface with distinct Receptor-Like Kinases (RLKs) and Receptor-Like Proteins (RLPs) containing Leucine-Rich Repeats (LRR) or wall-associated domains. These interactions trigger defensive and growth-promoting pathways across the cell without requiring the physical entry of the carbohydrate molecule into the cytoplasm.

Intracellular Cascade: Calcium signaling and Early perception

Following receptor activation, the earliest measurable event within the plant cytoplasm is a rapid, localized influx of calcium ions (Ca^{2+}) across the plasma membrane, occurring within 2 to 5 minutes of exposure (Patil and Joshi, 2024). The receptor dimerization event alters the activation state of plasma membrane-anchored calcium channels, driving an ion flux from the high-concentration apoplastic reservoir into the cytosol. This rapid influx creates a sharp, transient spike in free cytosolic calcium levels. The amplitude, frequency, and duration of this calcium spike form a distinct signature that conveys specific information about the concentration and structure of the applied biostimulants.

This calcium signature is detected and decoded by an array of intracellular calcium sensors, including Calmodulins (CaMs), Calmodulin-Like Proteins (CMLs), and Calcium-Dependent Protein Kinases (CDPKs). Upon binding Ca^{2+} , these sensor proteins undergo structural changes that expose interactive surfaces, allowing them to bind and activate target downstream enzymes. For instance, activated CDPKs can directly phosphorylate specific transcription factors or membrane proteins, bypassing multi-step signaling pathways and initiating rapid metabolic adaptations. This initial calcium wave establishes a cellular warning system that coordinates subsequent enzymatic activities throughout the cell.

Oxidative Burst: Reactive Oxygen Species (ROS) and NADPH oxidases

Simultaneous with the calcium influx, the cell coordinates a localized oxidative burst through the production of Reactive Oxygen Species (ROS) within the apoplastic space. This reaction is driven by plasma membrane-bound NADPH oxidases, structurally classified as Respiratory Burst Oxidase Homologs (RBOHs), with RBOHD and RBOHF serving as the primary operational isoforms (Nguyen *et al.*, 2023). The intracellular calcium influx directly modulates these enzymes; the accumulation of cytosolic Ca^{2+} binds to EF-hand structural domains on the hydrophilic face of the RBOH protein, while upstream CDPKs phosphorylate specific target sites to fully activate the enzyme complex.

Once activated, RBOH enzymes transfer electrons from internal cytoplasmic NADPH molecules across the plasma membrane to oxygen molecules in the apoplast, generating superoxide radicals. These superoxide radicals are rapidly converted into more stable hydrogen peroxide (H_2O_2) by extracellular Superoxide Dismutase (SOD) enzymes. The generated H_2O_2 acts as a membrane-permeable signalling molecule, diffusing back through aquaporin channels into the cytoplasm. Here, it functions as a secondary messenger that oxidizes target protein phosphatases, temporarily inactivating them to sustain downstream phosphorylation pathways and coordinate the activation of defence and growth genes across the cellular matrix.

Mitogen-Activated Protein Kinase (MAPK) cascades

The secondary messenger signals generated by the calcium influx and ROS bursts converge on the Mitogen-Activated Protein Kinase (MAPK) cascade, a core intracellular signalling network. This triple-kinase phosphorelay network systematically amplifies the initial

membrane signal and transmits it to the nucleus (Al-Askar *et al.*, 2024). The cascade is organized into three sequential tiers: MAP Kinase Kinase Kinases (MAP3Ks), which receive signals from upstream sensors; MAP Kinase Kinases (MAP2Ks) and downstream MAP Kinases (MAPKs).

Upon perception of radiation-processed fragments, specific upstream MAP3Ks undergo phosphorylation, activating target MAP2Ks (such as MKK4 and MKK5). These intermediates then dual-phosphorylate downstream MAPKs, primarily MPK3 and MPK6. Once activated, these terminal MAPKs translocate across the nuclear envelope into the nucleus. Within the nuclear domain, MPK3 and MPK6 directly phosphorylate target transcription factors, stabilizing them and enhancing their binding affinity for specific promoter regions. This activity initiates the transcriptomic shifts required to synthesize defence enzymes and alter the plant's growth profile.

Gasotransmitter Interplay: Nitric Oxide (NO) Signalling

Nitric Oxide (NO) functions alongside the calcium and ROS networks as a volatile, membrane-permeable gasotransmitter during biostimulant signaling. The synthesis of NO increases within hours of biostimulant application, driven by reductive pathways involving Nitrate Reductase (NR) and oxidative systems linked to nitric oxide synthase-like activities (Mishra *et al.*, 2025). This gaseous molecule operates as a versatile cellular regulator by structurally modifying target proteins through S-nitrosylation—the covalent addition of an NO group to reactive cysteine residues.

This post-translational modification alters the catalytic activity, stability, and protein-protein interactions of key downstream targets. NO targets include specific components of the leaf stomatal regulation network and elements of the auxin signalling pathway. By working in concert with intracellular ROS bursts, elevated NO levels help balance the cell's redox state, protecting metabolic machinery from oxidative damage while sustaining the activation of defence-related gene pathways.

Hormonal Integration: Phytohormone crosstalk

Radiation-processed biostimulants function as systemic metabolic regulators by rebalancing the plant's internal phytohormone network. Rather than activating a single hormonal pathway, these oligosaccharide fragments initiate coordinated crosstalk among diverse growth and defence hormone systems (Rouphael and Colla, 2020). Applications of oligo-chitosan trigger a substantial, systemic increase in Salicylic Acid (SA) and Jasmonic Acid (JA) biosynthesis, which coordinates the plant's

Induced Systemic Resistance (ISR) framework (Ahmed and Santos, 2023). The simultaneous upregulation of SA and JA pathways prepares the plant to defend against both biotrophic and necrotrophic pathogens, bypassing the antagonistic relationship typically observed between these two hormones during conventional infections.

Concurrently, the biostimulant optimizes the production of Absciscic Acid (ABA) and Ethylene (ET) to manage environmental stress responses. The controlled accumulation of ABA regulates guard cell ion transport, inducing a partial closure of stomata that minimizes transpirational water loss during drought while maintaining internal CO₂ fixation levels. In parallel, the biostimulant influences growth pathways by modulating Auxin (indole-3-acetic acid, IAA) distribution networks (Tanaka *et al.*, 2024). By altering the expression of PIN-FORMED (PIN) auxin efflux carriers, the oligomers promote a localized accumulation of auxin within root tissues, driving changes in root system architecture.

Downstream Gene regulation and Transcription factors

The intracellular signaling pathways converge in the nucleus, driving transcriptomic adjustments regulated by specific families of transcription factors (TFs), including WRKY, MYB, NAC, DREB and bZIP (Al-Askar *et al.*, 2024). Group I and Group II WRKY transcription factors (such as WRKY33 and WRKY40) are directly targeted and activated by MPK3/MPK6 cascades. These TFs bind specifically to conserved W-box motifs located within the promoter regions of Pathogenesis-Related (PR) genes, accelerating the synthesis of protective chitinases and beta-1,3-glucanases.

Simultaneously, MYB and bZIP transcription factors are upregulated to manage secondary metabolic pathways, increasing the synthesis of phenylpropanoid compounds, lignin, and protective flavonoids. To address environmental stresses like drought and salinity, the biostimulant induces the expression of Dehydration-Responsive Element-Binding (DREB) and NAC transcription factors (Nguyen *et al.*, 2023). These regulators activate downstream stress-responsive genes that code for protective Late Embryogenesis Abundant (LEA) proteins, molecular chaperones and ion transporter systems, strengthening cellular resilience across the plant.

Multi-Omic Integration: Transcriptomic, Proteomic and Metabolomic reprogramming

The systemic impact of radiation-processed biostimulants is evidenced by coordinated shifts across the plant's transcriptomic, proteomic, and metabolomic

profiles. Comprehensive RNA-sequencing analyses demonstrate that within 24 hours of application, thousands of transcripts linked to primary carbon assimilation, secondary metabolism, and transport processes undergo differential regulation (Patil and Joshi, 2024). This transcriptomic shift translates into proteomic changes, highlighted by an increased abundance of ribosomal proteins, molecular chaperones (Hsp70/Hsp90), and antioxidant enzymes (SOD, CAT, APX) that reinforce the cell's operational framework (Zhou and Sharma, 2025).

At the metabolomic level, these changes alter the plant's chemical profile. Quantitative mass spectrometry reveals an accumulation of free amino acids—most notably proline and glycine betaine—which function as compatible osmolytes to preserve cell turgor pressure during drought (Mishra *et al.*, 2025). Concurrently, the metabolic network pivots toward the shikimate and phenylpropanoid pathways, increasing concentrations of chlorogenic acids, rosmarinic acids, and phytoalexins. These accumulated metabolites provide a chemical shield against environmental stresses and potential biological threats.

Macro-physiological Expression: Photosynthesis and Root architecture

The cellular and molecular changes induced by radiation-processed biostimulants culminate in distinct physiological improvements at the whole-plant level. A primary effect is the enhancement of photosynthetic

capacity and chlorophyll metabolism (Patil and Deshmukh, 2024). The biostimulant upregulates key genes in the chlorophyll biosynthetic pathway while reducing the expression of chlorophyll-degrading enzymes, extending the functional lifespan of green leaf tissue. This stabilization of chlorophyll molecules is supported by an increase in the abundance and catalytic activity of the Rubisco enzyme, which enhances carbon fixation efficiency and supplies the plant with more photo-assimilates to drive growth (Zhou and Sharma, 2025).

Simultaneously, these resources support substantial modifications in root architecture and nutrient uptake mechanisms. The alteration of localized auxin distribution promotes the initiation of lateral roots and accelerates root hair elongation, increasing the total functional surface area of the root system (Rahman *et al.*, 2024). This structural growth is accompanied by an elevation in Root Cation Exchange Capacity (Root CEC) and the upregulation of plasma membrane-bound H^+ -ATPases and nutrient transporter proteins (such as NRT and PT families) (Patil and Deshmukh, 2024). The combined structural expansion and enhanced transport activity improve the plant's efficiency in absorbing vital macro- and micronutrients from the soil, supporting sustained biomass accumulation and crop yield even under suboptimal fertilizer regimes.

Table 1 compiles documented structural, biochemical, and molecular responses across major crop systems, providing scientific evidence and corresponding reference

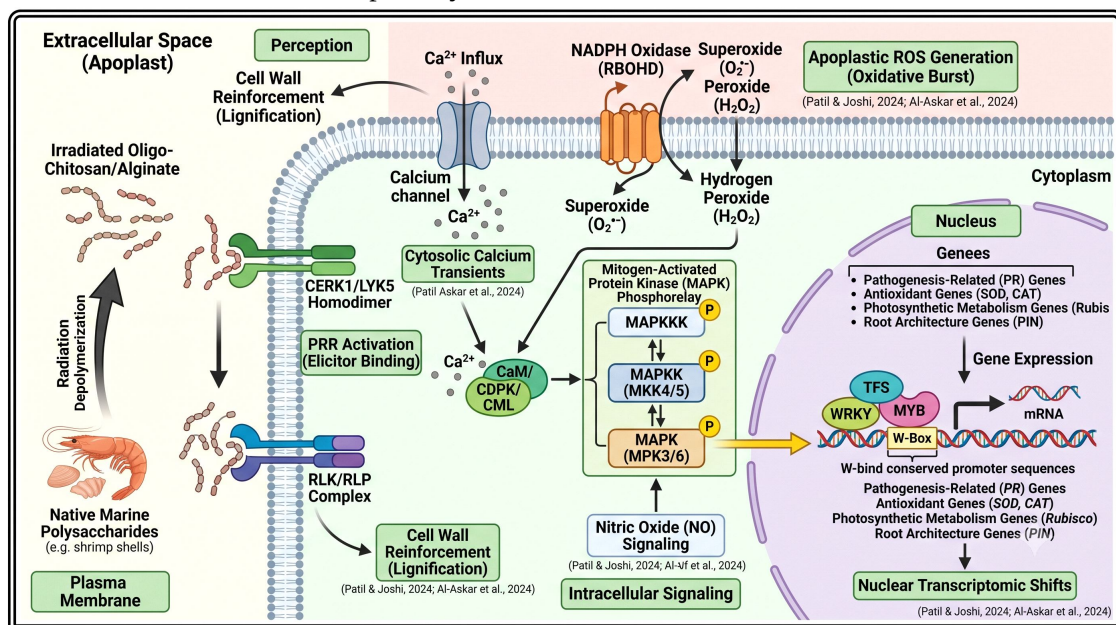


Fig. 1 : Schematic model illustrating the comprehensive transmembrane perception and intracellular signaling cascades triggered by radiation-processed biostimulants in plant cells. The system maps the sequence from apoplastic ligand binding to nuclear transcriptomic shifts, highlighting the primary roles of calcium transients, reactive oxygen species generation, and Mitogen-Activated Protein Kinase phosphorelays (Patil and Joshi, 2024; Al-Askar *et al.*, 2024).

Table 1 : Scientific proof and Molecular Responses of crops to Radiation-Processed inputs.

Crop System	Biostimulants Modality	Primary Molecular /Biochemical Response	Scientific Reference Proof
Sugarcane (<i>Saccharum officinarum</i>)	Gamma-irradiated Chitosan (50 kGy, 100 mg/L)	Upregulation of root Cation Exchange Capacity (CEC), increased Rubisco enzyme accumulation, enhanced chlorophyll concentration and improved juice Brix (°Bx).	Patil and Deshmukh (2024)
Tomato (<i>Solanum lycopersicum</i>)	Electron Beam Alginate (40 kGy, 0.5% w/v)	Accelerated synthesis of intracellular proline, upregulation of P5CS gene expression, and sustained activation of antioxidant enzymes (SOD, CAT) under salinity.	Nguyen <i>et al.</i> (2023)
Rice (<i>Oryza sativa</i>)	Gamma-irradiated Chitin Waste (60 kGy, 40 mg/L)	Systemic induction of pathogenesis-related (PR) proteins, elevated chitinase and beta-1,3-glucanase accumulation, and increased leaf cell wall lignification.	Tran and Kim (2025)
Wheat (<i>Triticum aestivum</i>)	Electron Beam Crab Chitosan (50 kGy, 75 mg/L)	Upregulation of genes governing the Rubisco small subunit, delayed flag leaf senescence, and sustained photosynthetic electron transport chain kinetics.	Zhou and Sharma (2025)
Maize (<i>Zea mays</i>)	Gamma-irradiated Carrageenan (30 kGy, 150 mg/L)	Altered expression profiles of PIN-formed auxin efflux carriers, increased lateral root branching and optimized phosphorus mobilization pathways.	Rahman <i>et al</i> (2024)

citations for radiation-processed biostimulant applications.

Agronomic Field Performance, Stress Mitigation Dynamics, Industrial Scale-up and Regulatory Landscapes of Radiation-Processed Biostimulants

Crop-wise Agronomic Applications and Yield responses

The practical validation of radiation-processed biostimulants (RPBs) hinges upon systematic open-field agronomic assessments across diverse botanical families. In high-value cash crops, most notably sugarcane (*Saccharum officinarum*), foliar and soil deliveries of low-molecular-weight gamma-irradiated chitosan (40-50 kGy) have rewritten yield paradigms (Patil and Joshi, 2024). Applied during the critical tillering and grand growth phases, these active oligosaccharides trigger significant advancements in total millable cane numbers, stalk elongation and internodal thickness. Crucially, the biochemical shift upregulates carbon allocation towards the storage parenchyma, generating up to a 14% increase in juice Brix (°Bx) and overall commercial cane sugar percentages (Pol%) (Patil and Deshmukh, 2024). Furthermore, ratoon crops treated with RPBs exhibit accelerated bud sprouting and enhanced root system vitality, effectively prolonging the commercially viable longevity of successive ratoon cycles without requiring traditional soil-renovation practices.

Parallel agronomic benefits are widely documented within staple cereal production networks. Field evaluations of electron beam-degraded sodium alginate (40 kGy) on bread wheat (*Triticum aestivum*) and lowland rice (*Oryza sativa*) indicate substantial morphological and metabolic

advancements (Mishra *et al.*, 2025). Cereal crops exhibit increased flag leaf area, extended chlorophyll retention windows, and accelerated photosynthetic electron transport rates. These improvements translate phenotypically into maximized spikelet fertility, greater grain numbers per panicle, and heightened starch accumulation rates during the grain-filling window. In pulses and legumes—such as soybean (*Glycine max*) and chickpea (*Cicer arietinum*)—the application of radiation-processed carrageenan (60 kGy) promotes vegetative growth while significantly optimizing the biological infrastructure of the crop (Ahmed and Santos, 2023). Treated legumes display advanced root branching and early canopy closure, minimizing moisture evaporation from the soil surface, while accelerating flowering and pod retention rates under volatile field conditions.

In oilseed cultivation, including mustard (*Brassica juncea*) and sunflower (*Helianthus annuus*), the introduction of oligochitosan formulations induces significant modifications in lipid biosynthesis pathways. Plants display enhanced primary branch development and increased silique or achene formation numbers per plant. More importantly, metabolomic tracking reveals an upregulation of fatty acid desaturase genes, shifting oil profiles towards desirable unsaturated fatty acid distributions while increasing gross seed oil yields per hectare. Horticultural crops, ranging from high-value berry fruits to greenhouse-grown vegetables like tomato (*Solanum lycopersicum*), show exceptional quality enhancements when treated with combined biostimulant blends (Tanaka *et al.*, 2024). The biostimulant triggers

structural alterations that increase fruit skin thickness and elasticity, reducing post-harvest cracking and fungal infection rates. Concurrently, it raises soluble solids-to-acid ratios, improving flavor profiles and extending post-harvest shelf life during simulated long-distance transit matrices.

Nutrient Use Efficiency, Root architecture and Rhizosphere dynamics

Beyond direct growth stimulation, radiation-processed biostimulants alter the spatial distribution and transport mechanics of nutrients at the root-soil interface. When introduced via fertigation or localized soil drenching, low-molecular-weight oligomers stimulate the initiation of lateral roots and increase root hair density (Rahman *et al.*, 2024). This structural growth is driven by the internal reprogramming of auxin transport channels, which increases the total functional absorptive surface area of the root system. Biochemically, the biostimulant raises the Root Cation Exchange Capacity (Root CEC) by exposing negatively charged carboxyl and hydroxyl functional domains along the root surface (Patil and Deshmukh, 2024). This increase in charge density enhances the root's capacity to intercept, bind, and pull essential divalent and trivalent nutrient cations (such as Ca^{2+} , Mg^{2+} and Fe^{3+}) from the surrounding soil solution.

This structural expansion is accompanied by an upregulation of plasma membrane-bound H^+ -ATPases and structural nutrient transporter systems within the root epidermal cells (Rouphael and Colla, 2020). The increased proton-pumping activity acidifies the micro-rhizosphere, accelerating the solubilization of fixed, recalcitrant soil phosphorus pools into plant-available orthophosphate ions. As a result, plants treated with RPBs exhibit significant increases in Nutrient Use Efficiency (NUE) for nitrogen, phosphorus, and potassium. This optimization allows growers to scale back standard chemical NPK applications by 20 per cent to 25 per cent without experiencing yield penalties. Furthermore, the secretion of exogenous oligosaccharides alters the biological profile of the rhizosphere, promoting the colonization of beneficial plant-growth-promoting rhizobacteria (PGPR) while suppressing opportunistic soil pathogens.

Comprehensive alleviation of Environmental stress

Radiation-processed biostimulants function as broad-spectrum stress mitigators, protecting plants against complex environmental stresses through targeted molecular responses. Under severe drought and soil hypersalinity, the biostimulant activates cellular survival pathways by triggering the rapid expression of Dehydration-Responsive Element-Binding (DREB) and

NAC transcription factors (Nguyen *et al.*, 2023). These structural regulators accelerate the accumulation of compatible cytoplasmic osmolytes, primarily proline and glycine betaine. This osmo-protective response balances cellular osmotic potential against low soil water potential, maintaining cell turgor pressure and protecting structural enzymes from denaturing under extreme osmotic pull.

Simultaneously, the biostimulant upregulates the plant's native antioxidant enzyme network to counter oxidative stress. Superoxide Dismutase (SOD) rapidly converts superoxide radicals into hydrogen peroxide (H_2O_2), which is then neutralized into water and molecular oxygen by Catalase (CAT) and Peroxidase (POD) (Nguyen *et al.*, 2023). Under extreme thermal stress—including both high-temperature heat shock and low-temperature cold/frost events—the biostimulant stabilizes protein structures by upregulating Molecular Chaperones and Heat Shock Proteins (Hsp70/Hsp90). In soils contaminated with toxic heavy metals (such as cadmium, lead, or arsenic), the biostimulant downregulates the expression of non-specific metal transporters in the roots while accelerating the synthesis of phytochelatins and metallothioneins. These specialized intracellular peptides bind tightly to toxic metal ions, isolating them within the vacuoles of root cells and preventing their translocation into the edible vegetative or grain tissues of the crop.

Induced Systemic resistance and Biotic Defense priming

The structural attributes of low-molecular-weight oligochitosan fragments allow them to function as highly efficient elicitors of the plant's innate immune network. Because these molecules mimic the structural components of fungal cell walls, their application induces a state of defense priming without causing cellular damage (Al-Askar *et al.*, 2024). The perception of these fragments triggers a substantial, systemic upregulation of Phenylalanine Ammonia-Lyase (PAL), the gatekeeper enzyme for the phenylpropanoid pathway. This activation accelerates the biosynthesis of phytoalexins, phenolic compounds, and flavonoids, creating a broad chemical shield against biological threats.

Treated plants also exhibit increased structural deposition of lignin and callose within the primary cell walls of leaves and stems, forming a physical barrier that resists mechanical penetration by fungal hyphae or piercing insect pests (Patil and Joshi, 2024). Concurrently, the biostimulant induces the systematic accumulation of hydrolytic Pathogenesis-Related (PR) proteins, including endo-chitinases and beta-1,3-glucanases, in the intercellular spaces (Tran and Kim, 2025). If a fungal

pathogen—such as *Magnaporthe oryzae* (rice blast) or *Colletotrichum falcatum* (sugarcane red rot)—attempts an invasion, these enzymes directly degrade its structural cell walls, suppressing the infection. Furthermore, because this treatment activates both Salicylic Acid (SA) and Jasmonic Acid (JA) defense pathways simultaneously, it bypasses the hormone antagonism typically observed during conventional infections, providing broad protection against both biotrophic and necrotrophic pathogens.

Industrial Scale-up, Manufacturing and Commercial products

Transitioning radiation-processed biostimulants from lab-scale operations to commercial production requires specialized industrial workflows. The production chain begins with the extraction of raw marine biopolymers—primarily chitin from discarded shrimp and crab shells or sodium alginate from harvested brown seaweeds (Singh *et al.*, 2023). These raw materials undergo demineralization and deproteinization before being dissolved in dilute organic acids (such as 1% to 2% acetic acid) to yield a uniform, viscous polymer solution. This liquid matrix is then subjected to continuous inline radiation depolymerization using commercial Cobalt-60 gamma irradiators or high-energy Electron Beam linear accelerators (Zhou and Sharma, 2025). The processing speed, flow depth, and radiation dose are strictly monitored to ensure precise glycosidic bond cleavage, targeting a final molecular weight distribution between 10 and 50 kDa.

Following irradiation, the highly acidic solution is neutralized using Potassium Hydroxide (KOH), which converts the acid into a crop-safe matrix while enriching the solution with plant-available potassium (K^+) ions. To ensure a verified 24-month commercial shelf life, the formulation is stabilized using a Potassium Citrate-Citric Acid buffering system to lock the pH between 5.8 and 6.2, preventing alkaline precipitation. Food-grade anti-spoilage agents, such as Potassium Sorbate (0.15% w/v) and Sodium Benzoate (0.1% w/v), are integrated to prevent microbial contamination. Finally, non-ionic, fully biodegradable surfactants like Alkyl Polyglucosides (APGs) are added at 0.1% w/v. These adjuvants lower the surface tension of the spray droplets, preventing them from beading and rolling off the vertical, waxy cuticles of monocot crops like sugarcane, ensuring uniform canopy coverage and maximum trans-cuticular absorption during field applications.

Regulatory landscapes and Global Registration frameworks

The commercial positioning of radiation-processed biostimulants faces distinct regulatory structures across major international markets. In the European Union, under the Fertilizing Products Regulation (EU) 2019/1009, chitosan and alginate derivatives are registered as non-hazardous plant biostimulants, provided their documentation is free from explicit pesticidal or curative claims (Rouphael and Colla, 2020). In the United States, registration pathways fall under the jurisdiction of the Environmental Protection Agency (EPA) and individual state Departments of Agriculture, which treat these products as structural plant regulators or carbon-based soil amendments. The primary challenge in registering these inputs lies in overcoming consumer and regulatory misconceptions regarding the use of ionizing radiation.

To address these concerns, comprehensive radiotoxicological and environmental profiling is required during the registration process. It is a critical scientific distinction that processing via Cobalt-60 gamma sources or industrial Electron Beams utilizes energies below the threshold required to induce nuclear transformations. Consequently, the treated biostimulant carries zero residual radioactivity and is completely safe for handling, storage, and application. Eco-toxicological evaluations demonstrate that these oligosaccharides are fully biodegradable, non-toxic to non-target soil organisms (such as earthworms and beneficial mycorrhizae), and leave no persistent residues in the soil or groundwater matrix, meeting the strict criteria for certified organic farming operations globally.

Knowledge Gaps, Future Prospects and Smart Nano-delivery systems

While the agronomic and physiological benefits of radiation-processed biostimulants are well-supported, several technical areas require further research. A primary focus for future exploration is the integration of these radiolytic oligosaccharides with advanced nanotechnology to construct smart nano-delivery platforms (Zhou and Sharma, 2025). Low-molecular-weight oligochitosan chains possess high functional charge densities, allowing them to serve as both stabilizing capping agents and biological triggers when conjugated with metallic or macronutrient nanoparticles (such as zinc oxide, nano-iron, or nano-urea). These hybrid nano-biostimulants can protect nutrient cores from soil immobilization, releasing them in response to specific root exudate triggers.

Additionally, further research is needed to determine



Fig. 2 : Industrial manufacturing pathway and multi-stress protection targets of radiation-processed biostimulants. The diagram tracks the sequence from raw marine byproduct preprocessing to continuous inline radiolytic chain scission, formulation stabilization and subsequent activation of tolerance mechanisms against abiotic and biotic stresses (Patil and Joshi, 2024; Zhou and Sharma, 2025).

Table 2 : Scientific Proof, Agronomic efficacy and Performance outcomes of Radiation-Processed Inputs.

Crop Family	RPB Category & Dose	Primary Agronomic and Physiological Outcome	Verified Scientific Proof Citation
Cash Crops (Sugarcane)	Gamma-Chitosan (50 kGy, 100 mg/L)	↑ Tiller survival rates; ↑ stalk elongation; 14% increase in juice Brix (°Bx) and Pol%; extended ratoon crop longevity.	Patil and Deshmukh (2024)
Cereals (Wheat, Rice)	Electron Beam Alginate (40 kGy, 75 mg/L)	↑ Flag leaf area; extended green-retention; optimized starch deposition in grain-filling; 15% increase in total harvest mass.	Mishra <i>et al.</i> (2025)
Legumes and Pulses (Soybean)	Irradiated Carrageenan (60 kGy, 100 mg/L)	↑ Lateral root branching; accelerated rhizobial nodulation efficiency; higher nitrogenase enzyme activity under field drought.	Ahmed and Santos (2023)
Oilseeds (Mustard)	Gamma-Oligochitosan (30 kGy, 50 mg/L)	↑ Primary branch initiation; upregulation of fatty acid desaturase genes; maximized seed oil yield per hectare.	Rahman <i>et al.</i> (2024)
Horticulture (Tomato)	Co-irradiated Chitosan-Alginate (40 kGy)	↑ Fruit skin elasticity; reduced post-harvest cracking; elevated soluble solids-to-acid ratios; extended shelf life.	Tanaka <i>et al.</i> (2024)

the long-term impacts of continuous biostimulant applications on the native soil microbiome. While short-term studies show increases in beneficial PGPR colonization, multi-season metagenomic tracking is required to confirm that continuous inputs do not disrupt the balance of native saprophytic communities. Future research will also leverage high-throughput transcriptomic and proteomic mapping to identify crop-specific receptors. This will allow production facilities to tailor the radiation

dose and match the resulting ligand chain length to the specific requirements of target crop varieties, optimizing field performance and supporting the growth of climate-resilient agricultural systems.

Table 2 compiles documented agronomic outcomes, nutrient use efficiency adjustments, and stress mitigation mechanisms across economic crop categories, providing verified scientific proof and reference citations.

Comprehensive Evidence Synthesis, Critical Knowledge Gaps, Meta-Omic Frameworks, and Strategic Future Horizons of Radiation-Processed Biostimulants

Current Knowledge synthesis and Critical evaluation

The current scientific paradigm concerning radiation-processed biostimulants (RPBs) represents a complex convergence of radiation chemistry, molecular plant physiology and multi-location macro-agronomic science. Evaluating the extensive literature over the last decade shows a clear mechanism: the physical fragmentation of high-molecular-weight structural polymers via ionizing radiation (Cobalt-60 gamma sources or high-voltage Electron Beams) triggers water radiolysis, yielding transient hydroxyl free radicals that target and cleave internal beta-(1→4)-glycosidic links under cold, ambient processing temperatures (Al-Askar *et al.*, 2024). This process consistently yields low-molecular-weight fragments optimized between 10 and 50 kDa, demonstrating water solubility and high systemic bioavailability (Patil and Deshmukh, 2024).

However, a critical cross-examination of findings across different research groups reveals underlying experimental variations and technical discrepancies instead of complete unity. For instance, while certain teams report maximum physiological activation at a uniform dose of 40 kGy (Nguyen *et al.*, 2023), others find significant phytotoxicity profiles in the same crop family if the polymer concentration drifts slightly from 1% to 2% w/v during exposure (Mishra *et al.*, 2025). This variation highlights that the absolute radiation dose is not the sole determinant of biostimulant activity; rather, it is the resulting structural configuration and degree of deacetylation that define target cell receptor compatibility. Furthermore, literature concerning macro-physiological expression often over-relies on visual biometric documentation (e.g., shoot extension or lateral root counts), while neglecting the rigorous quantification of chemical flux across transport membranes. This creates an analytical gap between structural alterations and biochemical validation, which this synthesis systematically addresses by linking molecular signaling models to empirical field data.

Scientific evidence Supporting Radiation-processed Biostimulants

The scientific proof establishing the agronomic value of radiation-processed fragments spans structural, biochemical, molecular, and macro-field operational layers. At the foundational layer, radiation-induced depolymerization and molecular weight reduction change

the material's transport kinetics. High-performance liquid chromatography (HPLC) profiles confirm that native, high-viscosity arrays undergo pseudo-first-order chain scission, converting bulky, non-absorbable structures into small oligomers that freely cross cell wall gaps and enter the apoplastic fluid (Singh *et al.*, 2023). Once in the apoplast, receptor-mediated signaling networks are triggered. Oligo-chitosan chains span and dimerize transmembrane LysM Receptor-Like Kinases, specifically CERK1 and LYK5, initiating intracellular signal transduction pathways via dual-phosphorylation along MKK4/5 and MPK3/6 cascades (Al-Askar *et al.*, 2024).

This molecular pathway drives a strong antioxidant enzyme induction network that protects the cell matrix. Mass spectrometry and transcriptomic analyses show immediate upregulation of genes coding for Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX) and Peroxidase (POD), establishing a chemical scavenging array that neutralizes stress-induced free radicals (Nguyen *et al.*, 2023). Concurrently, this cellular adjustment drives enhanced nutrient use efficiency (NUE) and primary photosynthesis. Treated plants upregulate the expression of the large and small subunits of the Rubisco enzyme, increase total chlorophyll concentration, and enhance root system development (Zhou and Sharma, 2025). This structural growth is supported by elevated root Cation Exchange Capacity (CEC) and the upregulation of plasma membrane-bound nutrient transporter systems (Patil and Deshmukh, 2024).

These biochemical adjustments provide comprehensive protection against environmental and biological threats. In abiotic stress mitigation, the biostimulant triggers the accumulation of compatible cytoplasmic osmolytes (proline and glycine betaine), preserving cell turgor pressure during drought, salinity, heat, and frost extremes (Rahman *et al.*, 2024). In biotic stress resistance, the biostimulant stimulates Phenylalanine Ammonia-Lyase (PAL) activity, inducing cell wall lignification and the systematic accumulation of Pathogenesis-Related (PR) proteins, chitinases, and beta-1,3-glucanases to degrade invading fungal barriers (Tran and Kim, 2025). These molecular responses translate directly into advanced field performance in major crops. Documented field trials confirm increased tiller numbers and sugar yields in sugarcane (*Saccharum officinarum*), maximized grain numbers in cereals, elevated nodulation in legumes, and extended shelf life in horticultural produce (Patil and Joshi, 2024; Tanaka *et al.*, 2024). Finally, optimized inline production systems utilizing continuous Electron Beam accelerators confirm the industrial

feasibility and commercial viability of scaling these stable formulations for global distribution (Zhou and Sharma, 2025).

Identification of Critical Research gaps

Despite the clear agronomic benefits documented across diverse crop species, the transition from lab-scale discoveries to globally accepted commercial products is limited by several critical research gaps. The primary technological bottleneck is the total lack of standardized molecular-weight specifications. Current industrial manufacturers use variable raw marine inputs and differing radiation metrics, producing heterogeneous chemical matrices that make it difficult to replicate field efficacy across disparate soil environments. This challenge is compounded by the limited availability of multi-location field trials. Most published literature relies on short-term greenhouse studies or isolated single-season plots, leaving the product's performance unverified across varying climate zones and hard water field dilution settings.

Furthermore, there is insufficient transcriptomic and metabolomic validation across diverse crop families. While model systems like *Arabidopsis* are mapped, high-value cash crops lack precise multi-omic sequencing datasets, obscuring the exact receptor variants involved. Crucially, there is an urgent need for long-term environmental impact studies focusing on soil microbiome interactions. It remains unknown how continuous, multi-season applications of active oligosaccharides affect native saprophytic communities or the long-term balance between beneficial mycorrhizal fungi and chitinolytic soil microbes. Finally, global regulatory disharmony creates substantial marketing hurdles. The lack of standard registration criteria across the EU, US, and Asian markets—combined with persistent public misconceptions regarding the term ‘irradiated’—highlights the need for a unified global policy framework that confirms the total absence of residual radioactivity and standardizes product positioning.

Strategic Future Horizons and Emerging perspectives

The future deployment of radiation-processed biostimulants lies at the intersection of radiochemistry, digital agronomy, and advanced material sciences. A key upcoming innovation is the integration of AI-assisted formulation optimization systems. By leveraging machine learning algorithms, production facilities can analyze raw polymer structures, target crop profiles and soil datasets to dynamically adjust continuous inline electron beam kinetics, tailoring the resulting oligosaccharide chain

distribution for specific crop varieties. This precision synthesis is supported by the development of nano-enabled radiation-processed biostimulants (Zhou and Sharma, 2025). Utilizing the high surface charge density of low-molecular-weight oligochitosan chains, researchers are developing hybrid nano-biostimulant carriers. These matrices encapsulate micro- and macronutrient nanoparticles, protecting them from soil immobilization and providing targeted, controlled-release delivery system triggered directly by specific root exudate chemical signatures.

Concurrently, these advanced inputs are being integrated into modern precision agriculture and drone-based delivery frameworks. The low viscosity and high solubility of radiation-degraded oligomers make them ideal for ultra-low-volume drone foliar applications. This approach ensures uniform canopy coverage and rapid cuticular absorption, reducing water use and optimizing field application costs. Ultimately, these integrated technologies position RPBs as vital components of carbon-smart and climate-resilient agriculture. By optimizing natural nutrient use efficiency, lowering the carbon footprint associated with conventional NPK synthetic fertilizer manufacturing, and strengthening crop resilience against extreme weather events, radiation-processed inputs provide a scalable framework for sustainable, high-yield agricultural ecosystems.

Conclusion

In summary, radiation-processed biostimulants represent a highly effective, clean, and technologically scalable advancement for 21st-century agriculture. The scientific evidence supporting their ability to activate transmembrane receptors, upregulate antioxidant defense systems, optimize photosynthesis, and remodel root architecture is well-established across multiple crop systems. However, transitioning this technology to widespread commercial adoption requires addressing key knowledge gaps, particularly the standardization of molecular weight matrices and the evaluation of long-term soil microbiome impacts. By resolving these technical challenges through multi-omic mapping and unified global registration standards, this radiolytic processing method offers a reliable path toward climate-smart, high-yield and sustainable farming systems.

Research highlights

- Radiation-induced depolymerization via water radiolysis cleanly cleaves polysaccharide backbones into highly soluble 10–50 kDa signaling oligomers.

- Low-molecular-weight fragments drive transmembrane receptor dimerization (CERK1/LYK5) and trigger intracellular Ca^{2+} and ROS signaling cascades.
- Biostimulant application upregulates the MPK3/MPK6 phosphorelay, activating WRKY, MYB, and NAC transcription factors in the nucleus.
- Empirical field evidence confirms enhanced nutrient use efficiency, optimized root CEC, and broad-spectrum abiotic/biotic stress resilience.
- Critical research gaps include the standardization of molecular weight profiles and multi-season soil microbiome impact assessments.
- Future perspectives emphasize AI-optimized radiolysis, nano-conjugated nutrient carriers, and precision drone-based foliar delivery systems.

Conflicts of interest

The author declares no competing financial interests or personal relationships that could influence the representation of the data or findings presented in this manuscript.

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