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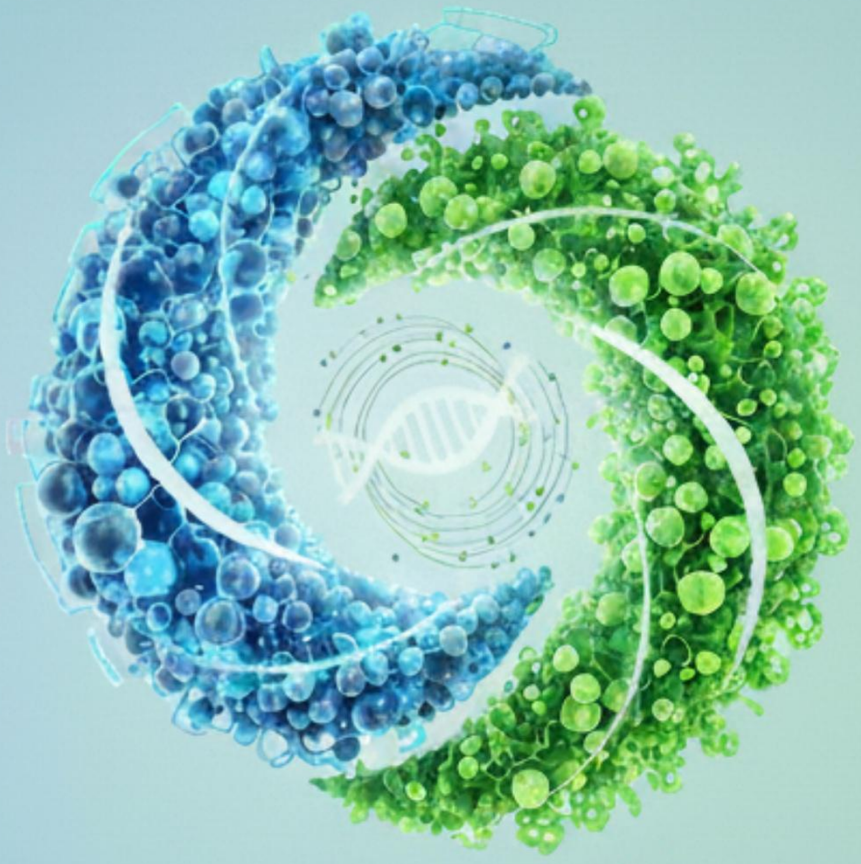
CONVERGING TECHNOLOGIES: NANOBIOTECHNOLOGY AND BIOMATERIAL INNOVATIONS



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PREFACE

The rapid advancement of science and technology is ushering in a transformative era where disciplines converge to create groundbreaking solutions. The book "Converging Technologies: Nanobiotechnology and Biomaterial Innovations" brings together emerging concepts, technological insights, and research developments at the interface of nanoscience, biotechnology, and material engineering. As global demand increases for sustainable medical systems, advanced diagnostics, smart therapeutics, and renewable biomaterials, interdisciplinary innovations are becoming essential for shaping the future.

Nanobiotechnology represents the application of nanoscale tools and techniques to biological systems, offering unprecedented capabilities in drug delivery, biosensing, tissue engineering, and environmental remediation. Meanwhile, biomaterial science has expanded significantly, moving beyond traditional polymers to include bioactive compounds, engineered tissues, natural macromolecules, and hybrid nano-biomaterials designed for specialized performance. When combined, these two domains open vast possibilities for healthcare, pharmaceuticals, regenerative medicine, and industrial sustainability.

This volume compiles chapters from researchers, academicians, and scientific professionals working across multiple fields. The content covers topics such as nano-enabled therapeutics, biodegradable materials, molecular targeting, scaffold design, biomedical devices, vaccine platforms, and smart biomaterial interfaces. Special attention is given to ethical considerations, safety assessments, and regulatory frameworks, recognizing that innovation must go hand-in-hand with responsibility.

A major goal of this book is to bridge conceptual knowledge with practical applications. By presenting both fundamental principles and real-world case studies, the volume seeks to provide a holistic perspective and encourage interdisciplinary collaboration.

We express our sincere gratitude to all contributing authors, reviewers, and those who supported the publication of this book. It is our hope that this text will inspire further exploration into converging technologies and foster innovation toward sustainable, health-oriented, and technologically empowered solutions for humanity.

- Editors

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NANOBIOTECHNOLOGY AND BIOSENSOR DEVELOPMENT

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Abstract:

Nanobiotechnology integrates nanotechnology with biological sciences to design, fabricate, and apply nanoscale materials for biomedical and analytical purposes. One of its most transformative applications is in biosensor development, where nanoscale materials enhance sensitivity, selectivity, and response time. This chapter explores the fundamental principles, types, and design strategies of nanobiosensors, emphasizing the role of nanomaterials—such as nanoparticles, carbon nanotubes, quantum dots, and graphene—in improving analytical performance. The integration of nanotechnology enables real-time, label-free, and multiplexed detection of biological molecules, with applications spanning medical diagnostics, environmental monitoring, and food safety. Advances in fabrication technologies and data analytics, particularly through microfluidics and artificial intelligence, are also discussed as emerging frontiers. The chapter concludes by addressing challenges and future perspectives in the clinical translation and commercialization of nanobiosensors.

Keywords- Nanobiotechnology**,** Biosensors**,** Nanomaterials**,** Signal Transduction**,** Biomedical Diagnostics

1. Introduction:

Nanobiotechnology lies at the intersection of nanoscience, biotechnology, and materials engineering, focusing on the manipulation of matter at the nanometer scale (10^{-9} m) for biological applications (Rai *et al.,* 2018). It has revolutionized biomedical diagnostics, drug delivery, and environmental sensing by enabling the design of devices and materials that interact precisely with biomolecules such as proteins, DNA, and cells (Bhalla *et al.,* 2016).

Biosensors, one of the most significant outcomes of nanobiotechnology, are analytical devices that convert a biological recognition event into a measurable signal (Turner, 2013). The integration of nanomaterials—such as gold nanoparticles, carbon nanotubes, and quantum dots—has enhanced biosensor sensitivity and reduced detection limits to the femtomolar or even attomolar range (Kumar *et al.,* 2019).

2. Principles of Biosensor Technology

2.1 Definition and Components

A biosensor consists of three primary components (Thévenot *et al.,* 2001):

1. **Bioreceptor:** a biological element that recognizes the target analyte (e.g., enzyme, antibody, nucleic acid).
2. **Transducer:** converts the recognition event into a measurable signal (e.g., optical, electrochemical, piezoelectric).
3. **Signal Processor:** amplifies and displays the output as readable data.

2.2 Working Mechanism

The general mechanism involves specific binding between the bioreceptor and analyte, generating a signal proportional to analyte concentration. Nanomaterials enhance signal generation by improving electron transfer, increasing surface area, and allowing better immobilization of bioreceptors (Singh *et al.,* 2017).

3. Role of Nanobiotechnology in Biosensor Development

Nanobiotechnology enhances biosensor performance by employing nanostructured materials that mimic biological systems and improve transduction efficiency (Salata, 2004).

3.1 Advantages of Nanomaterials in Biosensing

- **High surface-to-volume ratio**, enhancing analyte binding (Zhou *et al.,* 2015).
- **Unique optical and electrical properties** for sensitive signal transduction.
- **Facile functionalization** for biocompatibility and specificity.

Nanomaterials bridge the gap between biological recognition and electronic signal processing, enabling miniaturized, portable, and real-time detection systems.

4. Nanomaterials Used in Biosensor Fabrication

4.1 Gold Nanoparticles (AuNPs)

Gold nanoparticles are widely used due to their biocompatibility, surface plasmon resonance (SPR) properties, and ease of functionalization (Dykman & Khlebtsov, 2012). They enhance sensitivity in electrochemical and optical biosensors, allowing colorimetric detection without complex instrumentation.

4.2 Carbon Nanotubes (CNTs)

CNTs exhibit exceptional electrical conductivity and mechanical strength. Their high aspect ratio enables efficient electron transfer in electrochemical biosensors for glucose, cholesterol, and DNA detection (Gooding, 2005).

4.3 Quantum Dots (QDs)

Quantum dots are semiconductor nanocrystals with size-tunable fluorescence, allowing multiplexed bioimaging and detection (Alivisatos, 2004). They serve as fluorescent labels in optical biosensors for detecting proteins and nucleic acids.

4.4 Graphene and Graphene Oxide (GO)

Graphene, a single-atom-thick layer of carbon, offers excellent conductivity and mechanical flexibility, making it ideal for wearable biosensors and point-of-care devices (Novoselov *et al.,* 2012).

4.5 Magnetic Nanoparticles (MNPs)

MNPs allow easy separation and concentration of biomolecules under an external magnetic field, enabling preconcentration steps for ultra-sensitive detection (Laurent *et al.,* 2008).

5. Types of Nanobiosensors

5.1 Electrochemical Nanobiosensors

These biosensors detect current or potential changes resulting from redox reactions. CNTs and AuNPs improve electron transfer and stability (Wang, 2006). Applications include glucose monitoring and pathogen detection.

5.2 Optical Nanobiosensors

Optical sensors utilize fluorescence, luminescence, or SPR for detection. QDs and AuNPs are prominent nanomaterials due to their strong optical responses (Borisov & Wolfbeis, 2008).

5.3 Piezoelectric Nanobiosensors

Based on changes in mass or pressure, piezoelectric sensors—often made with ZnO nanostructures—detect binding events with high precision (Cheng *et al.,* 2010).

5.4 Magnetic Nanobiosensors

MNP-based sensors detect biomolecules through magnetic relaxation changes, providing label-free detection suitable for medical imaging and diagnostics (Issadore *et al.,* 2012).

6. Applications of Nanobiotechnology-Based Biosensors

6.1 Medical Diagnostics

Nanobiosensors enable rapid detection of biomarkers for diseases like cancer, diabetes, and infectious diseases (Singh *et al.,* 2017).

Example: AuNP-based lateral flow assays for COVID-19 antigen detection (Mahmoudi *et al.,* 2020).

6.2 Environmental Monitoring

Nanobiosensors detect heavy metals, pesticides, and microbial contaminants with high sensitivity in environmental samples (Zhao *et al.,* 2016).

6.3 Food Safety

Detection of foodborne pathogens such as *E. coli* and *Salmonella* is achieved using QD- and CNT-based biosensors (Viswanathan *et al.,* 2012).

6.4 Drug Discovery and Delivery

Nanobiotechnology aids in real-time monitoring of drug–target interactions and in designing nanosensors for controlled release (Rangnekar *et al.,* 2008).

7. Emerging Trends and Future Perspectives

Recent advancements involve microfluidic integration, wearable sensor platforms, and AI-driven biosensor data analysis (Chen *et al.,* 2021). Lab-on-a-chip devices now allow multiplexed detection with minimal sample volumes.

However, challenges remain in scalability, biocompatibility, and regulatory validation. Future research will focus on nanomaterial standardization and clinical translation for routine diagnostics (Wang & Gao, 2020).

Conclusion:

Nanobiotechnology has redefined biosensor technology, enabling ultra-sensitive, rapid, and miniaturized analytical tools for healthcare, environmental, and industrial applications. With continuous advances in material science, fabrication methods, and digital integration, nanobiosensors are poised to become integral components of precision diagnostics and personalized medicine.

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SEED PRIMING IN ONION:
A SUSTAINABLE APPROACH FOR ENHANCED PRODUCTIVITY

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Abstract:

Onion (*Allium cepa* L.) is one of the most economically important vegetable crops worldwide, yet its productivity is strongly constrained by poor seed vigour, short seed viability, and low, uneven germination. The rapid physiological deterioration of onion seeds, combined with environmental stresses such as salinity, drought, unsuitable temperatures, and nutrient imbalances, leads to weak seedling establishment and inconsistent field performance. Seed priming has emerged as an effective, low-cost, and sustainable pre-sowing technique to enhance seed metabolic activity prior to germination. This chapter summarizes the role of various priming methods including hydropriming, halopriming, osmopriming, nutrient priming, hormonal priming, biopriming, amino acid and sugar priming, solid matrix priming, magneto-priming, organic priming, and nanopriming in improving germination rate, germination uniformity, seedling vigour, stress tolerance, storability, and bulb yield in onion. Evidence showed that treatments such as GA_3 hormonal priming, PEG-based osmopriming, and nutrient priming significantly enhanced enzyme activation, membrane integrity, and physiological efficiency, resulting in superior field establishment of onion. This chapter further highlights future prospects including omics-based understanding of priming mechanisms, seed coating integration, and sustainable bio- and nano-formulations to improve the scalability and precision of priming technologies. Overall, seed priming represents a promising, eco-friendly strategy to overcome seed-related limitations and support higher productivity and resilience in onion cultivation.

Keywords: Onion (*Allium cepa* L.); Seed Priming; Germination; Seed Vigour; Osmopriming; Hormonal Priming; Stress Tolerance; Seedling Establishment.

Introduction:

Onion (*Allium cepa* L.) is second most important vegetable crops cultivated worldwide and belongs to the family *Amaryllidaceae*. It is grown in more than 170 countries, for domestic consumption and export. The crop is valued for its characteristic pungent flavor and aroma, which arise from sulfur-containing compounds, as well as for its nutritional and therapeutic properties. Onion bulbs are rich sources of carbohydrates, vitamins (particularly vitamin C and B-complex), minerals (such as calcium, phosphorus, and potassium), and various bioactive compounds including flavonoids, phenolics, and organosulfur constituents that contribute to human health and antioxidant defense (Pagano *et al.,* 2023).

In India, onion occupies a prominent position in the vegetable sector, being cultivated across diverse agro-climatic zones such as Maharashtra, Karnataka, Madhya Pradesh, Gujarat, and Rajasthan. The crop contributes substantially to both the national economy and export earnings due to its year-round demand and extensive culinary applications. Despite its commercial significance, onion productivity remains inconsistent, primarily due to poor seed quality, erratic germination, and non-uniform seedling establishment (Thirusendura Selvi & Saraswathy, 2018).

Onion seeds are small, short-lived, and highly sensitive to storage and environmental stresses. Rapid deterioration during storage results in loss of viability, reduced vigour, and delayed germination. Successful onion production, therefore, depends largely on obtaining uniform and vigourous seedlings capable of withstanding adverse field conditions. Conventional seed enhancement methods have shown limited success in overcoming these challenges.

Seed priming has emerged as an effective and eco-friendly pre-sowing strategy to improve germination rate, seedling vigour, and stress tolerance in onion and other seed-propagated crops (Pawar & Laware, 2018; Corbineau *et al.,* 2023). Seed priming involves controlled hydration of seeds to trigger pre-germinative metabolic activities without visible radicle protrusion, followed by re-drying to their initial moisture content. This process enhances the physiological and biochemical readiness of seeds, leading to faster and more uniform emergence under both optimal and stress conditions (Gebeyaw, 2020).

Given the increasing global demand for onion and the persistent constraints associated with seed quality and seedling establishment, exploring priming techniques for improving seed performance has become a major research focus. A comprehensive review of recent

progress in this field is essential to identify effective priming approaches, understand underlying mechanisms, and address open questions related to large-scale implementation.

Flowchart of the Seed Priming Procedure

Seed Selection:

Begin by choosing clean, healthy, and high-vigour onion seeds to ensure good priming results.

Soaking / Imbibition:

Seeds are placed in a priming solution such as plain water for hydropriming, salt solutions for halopriming, or PEG solutions for osmopriming and allowed to absorb moisture for a defined period. Temperature and aeration must be controlled to avoid radicle emergence. In onion, this hydration phase typically lasts between 6 and 24 hours, depending on the priming technique.

Metabolic Activation:

As seeds hydrate in a controlled manner, internal biochemical processes (including enzyme activation, DNA repair, and hormonal regulation) become active, which helps reduce the time required for germination after sowing.

Rinsing (when needed):

For treatments that involve salts or chemical solutions, seeds are washed thoroughly after soaking to remove any excess priming agent.

Drying:

Seeds are then dried back to their initial moisture level, either in the shade or under controlled drying conditions, to preserve their storability and handling quality.

Sowing:

Once dried, the primed seeds can be sown. Priming generally leads to quicker germination, better uniformity in seedling emergence, and improved seedling vigour, especially under stressful environmental conditions.

Problems in Onion Seed Germination

Onion (*Allium cepa* L.) is known for having inherently low seed vigour and short seed viability compared to many other vegetable crops. Poor and non-uniform germination are

among the major constraints affecting successful crop establishment and yield. Several physiological, biochemical, and environmental factors responsible for the germination-related problems.

1. Onion seeds have a short lifespan and deteriorate rapidly during storage. The seeds possess a thin seed coat and a high lipid content, which makes them prone to oxidative damage and rancidity during prolonged storage. This leads to a progressive decline in seed viability, vigour, and germination percentage. Studies have shown that even under favorable storage conditions, onion seed viability may drop significantly within 6–12 months (Thirusendura Selvi & Saraswathy, 2018).

2. The seed structure and composition contribute to poor germination performance. The presence of inhibitory compounds such as phenolics, coupled with low carbohydrate reserves and high levels of seed dormancy in some cultivars, can delay or reduce germination. Additionally, the small seed size limits nutrient availability to the developing embryo, affecting seedling vigour and early growth.

3. Environmental factors such as temperature, moisture, and salinity stress strongly influence germination. Onion seeds require narrow temperature ranges (20–25°C) for optimal germination, and deviations often result in delayed or uneven emergence. Under field conditions, unfavorable moisture regimes either drought or excess water further impair germination and root establishment. High salinity and osmotic stress also inhibit water uptake and enzyme activation necessary for germination and early metabolic activities.

Physiological seed aging causes loss of membrane integrity, reduced activity of antioxidant enzymes, and increased production of reactive oxygen species (ROS), which collectively impair cellular metabolism and delay germination (Pawar & Laware, 2018; Corbineau *et al.,* 2023). Reduced activity of hydrolytic enzymes such as α-amylase and protease in aged seeds results in slower mobilization of stored nutrients, affecting radicle emergence and seedling establishment.

Onion production faces multiple agronomic and environmental challenges that limit yield and quality. The crop is highly sensitive to temperature, photoperiod, and humidity; high temperatures cause bolting and small bulbs, while low temperatures delay vegetative growth. Irregular rainfall, waterlogging, and soil salinity lead to bulb rot and poor stand establishment. Nutrient deficiencies, particularly of nitrogen, sulfur, and micronutrients, further reduce bulb growth and storability. Major pests and diseases such as *Thrips tabaci*, *Alternaria* leaf blight, and *Fusarium* basal rot cause significant yield losses. Additionally,

poor curing, inadequate storage, and fluctuating market prices result in high post-harvest losses. The combined effects of these factors, along with poor seed germination and uneven crop establishment, lead to low and inconsistent productivity, highlighting the need for seed enhancement methods like seed priming to improve overall onion performance.

Types of Seed Priming and Their Impact on Onion Seed Germination, Vigour, Yield, etc.

Seed priming includes different pre-sowing treatments that slightly hydrate the seeds to restart important metabolic activities before planting. These methods use different agents such as water, salts, osmotic solutions like PEG, nutrients, plant hormones, beneficial microbes, amino acids, sugars, solid carriers, magnetic fields, organic extracts, and nanoparticles. Each type of priming works through different physiological and biochemical mechanisms for example, improving antioxidant activity, repairing cell membranes, balancing internal water levels, and activating enzymes while still preventing radicle emergence. Classifying priming into different types helps us understand how each method contributes to better germination, stronger seedling vigour, and higher stress tolerance. This classification is especially helpful for onion (*Allium cepa* L.) seeds, as priming greatly improves their germination, seedling establishment, and overall early growth performance.

1. Hydropriming

Hydropriming is the simplest and most eco-friendly seed priming method, involving soaking seeds in distilled water for a specified duration (usually 12-24 hours) followed by surface drying before sowing. It allows controlled hydration that activates metabolic processes such as enzyme synthesis, DNA repair, and energy metabolism without radicle emergence. In onion, hydropriming has been shown to improve germination percentage, germination speed, and uniformity while reducing mean germination time (Pagano *et al.,* 2023; Brar *et al.,* 2020). Thejeshwini *et al.,* (2019) reported better germination and early growth compared to unprimed seeds, while Muruli *et al.,* (2016) found higher vigour index in fresh seeds. Panghal *et al.,* (2023) observed that hydropriming delayed deterioration during storage and maintained seed quality. Although its effects are moderate compared to chemical priming, hydropriming remains a low-cost, safe, and effective technique for improving onion seed performance.

Type of Seed Priming	Agent Used + Concentration	Method / Duration	Key Results	Reference
1. Hydropriming	Distilled water (no chemical, plain water)	Soaking 12-24 h; surface drying	Improved germination %, speed, uniformity; reduced MGT; delayed deterioration; better early growth.	Pagano *et al.,* 2023; Brar *et al.,* 2020
2. Halopriming	KNO_3 (0.5-1%); NaCl (0.5-1%)	Soaking 12-24 h	Higher germination %, vigour, root-shoot length; reduced abnormal roots; better storability; improved establishment.	Tajbakhsh *et al.,* 2004, 2019; Brar *et al.,* 2020
3. Osmopriming	PEG 6000 (-1.0 MPa); Mannitol (osmotic solutions)	Soaking ~24 h	Increased germination (14-15%), uniformity; reduced MGT; improved stress tolerance and seedling vigour; delayed deterioration.	Singh *et al.,* 2017; Thejeshwini *et al.,* 2019
4. Nutrient Priming	$ZnSO_4$ (1%); KH_2PO_4 (1%); $CaCl_2$ (25-50 mM)	Soaking 12-24 h	Improved seedling length, vigour index, membrane integrity, bulb yield; reduced abnormal roots.	Brar *et al.,* 2020; Muruli *et al.,* 2016). Tajbakhsh *et al.,* 2004.
5. Hormonal Priming	GA_3 (50-100 ppm); IAA (100 ppm); Kinetin; Salicylic acid	Soaking 12-24 h	Higher germination (up to 21%), faster emergence, stronger vigour; enhanced enzyme activation; improved storability.	Khanduri 2010; Tejeshwini 2018; Muruli *et al.,* 2016
6. Biopriming	*Azotobacter, Trichoderma, Bacillus inocula*	Hydration with microbial suspension	Higher germination, enzyme activity, chlorophyll, antioxidant activity; improved storage vigour and stress tolerance.	Khanduri, 2010, Corbineau *et al.,* 2023.

7. Amino Acid Priming	Proline, Glycine, Glutamic acid, Aspartic acid, Tryptophan (10 mM)	Soaking 12-24 h	Germination up to 98.5%; higher vigour, chlorophyll and photosynthesis; improved stress tolerance and antioxidants.	Abdelkader *et al.,* 2023 and Shalaby *et al.,* 2023.
8. Sugar Priming	Trehalose (0.4 M); Raffinose (0.4 M)	Soaking several days at 15°C	Germination 30-50% under stress (vs 2% control); better uniformity; membrane stabilization; higher stress tolerance.	Horita and Saruyama (2006)
9. Solid Matrix Priming	Vermiculite or Micro-Cel E (no chemical concentration; inert matrix)	Controlled hydration with solid carrier	Improved germination rate, enzyme activity, vigour; reduced electrolyte leakage; better storability.	Khan *et al.,* 2003; Dhanush *et al.,* 2016
10. Magneto-priming	Magnetic field; $ZnSO_4$ (500 mg/L) or Ascorbic acid (50 mg/L)	Exposure to magnetic field for set duration	50% faster germination; 36% more seedling length; 58% higher vigour index; reduced MGT.	Abdel-Hady and Gadallah (2021)
11. Organic Priming	Vermicompost leachate (10%)	Soaking under drought stress (PEG 10%)	Germination ~90%; emergence ~81%; vigour index ~614; higher drought tolerance.	Islam *et al.,* (2023)
12. Nanopriming	ZnO, SiO_2, TiO_2 nanoparticles (low doses)	Soaking 12-24 h	Improved germination speed, vigour, ROS balance, antioxidant defense, and stress tolerance.	Pagano *et al.,* (2023) and Corbineau *et al.,* (2023)

2. Halopriming

Halopriming involves soaking seeds in salt solutions, commonly using potassium nitrate (KNO_3 0.5–1%) or sodium chloride (NaCl 0.5-1%). This treatment provides nitrate or ionic signals that regulate early metabolic activity and osmotic balance during imbibition. In onion, KNO_3 halopriming significantly enhances germination percentage, seedling vigour, and root–shoot elongation while reducing the occurrence of stunted root abnormalities (Tajbakhsh *et al.,* 2004). Brar *et al.,* (2020) found that halopriming improved the germination of aged seeds, maintaining higher vigour compared to untreated controls. Thejeshwini *et al.,* (2019) also reported improved bulb establishment and early growth in haloprimed seeds. Panghal *et al.,* (2023) demonstrated that haloprimed seeds maintained higher storability and vigour during storage. Overall, halopriming promotes rapid and uniform germination and enhances field performance by improving ion balance and nutrient uptake during early growth. Tajbakhsh *et al.,* (2004) demonstrated that $CaCl_2$ priming (50 mM) reduced abnormal root formation and increased root elongation. Nutrient priming helps strengthen seedling establishment and enhances yield attributes through improved nutrient availability during early growth.

3. Osmopriming

Osmopriming utilizes osmotic agents such as polyethylene glycol (PEG 6000) or mannitol to control water uptake during imbibition, maintaining the seed in a hydrated yet non-germinated state. PEG 6000 at −1.0 MPa for 24 hours has been widely used for onion priming. Osmoprimed seeds show enhanced germination by 14-15%, improved uniformity, reduced mean germination time, and enhanced stress tolerance (Singh *et al.,* 2017; Thejeshwini *et al.,* 2019). Under stressful environments (e.g., salinity or drought), osmopriming reduces abnormal seedlings and lipid peroxidation, leading to improved seedling vigour and field emergence (Brar *et al.,* 2020; Pagano *et al.,* 2023). Panghal *et al.,* (2023) noted that osmopriming also delays seed deterioration during storage. Thus, PEG-based osmopriming is one of the most effective priming techniques for improving onion germination, seedling vigour, and yield potential.

4. Nutrient Priming

Nutrient priming consists of soaking seeds in dilute nutrient solutions such as zinc sulphate ($ZnSO_4$ 1%), potassium dihydrogen phosphate (KH_2PO_4 1%), or calcium chloride ($CaCl_2$ 25–50 mM). These nutrients serve as cofactors for enzymatic activities and improve metabolic efficiency during germination. In onion, $ZnSO_4$ priming enhanced germination

rate, vigour index, seedling length, and bulb yield (Nissa *et al.,* 2024). KH_2PO_4 priming improved membrane integrity, post-storage germination, and seedling vigour (Brar *et al.,* 2020; Muruli *et al.,* 2016).

5. Hormonal Priming

Hormonal priming involves soaking seeds in plant growth regulator solutions such as gibberellic acid (GA_3 50–100 ppm), indole acetic acid (IAA 100 ppm), kinetin, or salicylic acid. Among these, GA_3 is the most commonly used and most effective for onion. GA_3-primed seeds show significantly higher germination percentage (up to 21%), faster emergence (27% increase), and enhanced vigour indices (Khanduri 2010; Tejeshwini 2018; Muruli *et al.,* 2016). GA_3 promotes enzyme activation (especially amylase and protease), resulting in faster mobilization of food reserves. Thejeshwini *et al.,* (2019) reported that GA_3 100 ppm priming produced the highest germination, seedling growth, and bulb yield, followed by KNO_3. Hormonal priming also maintains storability and seed viability under storage conditions (Panghal *et al.,* 2023). Hence, GA_3 priming is considered the most potent method for enhancing germination, vigour, and yield performance in onion.

6. Biopriming

Biopriming integrates biological agents such as *Azotobacter*, *Trichoderma*, or *Bacillus* species during seed hydration. This method enhances microbial colonization of the seed surface, improving nutrient uptake and protection against pathogens. Khanduri (2010) reported that *Azotobacter* inoculation combined with GA_3 enhanced germination and enzyme activity and maintained seed vigour during storage. Corbineau *et al.,* (2023) also confirmed that microbial biopriming improves chlorophyll content, antioxidant activity, and overall seedling performance. Bioprimed onion seeds demonstrate higher field emergence, stress tolerance, and post-storage viability compared to untreated seeds.

7. Amino Acid Priming

Amino acid priming (a subset of biostimulant priming) involves soaking seeds in amino acid solutions such as proline, glycine, glutamic acid, aspartic acid, or tryptophan (usually 10 mM). These organic molecules act as osmoprotectants and signaling compounds that enhance stress resistance and metabolic efficiency. Abdelkader *et al.,* (2023) and Shalaby *et al.,* (2023) observed significant improvements in germination rate (up to 98.5%), vigour index, chlorophyll content, and photosynthetic efficiency in onion seedlings primed with amino acids. Proline and glutamine were particularly effective in promoting early growth,

antioxidant activity, and stress tolerance, making this method valuable for improving both germination and physiological quality under stress conditions.

8. Sugar Priming

Sugar priming involves soaking seeds in sugar solutions such as trehalose or raffinose (0.4 M) for several days at 15 °C. These sugars stabilize cellular membranes and proteins during imbibition, particularly under stress. Horita and Saruyama (2006) found that sugar-primed onion seeds showed 30–50% germination under salinity and drought stress compared to only 2% in unprimed controls. The treatment also produced uniform germination and improved stress tolerance. Sugar priming is particularly useful for improving germination under suboptimal temperature and osmotic conditions.

9. Solid Matrix Priming

Solid matrix priming (SMP) uses inert carriers such as vermiculite or Micro-Cel E mixed with water and seeds in a defined ratio to maintain optimal moisture levels during conditioning. This technique facilitates slow hydration, ensuring uniform priming across seeds. In onion, SMP has been shown to improve germination rate, seedling vigour, metabolic enzyme activity, and ACC oxidase activity while reducing electrolyte leakage (Khan *et al.,* 2003; Dhanush *et al.,* 2016). It also improves storability and reduces seed deterioration during storage, making it effective for both fresh and carryover seed lots.

10. Magneto-Priming

Magneto-priming exposes seeds to magnetic fields alone or in combination with chemical treatments such as $ZnSO_4$ (500 mg L^{-1}) or ascorbic acid (50 mg L^{-1}). This physical priming technique enhances ion mobility and enzymatic activation. Abdel-Hady and Gadallah (2021) demonstrated that magneto-primed onion seeds showed a 50% increase in germination speed, 36% increase in seedling length, and 58% increase in vigour index compared with control seeds. The treatment also shortened mean germination time, indicating its potential for stimulating early seedling growth and vigour.

11. Organic Priming (Vermicompost Leachate Priming)

Organic priming uses natural extracts such as vermicompost leachate, which contains humic substances and plant growth-promoting compounds. Islam *et al.,* (2023) reported that priming onion seeds with 10% vermicompost leach solution under drought stress (PEG 10%) improved germination (~90%), field emergence (~81%), vigour index (~614), and stress tolerance index (~84%). Organic priming enhances seedling establishment and resilience under abiotic stress while being environmentally sustainable.

12. Nanopriming

Nanopriming is an advanced priming technique using nanoparticles such as ZnO, SiO_2, or TiO_2 at low concentrations. These nanoparticles modulate reactive oxygen species (ROS), enhance antioxidant defense, and promote nutrient uptake. Although still in early stages for onion, Pagano *et al.,* (2023) and Corbineau *et al.,* (2023) highlighted its potential to improve vigour index, germination speed, and stress tolerance, suggesting a promising direction for future research.

Future Aspects of Seed Priming Strategies in Onion

- Future research on seed priming strategies in onion and related crops is expected to move toward precision, sustainability, and molecular understanding of the priming process. Although conventional methods such as hydro-, halo-, and osmopriming have proven effective, there remains a need to optimize treatment duration, concentration, and environmental conditions for different onion cultivars and seed ages to achieve consistent results.
- The integration of omics technologies (transcriptomics, proteomics, and metabolomics) can unravel the molecular mechanisms underlying seed metabolism reactivation and stress memory during priming, leading to more targeted and genotype-specific protocols. Emerging approaches such as nanopriming, magneto-priming, and biostimulant-based priming (using amino acids, humic substances, or beneficial microbes) show great potential to enhance stress tolerance, seedling vigour, and nutrient-use efficiency under changing climatic conditions.
- Furthermore, combining priming with seed coating or encapsulation technologies could provide long-lasting protection and controlled activation of priming agents. The development of eco-friendly, low-cost formulations using organic and biodegradable materials will align priming techniques with sustainable agricultural goals.
- Future studies should also focus on field validation, scalability, and storage stability of primed seeds to bridge the gap between laboratory success and commercial application. Overall, the next generation of seed priming strategies will likely be characterized by integrated, multi-functional, and environmentally sustainable approaches that enhance both seed performance and crop productivity in onion cultivation.
- However, despite these proven benefits, standardization across cultivars and environments remains a challenge. The optimum duration, concentration, and drying

conditions must be carefully optimized for each priming agent and genotype to achieve reproducible results. Future work should emphasize molecular and biochemical characterization of priming-induced changes using transcriptomic, proteomic, and metabolomic approaches to understand the underlying mechanisms of "seed memory." Integrating advanced techniques such as nanopriming, bioformulation-based priming, and encapsulated seed coatings can further enhance the efficiency, storability, and stress resilience of onion seeds.

Conclusion:

Seed priming represents one of the most promising pre-sowing physiological enhancement techniques for improving seed performance in onion (*Allium cepa* L.), a crop that faces serious challenges related to poor seed vigour, short longevity, and erratic germination. Through partial hydration, priming initiates early metabolic and biochemical processes such as enzyme activation, repair of damaged nucleic acids, synthesis of proteins, and mobilization of food reserves, all of which prepare the seed for rapid and uniform germination once sown. Extensive research evidence demonstrates that priming not only improves germination percentage, rate, and uniformity, but also enhances seedling vigour, stress tolerance, field establishment, and ultimately bulb yield.

Among the different methods, hormonal priming with gibberellic acid (GA_3 at 50-100 ppm) has consistently provided the highest germination and vigour indices by accelerating enzymatic and hormonal activation during imbibition. Osmopriming with polyethylene glycol (PEG 6000 at –1.0 MPa) effectively regulates water uptake, enhances stress resistance, and reduces abnormal seedling formation, leading to improved uniform emergence under field conditions. Similarly, nutrient priming using $ZnSO_4$ or KH_2PO_4 improves membrane stability, seedling growth, and yield attributes through improved nutrient availability during the early stages of germination. Halopriming with KNO_3, hydropriming with water, and biopriming using beneficial microbes also contribute positively by enhancing vigour and maintaining viability during storage. Recent innovations such as amino acid priming, sugar priming, magneto-priming, and organic priming with vermicompost leachate have shown additional potential for improving physiological efficiency, antioxidant activity, and abiotic stress tolerance in onion seedlings.

Beyond the laboratory, priming has significant practical implications. It enables uniform seedling establishment, a crucial factor for achieving high bulb uniformity, early maturity, and increased productivity. Priming also rejuvenates aged or low-vigour seeds, allowing

seed companies and farmers to extend seed lifespan and reduce losses associated with poor seed quality. Furthermore, the eco-friendly nature of priming especially hydro- and organic-based treatments aligns well with the goals of sustainable and low-input agriculture.

Seed priming serves as a powerful, scientifically validated, and practically feasible strategy for enhancing the germination potential, vigour, and yield of onion. With continued refinement and integration of modern biotechnological and nanotechnological tools, priming can play a pivotal role in sustainable onion production, seed industry improvement, and climate-resilient agriculture in the years ahead.

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GENOMIC APPROACHES IN PLANT DISEASE DIAGNOSIS, SURVEILLANCE, AND MANAGEMENT

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Abstract:

Advances in genomic technologies have transformed our understanding of plant diseases and reshaped the way pathogens are detected, monitored, and managed. Traditional diagnostic tools, though valuable, often fall short in identifying emerging, fast-evolving or morphologically similar pathogens. Genomic approaches now bridge these gaps by enabling precise, rapid, and high-resolution detection of causal agents directly from plant tissues and environmental samples. High-throughput sequencing, molecular markers, and gene expression profiling have expanded our ability to trace pathogen evolution, uncover host–pathogen interactions, and predict disease outbreaks with greater accuracy. These tools also support the development of resistant crop varieties and guide evidence-based disease management strategies in modern agriculture. This chapter synthesizes the major genomic techniques used in pathogen identification, disease surveillance, functional analysis of host responses, and the integration of these tools into crop improvement programs. By bringing together current methodologies and their practical applications, the chapter highlights how genomics continues to strengthen plant health monitoring and sustainable disease management under changing agricultural and climatic conditions.

Keywords: Genomics, Diagnostics, host–Pathogen Interactions, Tools, Plant Diseases

Introduction:

Plant diseases continue to threaten global food security, agricultural productivity, and the economic stability of farming communities. With increasing globalization, climate variability, and intensification of crop production systems, the frequency and impact of emerging and re-emerging plant pathogens have become more pronounced. Many pathogens spread silently, remain undetected during early infection stages, or exhibit high genetic variability that challenges conventional identification approaches. These complexities demand advanced, highly sensitive, and reliable diagnostic and monitoring tools that can keep pace with rapidly evolving plant health challenges.

Genomic technologies have revolutionized the field of plant pathology by enabling direct, precise, and comprehensive investigation of pathogens and host plants at the molecular level. Unlike traditional methods that rely on morphological traits, culture-based identification, or biochemical characteristics, genomic approaches allow detection of pathogens directly from infected tissues, soil, water, and vector samples—even when organisms are unculturable or present at very low concentrations. Tools such as PCR, qRT-PCR, DNA barcoding, whole-genome sequencing (WGS), metagenomics, transcriptomics, and molecular marker-based analyses have fundamentally transformed how diseases are diagnosed, how pathogen populations are tracked, and how host responses are understood. The integration of genomics into plant disease surveillance has also enhanced our ability to monitor pathogen movement across regions, identify new strains or variants, and uncover genetic signatures associated with virulence, host specificity, and fungicide or pesticide resistance. These insights are vital for developing predictive surveillance systems and guiding rapid decision-making during disease outbreaks. Likewise, genomic information has accelerated crop improvement programs by enabling marker-assisted selection, genomic selection, and gene-editing strategies aimed at developing resilient crop varieties capable of withstanding diverse biotic stresses.

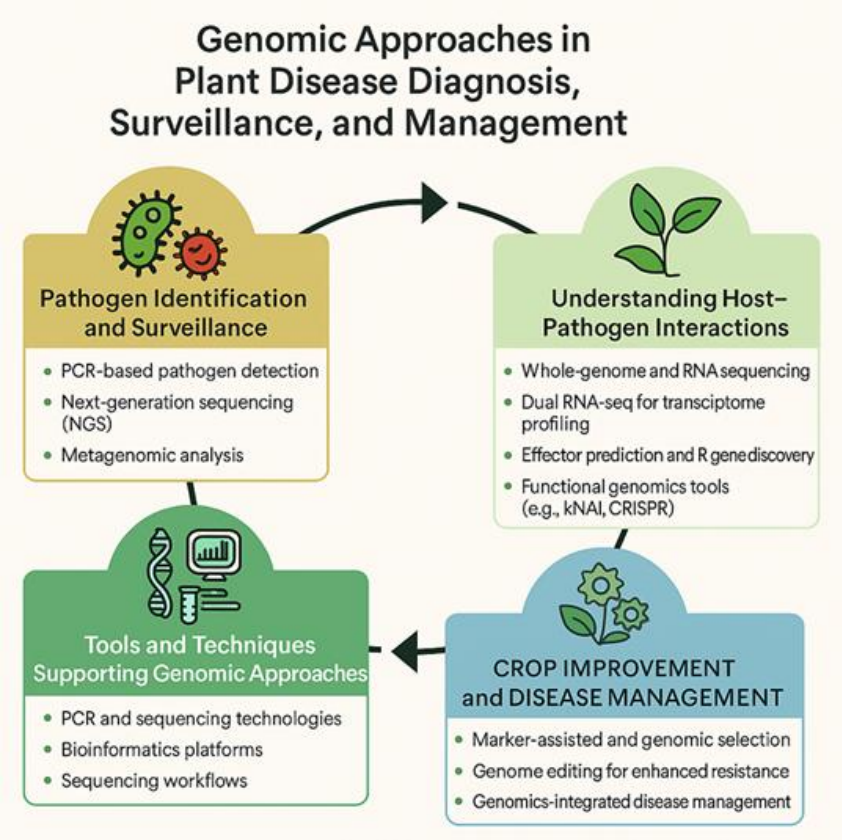

Figure 1: Conceptual overview of genomic approaches in plant disease diagnosis, surveillance, and management

As agriculture moves toward data-driven, precision-based approaches, genomics has emerged as a powerful foundation for sustainable disease management. This chapter provides an in-depth overview of major genomic tools and their applications in plant disease diagnosis, pathogen surveillance, understanding host–pathogen interactions, and crop improvement (Fig.1). It also highlights how these tools collectively contribute to modern plant health monitoring frameworks and long-term disease resilience.

Pathogen Identification and Surveillance

Pathogen identification and surveillance form the backbone of modern plant disease diagnostics and management systems. With increasing globalization, climate change, and movement of planting materials, new and emerging pathogens are spreading more rapidly than ever before, making early and accurate identification crucial for preventing large-scale crop losses. Traditionally, plant pathogens were identified based on morphological and cultural characteristics, such as fungal spore morphology, bacterial colony appearance, or symptom-based diagnosis; however, these approaches are slow, subjective, and often insufficient for distinguishing morphologically similar or fastidious pathogens. To overcome these challenges, molecular diagnostics including PCR, qPCR, RT-PCR, nested and multiplex PCR, DNA barcoding, LAMP, and next-generation sequencing (NGS) have revolutionized pathogen identification by providing high specificity, sensitivity, and the ability to detect pathogens even at very low titers or during latent infections (Table.1). These tools enable strain-level identification, detection of mixed infections, and confirmation of obligate or unculturable pathogens, which is not possible through conventional methods.

Alongside identification, continuous surveillance plays a critical role in monitoring pathogen distribution, tracking disease outbreaks, and understanding the epidemiology of emerging threats. Modern surveillance integrates field surveys, sentinel plots, environmental DNA (eDNA) monitoring, remote sensing, drone-based disease scouting, and digital disease reporting platforms to generate real-time data on pathogen occurrence. NGS and metagenomics-based surveillance allow unbiased detection of all microorganisms present in a sample, enabling early detection of novel pathogens before symptoms appear. Furthermore, genomic surveillance where pathogen genomes are sequenced across time and locations supports epidemiological tracing by identifying introduction routes, mutation patterns, population diversity, and evolution of virulence or resistance genes.

Table 1: Genomic Tools for Pathogen Identification and Surveillance

Tool / Approach	Principle / Basis	What It Detects	Advantages	Example Application
PCR / qPCR	Amplification of pathogen-specific DNA regions	Fungi, bacteria, DNA viruses	Highly sensitive and specific; quantitative	Detection of *Fusarium oxysporum*, *Ralstonia solanacearum*, Begomoviruses
RT-PCR	Amplifies RNA after reverse transcription	RNA viruses, pathogen transcripts	Detects active infection; very sensitive	Diagnosis of TMV, CMV, MYMIV/MYMIV
DNA Barcoding (ITS, rDNA, COI)	Sequencing of conserved-locus barcode regions	Broad range of fungi, bacteria, insects	Universal system, species-level identification	Identifying *Pseudocercospora*, *Alternaria*, *Phytophthora*
LAMP / RPA	Isothermal amplification	All pathogen types depending on primers	Rapid (30–45 min), field-usable	On-site detection of *Xanthomonas*, *Phytophthora infestans*
NGS / Metagenomics	Sequencing all DNA/RNA in a sample	Entire microbial communities	Detects new, mixed, and unknown pathogens	Discovery of wheat blast in Bangladesh; soil microbiome analysis
Environmental DNA (eDNA)	DNA isolated from soil, water, air	Pathogens present in environment	Non-invasive, early detection	Monitoring *Phytophthora* in irrigation water
Remote Sensing & Drones	Spectral imaging detects stress signatures	Disease symptoms, crop stress	Large-scale, real-time monitoring	Mapping viral disease spread in soybean fields
Genomic Surveillance	Whole-genome sequencing over time	Pathogen evolution & movement	Tracks mutations, identifies outbreak sources	Tracking wheat stem rust race Ug99 movement
Digital Surveillance Systems	Mobile apps, IoT sensors, disease reporting	Field disease occurrence	Real-time alerts & early warnings	Plantix, e-Pest Surveillance System (India)

This integration of diagnostics and surveillance provides early warning signals, supports rapid disease containment, guides quarantine decisions, and strengthens national biosecurity by preventing the spread of exotic pathogens through seed, nursery materials, or trade.

Overall, pathogen identification and surveillance together create an effective system for disease prevention and management. They enable timely interventions such as roguing infected plants, restricting movement of planting material, applying targeted fungicides, and advising farmers through early warning bulletins. As agriculture moves toward precision plant health, combining molecular diagnostics, genomic tools, and digital surveillance networks will be essential for safeguarding crop productivity and ensuring food security.

Understanding Host Pathogen Interactions

Understanding host pathogen interactions is central to developing effective, long-term solutions for plant disease management. When a pathogen encounters a host plant, a complex molecular dialogue begins—one that determines whether the infection will succeed or be halted by the plant's defense machinery. Genomic technologies have dramatically advanced our capacity to decipher these interactions at cellular, biochemical, and molecular levels. By integrating genomics, transcriptomics, proteomics, and metabolomics, researchers can now visualize how plants perceive pathogens, activate defense responses, and remodel physiological pathways during infection.

At the heart of this interaction is the plant immune system, which operates through two interconnected layers. The first is pattern-triggered immunity (PTI), where plant receptors recognize conserved pathogen-associated molecular patterns such as flagellin, chitin, or elongation factor Tu. Successful pathogens often evolve effector molecules to suppress this basal defense, leading to the second layer known as effector-triggered immunity (ETI). ETI is mediated by plant resistance (R) genes that detect pathogen effectors and activate strong defense reactions, such as hypersensitive response, reactive oxygen species (ROS) production, and defense gene induction. Genomic sequencing of both hosts and pathogens enables precise identification of these receptors, effectors, and downstream signaling components (Table.2).

Pathogens including fungi, bacteria, viruses, nematodes, and oomycetes—deploy a diverse arsenal of factors like secreted enzymes, toxins, small RNAs, and protein effectors to colonize host tissues. Genomics assists in pinpointing these virulence determinants by

enabling whole-genome comparative analyses, effector prediction pipelines, and transcriptomic profiling during infection. For instance, RNA-seq–based expression studies reveal which plant genes are up- or downregulated during infection, providing insights into the dynamic interplay between plant defense pathways such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) signaling networks.

Similarly, functional genomics approaches such as virus-induced gene silencing (VIGS), CRISPR/Cas-mediated gene editing, and overexpression studies validate the roles of candidate genes in immunity or susceptibility. These tools help determine how specific gene families like NB-LRR proteins, transcription factors, pathogen recognition receptors (PRRs), and secondary metabolite biosynthesis enzymes contribute to disease resistance. Collectively, such genomic investigations provide a comprehensive understanding of how host genetic architecture shapes susceptibility or resilience to pathogens (Table.3).

With the growing complexity of plant diseases caused by mixed infections, vector-borne pathogens, and rapidly evolving strains, the study of host pathogen interactions provides critical predictive power. Integrating host and pathogen genomes allows researchers to model co-evolutionary patterns, detect adaptive mutations, and identify molecular arms-race signatures that influence disease epidemiology. These insights are indispensable for designing future disease-resilient crops, deploying R genes strategically, and ensuring long-term stability of resistance in the field.

Table 2: Genomic Tools for Studying Host–Pathogen Interactions and Their Applications

Genomic Approach	What It Reveals	Key Methods / Tools	Applications in Host–Pathogen Studies	References
Whole-Genome Sequencing (WGS)	Genome architecture of host or pathogen	Illumina, PacBio, Nanopore	R-gene discovery, effector identification, strain evolution	Jones *et al.,* 2014; Goodwin *et al.,* 2011
RNA Sequencing (Transcriptomics)	Differential gene expression	RNA-seq, DEG analysis	Defense pathway profiling, pathogen transcript analysis	Wang *et al.,* 2009; Zou *et al.,* 2019
Small RNA	Regulatory	sRNA-seq	Discovery of	Weiberg & Jin,

Sequencing	RNAs in immunity		miRNAs/siRNAs involved in defense	2015
Proteomics	Protein abundance and modifications	LC-MS/MS, MALDI-TOF	Effector proteins, defense enzymes	van Bentem & Hirt, 2009; Delaurent *et al.,* 2021
Metabolomics	Defense metabolites and phytoalexins	GC-MS, LC-MS	Identification of SA, JA, ET pathway metabolites	Alseekh & Fernie, 2018
VIGS / RNAi	Functional gene validation	TRV-based VIGS, RNAi constructs	Identifying host resistance and susceptibility genes	Burch-Smith *et al.,* 2004
CRISPR/Cas Genome Editing	Targeted gene modification	Cas9, Cas12a	Engineering disease-resistant crops	Langner *et al.,* 2018; Zaidi *et al.,* 2018
Dual RNA-seq	Simultaneous host + pathogen expression	Dual RNA profiling	Revealing molecular dialogue during infection	Westermann *et al.,* 2017
Effector Prediction Pipelines	Secreted pathogen effectors	SignalP, EffectorP	Characterizing virulence mechanisms	Sperschneider *et al.,* 2016

Table 3: Key Genes Involved in Host Immunity and Their Genomic Functions

Gene / Gene Family	Type	Function in Defense	Genomic Insight	References
NB-LRR (NLR) Genes	Resistance genes	Recognize pathogen effectors; trigger ETI	Genome sequencing reveals clusters and allelic diversity	Jones & Dangl, 2006; Meyers *et al.,* 2003
PRRs (e.g., FLS2, EFR)	Pattern-recognition receptors	Detect PAMPs; activate PTI	Conserved receptor domains identified through genome comparisons	Zipfel, 2014; Gómez-Gómez & Boller, 2000
WRKY Transcription Factors	Regulatory genes	Modulate SA/JA/ET signaling	Transcriptomics shows WRKY induction during infection	Pandey & Somssich, 2009
PAL, CHS, DFR, FLS (Phenylpropanoid Pathway)	Secondary metabolism	Structural and chemical defenses; lignin & flavonoid biosynthesis	Upregulated during infection in RNA-seq datasets	Dixon & Paiva, 1995; Vogt, 2010
MAPKs (MPK3, MPK6)	Signaling proteins	Transduce immune signals	Functional genomics reveals key roles in defense cascades	Meng & Zhang, 2013
RPM1, RPS2, RPP family	Classical R genes	Recognize pathogen effectors	WGS identifies allelic variants linked to resistance	Bent *et al.,* 1994; Grant *et al.,* 1995

Crop Improvement and Disease Management

Ensuring sustainable crop production under increasing biotic pressures requires the integration of genomic knowledge into breeding and disease management programs. Genomic tools offer unprecedented resolution for identifying resistance genes, predicting plant responses to pathogens, and accelerating the development of resilient crop varieties.

These approaches complement traditional plant breeding by enabling precise selection, faster trait introgression, and a deeper understanding of host–pathogen interactions.

The incorporation of genomic-assisted breeding strategies such as marker-assisted selection (MAS), genomic selection (GS), and genome editing as significantly strengthened modern agriculture. Unlike phenotypic screening, which is influenced by environmental fluctuations and disease pressure, genomic tools provide stable and reliable markers linked to resistance traits. High-density SNP arrays, RAD-seq, and whole-genome resequencing have greatly expanded the marker repertoire for identifying quantitative trait loci (QTLs) associated with tolerance or resistance to fungal, bacterial, viral, and nematode pathogens (Table.4).

Furthermore, genomic knowledge guides the deployment of disease management algorithms, including early-warning systems, pathogen surveillance models, predictive epidemiology, and host resistance stewardship. When combined with integrated disease management (IDM) practices such as biological control, sanitation, cultural practices, and judicious use of pesticides genomic approaches improve the precision and effectiveness of field-level decisions (Table.5).

Genome editing technologies, especially CRISPR/Cas systems, have opened the possibility of modifying susceptibility (S) genes, enhancing immune pathways, and stacking broad-spectrum resistance traits without altering the agronomic performance of the crop. Gene-edited varieties with increased resistance to powdery mildew, bacterial blight, and viral pathogens have already demonstrated the potential of this technology to address emerging plant health challenges.

In addition to host improvement, genomics supports the design of RNAi-based constructs, dsRNA sprays, and CRISPR-based pathogen-targeting strategies that directly interfere with pathogen survival or virulence. These technologies represent the next generation of environmentally safe plant disease management tools, with the advantage of specificity and overall, integrating genomics with crop improvement and disease management is transforming the agricultural landscape by enabling faster, smarter, and more sustainable solutions to plant disease threats.

Table 4: Genomic Approaches Used in Crop Improvement for Disease Resistance

Genomic Approach	Mechanism	Application in Crop Improvement	Examples	Key References
Marker-Assisted Selection (MAS)	Uses molecular markers linked to resistance loci	Accelerates introgression of R genes & QTLs	Xa21 for bacterial blight in rice; Rpv3 for downy mildew in grape	Collard & Mackill, 2008
Genomic Selection (GS)	Predicts breeding values using genome-wide markers	Improves polygenic disease resistance	Rust resistance in wheat; blight tolerance in maize	Crossa *et al.,* 2017
QTL Mapping / GWAS	Identifies genomic regions associated with resistance	Discovery of new R genes & resistance loci	QTLs for late blight (potato), root rot (soybean)	Brachi *et al.,* 2011
Genome Editing (CRISPR/Cas)	Targeted modification of resistance or susceptibility genes	Engineering durable resistance	Knocking out MLO gene for powdery mildew resistance in wheat/barley	Wang *et al.,* 2014; Zaidi *et al.,* 2018
Genomic Resequencing	High-resolution SNP discovery	Identifying resistant germplasm	Rice blast-resistant landraces; soybean rust-resistant accessions	Varshney *et al.,* 2009
RNA Interference (RNAi)	Silencing pathogen or host susceptibility genes	Transgenic & spray-induced resistance	RNAi-based resistance to MYMV, root-knot nematodes	Koch & Kogel, 2014

Table 5: Genomics-Integrated Disease Management Strategies

Strategy	Genomic Basis	Outcome	Examples	References
Pathogen Surveillance Using Genomics	WGS, metagenomics identify variants & track spread	Early detection & outbreak management	Tracking wheat blast *Magnaporthe oryzae* spread in Asia	Islam *et al.,* 2016
Predictive Epidemiology Models	Uses genomic data + climatic data	Forecasts disease risk	Late blight prediction using pathogen genotype data	Savary *et al.,* 2019
R-gene Stewardship	Genomics reveals R-gene diversity & durability	Minimizes resistance breakdown	Strategic deployment of R genes in potato, rice	McDonald & Linde, 2002
RNA-based Disease Control	dsRNA sprays or host-induced gene silencing	Blocks pathogen growth	RNA-spray control of *Botrytis cinerea*	Koch *et al.,* 2016
CRISPR-Based Pathogen Targeting	Direct editing of pathogen genomes	Reduces virulence	CRISPR targeting of viral genomes in cassava, tobacco	Ali *et al.,* 2015

Tools and Techniques Supporting Genomic Approaches

Modern genomic approaches rely on a wide array of laboratory-based and computational tools that enable accurate detection, quantification, and characterization of plant pathogens and host responses. These tools form the technological backbone of advanced plant disease diagnosis, pathogen surveillance, and crop improvement programs. From PCR-based amplification to whole-genome sequencing and high-throughput bioinformatics workflows, these techniques together enable rapid, sensitive, and detailed genomic analyses at scales unimaginable in traditional plant pathology.

Among the most fundamental molecular tools is the Polymerase Chain Reaction (PCR) and its derivatives, including reverse transcription PCR (RT-PCR), quantitative real-time PCR

(qPCR), multiplex PCR, and loop-mediated isothermal amplification (LAMP). These techniques amplify specific DNA or RNA sequences, enabling detection of low-level infections, confirmation of pathogen species, and assessment of gene expression patterns during host–pathogen interactions. PCR-based detection remains the frontline diagnostic tool because of its sensitivity, speed, and compatibility with diverse sample types (Table 6).

The advent of Next-Generation Sequencing (NGS) has revolutionized plant disease research by enabling comprehensive genome, transcriptome, and metagenome analyses at high throughput. Platforms such as Illumina, Oxford Nanopore, and PacBio offer varied read lengths, accuracy levels, and sequencing capacities, allowing researchers to select appropriate tools based on study objectives. Whole-genome sequencing supports effector discovery, R-gene identification, phylogenetic reconstruction, and pathogen outbreak tracking. Meanwhile, RNA-seq and small RNA sequencing provide insights into differential gene expression, regulatory small RNAs, and transcriptional reprogramming under infection (Table 7).

In parallel, powerful bioinformatics platforms have emerged to interpret the enormous volumes of data generated through sequencing (Table. 8). Tools for quality filtering, genome assembly, gene prediction, variant calling, phylogenomics, and pathway analysis are essential for deriving biological meaning from raw sequence data. Integrated workflows—built using platforms like Galaxy, Geneious, CLC Genomics Workbench, and custom pipelines—enable researchers to analyze genomic datasets with accuracy and reproducibility.

Together, these tools form a comprehensive ecosystem that supports genomic studies in plant disease diagnosis, surveillance, and management. Their integration ensures a robust framework for high-resolution understanding of plant–pathogen interactions and accelerates the development of disease-resistant crop varieties.

Table 6: Genomic Approaches and techniques used in Plant Disease Studies

Main Approach	Overview (What it Detects)	Key Techniques / Methods	Applications
1. DNA-Based Approaches	Detect pathogen **DNA** directly from infected tissue	PCR, qPCR, Nested PCR, Multiplex PCR, LAMP, RPA, DNA barcoding, Microarray	Detection of fungal, bacterial & DNA viral pathogens; species confirmation; strain-level identification

2. RNA-Based Approaches	Detect **RNA** genomes (viruses) or pathogen transcripts	RT-PCR, RT-qPCR, RNA-seq, Small RNA profiling	Virus and viroid detection; monitoring pathogen gene expression; early infection detection
3. NGS-Based Approaches	Sequence entire genomes or mixed microbial communities	Whole Genome Sequencing (WGS), Metagenomics, Amplicon sequencing (ITS/16S), Targeted enrichment	Detection of unknown/unknown pathogens; pathogen evolution; mixed infections
4. Marker-Based Approaches	Use genetic polymorphic markers for characterization	SSR, ISSR, RAPD, AFLP, SNP genotyping, MLST, RFLP	Strain differentiation, population genetics, epidemiology studies
5. CRISPR-Based Approaches	CRISPR enzymes recognize pathogen nucleic acids with high specificity	Cas12 (DNA detection), Cas13 (RNA detection), DETECTR, SHERLOCK	Rapid, field-level pathogen detection; high sensitivity viral & bacterial diagnosis
6. Nanotechnology & Biosensor Approaches	Detect nucleic acids using sensor platforms and nanoparticles	DNA biosensors, electrochemical sensors, optical sensors, gold nanoparticle assays, microfluidic chips	On-site diagnostics; rapid low-cost detection; portable disease sensors
7. Immuno-Genomic Approaches	Combine immunocapture with nucleic acid amplification	Immunocapture-PCR, ELISA-PCR	Detection of low-abundance viruses and bacteria; improved sensitivity

Table 7: Major NGS Sequencing Platforms Used in Plant Genomics and Pathogen Diagnostics

Sequencing Platform	Type / Read Length	Strengths	Applications	References
Illumina (MiSeq, NextSeq, NovaSeq)	Short reads (75–300 bp)	High accuracy, cost-effective	WGS, metagenomics, RNA-seq	Bentley *et al.,* 2008
PacBio Sequel II	Long reads (10–25 kb, HiFi reads 99% accuracy)	Resolves complex repeats, high accuracy with HiFi	Structural variation, effector gene discovery	Wenger *et al.,* 2019
Oxford Nanopore (MinION, GridION, PromethION)	Ultra-long reads (10 kb–2 Mb)	Portable, real-time sequencing	Rapid pathogen identification, field surveillance	Jain *et al.,* 2016
BGI/MGISEQ	Short-read NGS	High throughput sequencing	Whole-genome resequencing	Huang *et al.,* 2012
Sanger Sequencing	Chain termination method	Gold standard accuracy	Barcode sequencing, confirmation of PCR amplicons	Sanger, 1977

Table 8: Bioinformatics Tools and Pipelines Supporting Plant Genomic Analyses

Bioinformatics Tool / Platform	Function	Use in Plant Disease & Genomics	References
FastQC	Checks sequence quality	Raw read quality assessment	Andrews, 2010
Trimmomatic	Trims low-quality reads & adapters	Preprocessing of NGS reads	Bolger *et al.,* 2014
SPAdes / Velvet	De novo genome assembly	Assembly of pathogen genomes	Bankevich *et al.,* 2012

Bowtie2 / HISAT2	Read alignment	RNA-seq alignment for gene expression	Langmead & Salzberg, 2012
SAMtools / BCFtools	Variant calling & processing	SNP/INDEL detection in host/pathogen	Li *et al.,* 2009
Geneious / CLC Workbench	Integrated GUI platform	Multi-step genomic workflows	Kearse *et al.,* 2012
BLAST / InterProScan	Gene annotation	Pathogen effector and R-gene prediction	Altschul *et al.,* 1990
KEGG & GO Analysis Tools	Pathway & functional annotation	Understanding host immune pathways	Kanehisa *et al.,* 2000
MEGA / IQ-TREE / RAxML	Phylogenetic analysis	Tracing pathogen evolution	Kumar *et al.,* 2018

Conclusion:

Advances in genomic technologies have fundamentally reshaped the landscape of plant disease research, enabling deeper, faster, and more accurate insights than ever before. From diagnosing pathogens at the molecular level to deciphering complex host–pathogen interactions and guiding resistant crop development, genomics now serves as the cornerstone of modern plant health management. Traditional approaches though historically valuable are increasingly supplemented and strengthened by high-resolution genomic tools that detect pathogens early, track their evolution, and provide actionable information for effective disease control.

The integration of sequencing technologies, PCR-based diagnostics, transcriptomics, and bioinformatics has greatly enhanced our understanding of pathogen diversity, virulence mechanisms, and population dynamics across diverse agroecosystems. Such insights make it possible to establish robust surveillance networks, identify emerging strains before they become widespread, and predict disease risks with higher confidence. At the same time, comprehensive genomic knowledge of host defense pathways and resistance genes empowers breeders to design resilient crop varieties through marker-assisted selection, genomic selection, and precise genome editing.

As agriculture moves toward sustainability and precision-based decision-making, genomics offers tools that are not only powerful but also adaptable to field conditions, including portable sequencing devices, real-time data analytics, and RNA-based biocontrol strategies.

The coupling of these innovations with integrated disease management practices ensures more resilient farming systems capable of withstanding future disease pressures driven by climate change, globalization, and evolving pathogen populations.

In essence, genomic approaches are no longer specialized tools confined to research laboratories they have become essential components of plant disease diagnosis, surveillance, and management frameworks globally. Continued investment in sequencing infrastructure, high-quality reference genomes, bioinformatics capacity, and translational breeding programs will be critical for harnessing the full potential of these technologies. By bridging scientific innovation with practical disease management, genomics paves the way for sustainable crop production and long-term agricultural security in an increasingly complex and dynamic plant health landscape.

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PROTEIN MISFOLDING, ER STRESS, AND MITOCHONDRIAL DYSFUNCTION IN ARSENIC-LINKED CELLULAR INJURY

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Abstract:

Arsenic is a globally pervasive environmental toxicant, with chronic exposure linked to multiorgan pathologies including hepatic, neurological, renal, cardiovascular, metabolic, and carcinogenic outcomes. Among the diverse molecular lesions induced by arsenic, disruption of proteostasis—encompassing protein misfolding, aggregation, endoplasmic reticulum (ER) stress, and mitochondrial dysfunction—represents a central axis of toxicity across organ systems. Arsenic's strong thiophilic reactivity leads to covalent modification of protein cysteine residues, distortion of zinc-finger motifs, and disruption of metalloprotein active sites. These modifications trigger widespread misfolding, destabilization of protein tertiary structures, and overload of chaperone systems. Misfolded proteins accumulate in the ER lumen, activating the unfolded protein response (UPR) through the PERK, IRE1α, and ATF6 signaling pathways. Prolonged UPR signaling leads to inhibition of global translation, increased CHOP-mediated apoptosis, altered ER-associated degradation (ERAD), and dysregulated autophagy. Concurrently, arsenic impairs mitochondrial bioenergetics by inhibiting pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and electron transport chain complexes, producing excessive reactive oxygen species (ROS) and disrupting mitochondrial dynamics and membrane potential ($\Delta\Psi m$). ER stress and mitochondrial dysfunction are mechanistically intertwined via mitochondria-associated ER membranes (MAMs), where Ca^{2+} flux and redox cycling generate feed-forward amplification loops that exacerbate proteotoxicity and oxidative injury. These molecular cascades converge to produce cytotoxicity, inflammation, fibrosis, and carcinogenesis. This chapter synthesizes high-resolution mechanistic insights into protein misfolding, ER stress, and mitochondrial dysfunction in arsenic toxicity, highlighting implications for biomarker development, therapeutic targeting, and public health—particularly in high-exposure regions such as Bihar, India.

1. Introduction:

Arsenic contamination poses a substantial global health burden, affecting an estimated 200 million people, especially in South Asia, including Bangladesh, Nepal, and India (World Health Organization [WHO], 2022). In India, the state of Bihar is among the worst affected; groundwater surveys reveal arsenic concentrations exceeding 50–200 μg/L—far above the WHO limit of 10 μg/L, exposing millions to long-term toxicity (Kumar *et al.,* 2021; Central Ground Water Board [CGWB], 2024). Chronic arsenic exposure has been linked to numerous diseases, including skin carcinomas, hepatotoxicity, cardiovascular disorders, diabetes, neurodegeneration, and immune dysregulation (Naujokas *et al.,* 2013; NIEHS, n.d.).

Historically, arsenic toxicity was attributed primarily to oxidative stress and DNA damage. However, recent research reveals a more complex landscape: arsenic exerts profound effects on protein structure, protein quality control pathways, and mitochondrial function (Shen *et al.,* 2013; Ganie *et al.,* 2023). These effects compromise the fundamental proteostasis network that maintains cellular homeostasis.

Proteostasis collapse is now recognized as a molecular hallmark of arsenic toxicity, with three intimately linked processes:

1. Protein misfolding and aggregation
2. ER stress and maladaptive activation of the UPR
3. Mitochondrial bioenergetic failure and ROS-driven apoptosis

Together, these disruptions form a toxic triad that drives tissue injury and disease progression.

This chapter provides a comprehensive, mechanistic examination of these pathways and their contribution to arsenic-induced pathology.

2. Arsenic Chemistry and Reactivity

Major Environmental and Toxicological Forms of Arsenic

Arsenic exists in several oxidation states, with inorganic arsenite (As^{3+}) and arsenate (As^{5+}) being the most relevant to human toxicity.

- **Arsenite (As^{3+})**: Highly reactive, binds to protein thiols, and disrupts protein folding and enzyme activity (Shen *et al.,* 2013).
- **Arsenate (As^{5+})**: Mimics phosphate and interferes with ATP-producing reactions, but is less potent than As^{3+}.

After ingestion, inorganic arsenic undergoes hepatic biomethylation via AS3MT, producing monomethylated (MMA) and dimethylated (DMA) metabolites (Douillet & Thomas, 2023).

Importantly, the trivalent methylated intermediates (MMA^{3+}, DMA^{3+}) are more toxic than inorganic As^{5+}.

Thiol Affinity and Covalent Modification of Proteins

The cornerstone of arsenic's molecular toxicity is its high affinity for sulfhydryl (–SH) groups of cysteine. This enables As^{3+} to:

- Form **dithioarsenite complexes** with vicinal cysteines
- Lock proteins in misfolded conformations
- Inactivate thiol-dependent enzymes (e.g., PDH, AKGDH)
- Disrupt structural disulfide bonds

Arsenic binds strongly to reduced thiols in:

- **Lipoamide-containing complexes** (e.g., PDH, α-KGDH)
- **Transcriptional zinc-finger proteins**, replacing Zn^{2+}
- **Antioxidant molecules** (glutathione, thioredoxin)

The formation of arsenic–thiol adducts is often irreversible under physiological conditions.

Oxidative Stress and Protein Oxidation

Arsenic-induced ROS originate from:

Mitochondrial ETC leakage, NADPH oxidase activation, Impaired antioxidant defenses

ROS cause: Protein carbonylation, Methionine sulfoxidation, Incorrect disulfide bond formation

These modifications destabilize protein folding landscapes and increase aggregation propensity.

Disruption of Protein Folding Thermodynamics

Arsenic lowers the free energy difference between native and unfolded states by:

- Reducing intramolecular hydrogen bonding
- Disrupting hydrophobic core packing
- Perturbing disulfide bond formation in the ER

These effects shift proteins toward molten globule intermediates, which are prone to aggregation.

Implications for Proteostasis

Arsenic perturbs proteostasis by:

- Increasing the burden of misfolded proteins
- Reducing ER chaperone availability
- Impairing ER redox balance
- Inhibiting proteasomal degradation

This creates a cellular environment primed for ER overload, UPR activation, and mitochondrial destabilization.

3. Protein Misfolding Mechanisms

Direct Thiol Binding and Conformational Distortion

Arsenite (As^{3+}) forms strong coordinate covalent bonds with cysteine thiolates, particularly when cysteines occur as vicinal pairs (adjacent residues). This interaction induces:

- Conformational rigidity due to crosslinking
- Disruption of intramolecular disulfide bonds, essential in ER protein folding
- Steric hindrance preventing proper tertiary structure formation
- Loss of catalytic activity in enzymes with thiol-dependent active sites

Examples of highly susceptible proteins include:

- **Lipoamide-containing enzymes:**
  - Pyruvate dehydrogenase (PDH)
  - α-ketoglutarate dehydrogenase (α-KGDH)

These complexes rely on reduced lipoamide cofactors, which are prime targets for As^{3+} (Park *et al.,* 2010).

- **Zinc-finger proteins:** Arsenic can displace Zn^{2+}, destabilizing DNA-binding domains (Chen & Sule, 2015).
- **Thioredoxin and glutaredoxin:** Their oxidation impairs cellular redox buffering capacity.

The binding of As^{3+} to reduced thiols produces inorganic and methylated thioarsenite complexes, which trap proteins in misfolded intermediate states.

Oxidative Protein Damage and Aggregation

Arsenic-induced reactive oxygen species (ROS) cause:

- **Protein carbonylation** (irreversible damage)
- **S-sulfonation and S-nitrosylation** of cysteines
- **Tyrosine nitration**
- **Methionine sulfoxide formation**

These posttranslational modifications (PTMs) lead to:

- **Disruption of secondary structures**
- **Increased β-sheet content**, promoting protein aggregation — similar to amyloidogenic pathways
- **Reduced chaperone affinity**, limiting refolding attempts

Proteomic studies show arsenic increases aggregation-prone proteins such as:

- α-synuclein
- Tau
- SOD1
- Heat shock proteins (due to overload)

Such aggregates overload the ER and the cytosolic proteasome system, accelerating cell death.

Failure of Molecular Chaperone Systems

Arsenic exposure reduces or impairs major chaperones, including:

- **BiP/GRP78** (ER chaperone)
- **Hsp70/Hsp40** (cytosolic folding machinery)
- **PDI family (PDI, ERp57, ERp72)** required for disulfide bond isomerization

Mechanisms of chaperone failure:

Chaperone proteins themselves become oxidatively modified and lose affinity for unfolded substrates. Overwhelming influx of misfolded proteins sequesters chaperones into irreversible complexes. Arsenic disrupts ATP-dependent folding cycles, as mitochondrial inhibition reduces ATP supply.

This chaperone exhaustion is a critical trigger for the ER stress response.

Disruption of Disulfide Bond Formation in the ER

The ER lumen provides an oxidizing environment enabling correct disulfide bond formation, facilitated by:

- **Protein disulfide isomerase (PDI)**
- **Ero1α/β**
- **GSH/GSSG cycling**

Arsenic disrupts this system by:

- Inhibiting PDI activity via thiol binding
- Increasing ER ROS, leading to non-native disulfide pairing
- Altering the GSH/GSSG ratio

The result is a buildup of misfolded, disulfide-rich proteins unable to exit the ER.

4. ER Stress and UPR

The PERK Pathway: Translational Repression and ATF4 Activation: Activation Mechanism - Under normal conditions, PERK is bound by BiP/GRP78. Misfolded proteins recruit BiP away, allowing PERK to homodimerize and autophosphorylate.

PERK Signalling Cascade

Activated PERK phosphorylates:

- eIF2α (Ser51) → inhibits global translation
- Reduces ER protein load
- Selectively increases translation of ATF4 mRNA

ATF4 Target Genes

ATF4 upregulates:

- **CHOP (GADD153)** — pro-apoptotic transcription factor
- **GADD34** — feedback dephosphorylates eIF2α
- **ASNS, xCT** — amino acid metabolism
- **HO-1, NQO1** — antioxidant defense

Arsenic and CHOP-Mediated Apoptosis

Arsenic strongly induces CHOP expression (Liu *et al.,* 2022), leading to:

- Downregulation of anti-apoptotic Bcl-2
- Upregulation of pro-apoptotic BIM
- Increased oxidative stress
- Sensitization of mitochondria to cytochrome c release

CHOP is a major determinant of arsenic-induced cell death.

The IRE1α Pathway: Stress Signaling and XBP1 Splicing

IRE1α is the most evolutionarily conserved UPR sensor.

Dual Function Kinase–Endoribonuclease

Upon activation:

- IRE1α oligomerizes and autophosphorylates
- Activates its RNase domain
- Cleaves XBP1u mRNA → producing XBP1s, a potent transcription factor

Functions of XBP1s

XBP1s enhances:

- ERAD components
- Lipid synthesis
- ER biogenesis
- Chaperone expression

Pro-apoptotic Outputs

Under prolonged arsenic exposure:

- IRE1α recruits **TRAF2**
- Activates **ASK1 → JNK**
- Promotes apoptosis and inflammation

IRE1α becomes a pro-death signal under chronic arsenic stress.

ATF6 Pathway: ER Expansion and Chaperone Upregulation

ATF6 is transported to the Golgi under ER stress, where S1P and S2P proteases release the transcriptionally active ATF6(N).

ATF6(N) upregulates:

- BiP/GRP78
- Calreticulin
- ERAD genes
- XBP1

Under arsenic stress, ATF6 supports early adaptation but later contributes to apoptosis when ER stress becomes unresolvable.

Conclusion:

Arsenic induces a coordinated collapse of proteostasis and mitochondrial integrity. Understanding ER–mitochondrial coupling and redox biology enables biomarker development and therapeutic interventions, especially vital for high-risk regions such as Bihar, India.

Arsenic-induced cellular injury is a complex, multifaceted process characterized by the convergence of protein misfolding, ER stress, and mitochondrial dysfunction. These interconnected mechanisms drive toxic outcomes in multiple organs, particularly the liver, kidney, skin, and nervous system. Arsenic's thiophilic reactivity destabilizes proteins directly, while its oxidative and metabolic effects propagate misfolding and aggregation. ER stress responses—mediated by PERK, IRE1α, and ATF6—initially attempt to restore proteostasis but ultimately promote apoptosis through CHOP and JNK pathways when stress becomes chronic. Mitochondrial dysfunction, characterized by ETC inhibition, ROS amplification, $\Delta\Psi m$ collapse, and apoptotic signalling, exacerbates ER stress through reciprocal ROS and Ca^{2+} crosstalk at MAM interfaces.

Understanding these molecular events provides a mechanistic framework for identifying biomarkers, developing therapies, and implementing public health measures—particularly in arsenic-endemic regions such as Bihar, India. Future research should integrate **multi-**omics profiling, high-resolution proteomics, and systems biology modeling to map the full landscape of arsenic-induced proteostasis collapse. Ultimately, targeting ER–mitochondria communication, enhancing chaperone function, restoring redox balance, and improving mitochondrial resilience represent promising strategies for mitigating the burden of arsenic toxicity.

Figures:

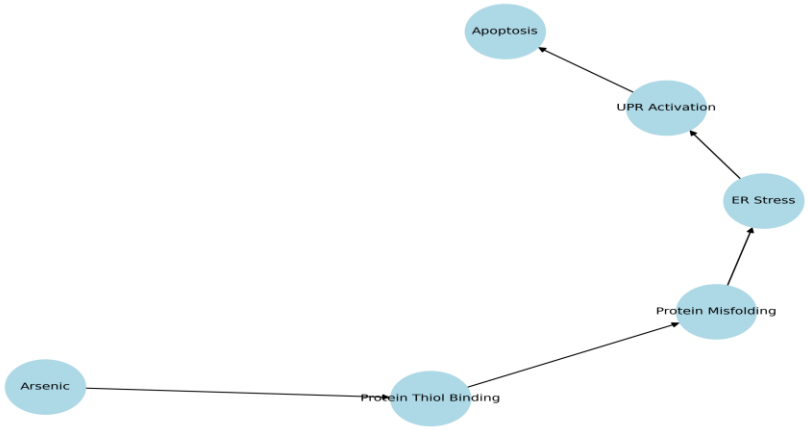

Figure 1: Arsenic-induced protein misfolding and ER stress.

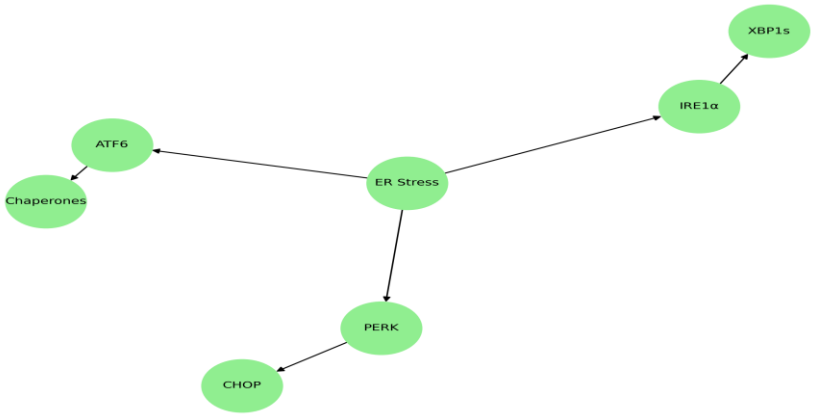

Figure 2: UPR activation pathways.

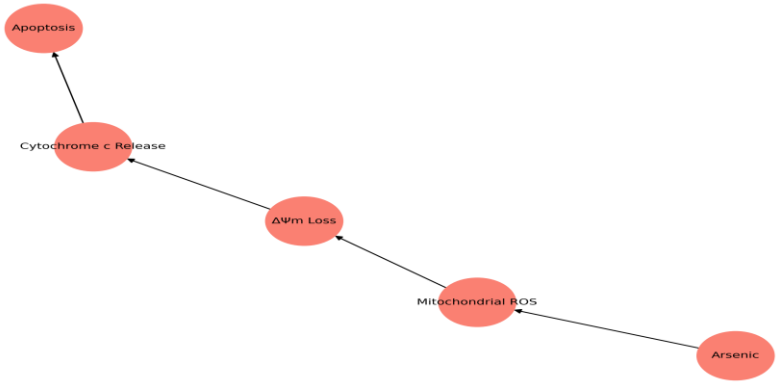

Figure 3. Mitochondrial dysfunction and apoptotic activation

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FERMENTED FISH PRODUCTS OF NORTHEAST INDIA: A TASTE OF TRADITION

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Abstract:

The north-east India is a territory of high ethnic diversity and traditional food culture, where a great number of different fermented fish products are presented. Foods like Shidol, Ngari, Hentak and Napham, were deeply entrenched in indigenous knowledge as they were the best examples of natural methods of fermentation to preserve fish without the aid of modern refrigeration. Many tribal communities rely heavily on these products as a part of local diet. These fermented delicacies are not only considered as a source of food but also, they represent the culture of this region. The natural microflora and fermentation process commonly done by sun drying or pit storing techniques, contribute towards complexity of flavour, shelf life and nutrition profile of the fish. Despite their strong smell and characteristically fishy flavor, these fermented fish products have a huge nutritional and functional significance. They are a good source of proteins, vital fatty acids, vitamins, and probiotics, which make them relevant to gut health and food security. Ecological intelligence is involved in the production of these fermented products: not much waste, local materials and labour are involved, which makes these millenary practices highly topical in the modern context of building sustainable and resilient food systems. As the world has started showing interest in fermented and probiotic rich foods, bioactive components and microbial interactions of fish fermentations of Northeast India are becoming a subject of scientific research and commercial exploitation.

1. Introduction:

A land of remarkable ecological diversity, indigenous knowledge and ethnic richness, northeast India, which is termed as the cultural mosaic of the country by most, is one great region. It is located in the north east of India. With more than hundreds of indigenous communities, Northeast India is notable with its integrated traditions, oral stories and

sustainable lifestyles that are deeply associated with nature. Food traditions are not just about survival, the tradition is associated with other aspects of being, such as identity, memory of the past, change of seasons, and customs. Fermentation is one of the most important arts of culinary in many regions of the Northeast, and that is what encompasses this strong bond. Fermentation is practised in many food varieties which include bamboo shoots, soybeans, vegetables, and meats amongst others. Of these, some foods that stand out the most are fermented fish, because of the availability of freshwater resources and the presence of fish in the culture and day to day foods consumed by people particularly when camped along rivers, lakes and wetlands. Production of fermented fish products such as the Ngari and Hentak in Manipur, Shidol in Tripura and Assam, Tungtap in Meghalaya, and Napham in Assam depend on time-tested methodologies which involves utilization of natural microflora on sun-dried fish along with ancient method of preservation. These products normally are kept in containers made of bamboo or earthen pots or even buried in earthen pits depending on the ethnic group and the surrounding climate. Many of the skills are based on oral traditions of preparation which have been passed on through several generations and they do also indicate not only food preparations of the time but also the knowledge of the microbial ecology well before the science today could define them.

Fermentation is latin in origin and it comes out of a latin verb although it is used to mean seethe or boil. One of the oldest ways of preservation of food is fermentation; it is one of the most ancient and very old methods of preserving a food. Fermentation is usually characterized by disintegration of complicated protein molecules in fish through the action of organic catalyst and enzymes into simple molecules. It is a cost-effective procedure since it is not expensive. The cost of production has very few expenses since the producers can ferment fish using locally available materials, unlike other preservation methods that require elaborate machines like canning or freezing among others. Less energy is used in comparison to refrigeration processes and this is sustainable to the environment, and the entire fish is used enhancing sustainable fisheries. Most importantly, the fermented fish products, on most occasions sustain flavour, aroma, texture and quality to name but a few attributes, and retains its nutritive values at ambient temperature, which greatly retards against spoilage. In fermentation, sugars become organic acids as they are broken down to organic acids by microorganisms, including lactic acid bacteria. This conversion brings down the ph level of the fish into an acidic environment which is unfavorable to the growth

of a lot of bacteria which causes spoilages. Fish products that have undergone the fermentation process therefore possess longer shelf life that may take weeks, months or even years without requiring refrigeration.

The fermented food also has different health benefits due to its boosted nutritional characteristics and probiotic features. Bioactive peptides; This type of organic substance becomes physiologically active when passing through an enzymatic or chemical reaction and that makes it block and cause an immune system response to lower the inflammation; they are produced as a result of the fermentation process. Some bioactive peptides possess angiotensin - converting enzyme inhibitor effect that is useful in reducing blood pressure in the same way that antihypertensive drug can achieve. A variety of probiotic microbes is lactic acid bacteria that aid in the maintenance of a normal microbiome in the gut by preventing the proliferation of pathogens. Probiotics also aids in digestion, performance of the immune system and offer advantages in a number of intestinal ailments like constipation and necessary to treat IBS (irritable bowel syndrome).

Northeast of India is popularly associated with a big producer of fermented fish products in India. Considering the diversity of fermented fish products in various states and communities, it is necessary to examine such as objects of scientific andinguistic interest as well as ethnic delicacies. Full of local ecology, tradition, each product is a unique story of innovation, adaptation and resilience of culture. The present paper is an exploration of the regional diversity of fermented fish preparations across Northeast India, their preparation methods, microbiological profile, nutritional value and issues on the conditions of hygiene and quality levels in the traditional contexts. With this discovery, we shall note that it is very important to conserve these ancient practices alongside promoting sustainable innovation and documentation to the next generation.

I. Ethnic Fermented Fish products: A Regional Perspective

The ethnic communities in Northeast, India are very diverse as exemplified by the fermented fish productions in the region. Individual states have evolved different traditional ways of fish fermenting that have been influenced by the area where they are located, availability of resources and the nature of people. Although the basic base is still the same ingredient- fish, the methods, duration of fermentation, additives and end flavours show much diversity. These foods are mostly part of the local diet and are used as a seasoning as well as a basic ingredient in every meal. This section will offer a

geographical description of the most well-known fermented fish products, and methods of preparation applied traditionally.

1.1 Shidol

One of the most liked delicacies consumed by the Assamese and Tripuris. Shidol or synonymously known as Shidal is a non-salted, sticky and a semi-fermented product. It is popularly drunk amongst several states in the northeast, and is frequently named differently, as per region. Shidol looks solid in the form of the fish yet it is soft and easy to mould almost like paste. Fermented shidol is not used directly; however, it usually gets prepared in its form in a chutney, or a sauce like preparation known as, shidol bhorta, usually eaten as a side dish with rice. Shidol is only made of Puntius sophore which is commonly called as stigma barb, spotfin swamp barb or the pool barb or even other kinds of fish- Gangetic hairfin anchovy (Setipinna phasa), Indian river shad (Gudusia chapra). Procedure of production of Shidol does not include activities such as cooking or pasteurisation to kill microorganisms. Shidol is stored at ambient temperature up to the time it is consumed, this and a salt free concentration create vey high microbiological risks.

Preparation technique

The raw material needed is fish, matka, edible oil, cover paste and clay seal.

a. Basic raw material (Fish); preferably dry, large size, Uniform sized and of no insect infestation. Containers used are mutka (earthen pot) narrow necked, round bottom pots. The pot enters in processing with the help of an edible oil, after the absorption, the inner side of the mutka is covered with the help of oil and dried in the sun. This activity is done until the pot is saturated with oil that has no space of air vents that remain open.
b. Oil; The best oil would be fish oil and this is extracted out of the fish entrails. The first one is to avoid air permeability due to the small holes, to minimize desiccation that arises as a result of evaporation and seepage.
c. Cover paste: this is done by pounding of dried fish preferably broken or small parts of sundried puntius spp. Cover leaf: plant leaves with wide surface areas like banana leaves or bottle gourd leaves are placed on top of the cover pot to temporarily keep insects and flies at bay. The newspaper and polythene bag has been commercialised in the mass production.

d. Clay seal; mixture of fine soil with water then thickened to form a clay like compound applied on the mouth region of the mutka as the closing seal. This will prevent the infestation by insect and the condition to be anaerobic within the pot.

1.2. The villagers of the state still used a conventional technique that requires extraction of fish oil out of the fish entrails. Preparation of shidols in a more cost effective and less harmful process is observed in numerous states composing Northeast, India. It is done by; descaling, de gutting of raw fresh fish (Puntius spp.), washing followed by semi dried by the sun at least 50 percent moisture is retained in fish, then these semi dried fishes are stuffed in oil processed mutkas. The belly piece of the earthen pots are kept buried into the ground up to the rim of the mutka to hold it upright and so as to be packed with minimum packing pressures, at this time a cover leaf is put on inverted position over cover paste. The mutka is brought out of the ground after a week and cover leaf is removed and mutka is finally covered with a thick paste of clay on top of the cover paste. The mutka used is stored in a dry and shady place, clay seal is likely to crack hence, by chance the suspected second coating of thick clay is practiced until it is crack free. The mutka is stored un-touched during 3-4 months. The clay seal and the covering paste that has festered is removed carefully on the day of the harvest and the final product pulled out layer wise.

1.3. Ngari

The fact is; Ngari is a fermented fish product native to the Manipurian people. Fermented fish processing is a manmade activity that has been inherited generation to generation. Ngari is made out of spotfin swamp barb (Puntius sophore), This spiced up and highly proteinous delicacy- prepared out of freshwater fish, left to ferment in the sun over weeks is not simply a food but a symbol of culture. Respected due to its strong odor and savory level of umami, Ngari adds a specific taste to Manipuri food that is reminiscent of ancient techniques, local wisdom and intimate relationship with the land and the rivers of the Northeast of India. Despite being mixed in chutneys or stewed in the spicy stews, Ngari remains central to the constitution of Manipur cuisine. It is majorly prepared on a household basis, by traditional means. It is anaerobically fermented and no starter has been used. Fermentation is a natural phenomenon whose duration may vary to many months and depending on the ambient conditions and microbial activity, the product is very fragrant and flavorful. Ngari is easily located in the local market and continues to be found in the set up of most Manipuri homes due to its flavor and also because of its nutrition and cultural values attached to it.

1.4 Preparation technique

Fishes (the sun-dried ones), prior to fermentation are cleaned in water with the aid of porous bamboo baskets and left to drain and dry (24-48 hrs.). Washed drained fishes are pressed the following day which implies that pressure is exerted on them by stepping on them. Head and bones provided in the fishes that are likely to be broken down are also done alongside covering gunny sessions to ensure that the extra water is taken out. The fermentation is considered to be triggered by the oil leakage of head happening under pressure. The process makes use of earthen pots. The older the pot the better the productThe inner surface of the pot is covered with oil but preferably mustard oil which is soaked in the pot and then dried once more another layer of oil is again rubbed and then dried. The coating of oil is necessary so as to trap fermentation in and out of the pot which means that the pot, saturated with Air vents, is assumed to make the anaerobic ambiance inside the pot during the fermentation process. The oil also keeps the fish free of clinging to the inside surface of the pot that facilitates the easy removal of the product. Dried fish, which is pressed, is packed inside the pot with the aid of force and pressure. Pots are also sealed after they are packed and air is clamped using dough like paste prepared using trash and fish/fish powder. It is then wrapped with polythene or cover leaf. A final seal is given by plastering a thick coating of clay on the earthen pot as the final seal till maturation. Fermentation is done in the seal pot at ambient temperature between 4 to 12 months.

1.5 Hentak

Hentak is one of a kind fermented fish product that is endemic to the culinary and cultural history of Manipur, a state located in the northeastern region of India. Hentak is unlike other types of fermented fish in that it is specially prepared by mixing sun-dried fish not only those belonging to the family of Puntius, but also using a local plant ingredient such as petioles of Alocasia macrorrhizos (Giant Taro). The mixture is pressed into little cakes, and fermented under natural conditions, generally at the house-hold level. Hentak is well known with its pungent aroma and rich taste that is mostly used as a side dish or flavoring ingredient in native dishes. It is not sold at commercial markets that is why its replacement was on its role as a home based heritage food and a means to supplement the dietary protein intake. It is one of the very popular ethnic, fermented fish products in Manipur. Hentak is a fermented fish paste with a pungent smell that is made into a paste in Meitei families and extremely smelly (with a wonderful umami taste). Small quantities of onions are sometimes employed during the process of production. It can be properly used as a side

dish or condiment and is mostly accompanied with rice and vegetable preparations made simple. Preparation technique

Crushed fishes. The fishes are prepared sun dried and pounded into powder. Alocassia macrorrhizos petioles are sliced into pieces and rinsed with water and placed in the sun an hour. The pieces, which are then sliced and fish in the ratio of weight get pulverized to form a paste. The past is shaped into small balls and set to ferment in muddy pots; fermentation takes roughly 15 to 20 days. The balls are then Harden after a few months of time in storage and added to morsels of water and propounded to paste that is put into balls form and stored into reserved food. Onion may be substituted with *A. macrorrhizos* at some preparation of hentak and even though of lower quality and shorter shelf life. Apart from boosting shelf life of the product, the fermentation process actually gives the product its distinctive pungent smell and its hot flavor that makes it a treasured side dish in the traditional Meitei cuisine. Clay pots are also important in creating the optimal balance of moisture and temperature within them such that indigenous microorganisms can grow and propel the natural fermentation. When the paste is stored, it slowly changes biochemically after which it develops the flavour complex compounds and also attains increased digestibility. The consumption of hentak is normally small as people find it with strong taste, therefore, it is usually cooked with steamed rice or vegetables. Although it does not have a commercial production, its contribution to households food system is intense at least in lean periods or when fresh fish is out of sight. Its manufacturing is based on the strong insight into the local supply of ingredients, seasons, and sustainability of food storage. The introduction of such plant substances as Alocasia macrorrhizos help to thicken and add a variety to the taste but also helps demonstrate the historical tradition of working with the flora that is available at the area. Its pungent smell cannot be appreciated by foreign taste buds, but to the Manipuris, Hentak has been a beloved condiment since time immemorial, which is able to delight kitchen suppers and ensure food security even in places where refrigeration and other advanced techniques of preservation are not available.

Conclusion:

The fermented fish products of Northeast India serve much more than mere preserved foods, they are active accompaniments of cultural identity and traditional knowledge and sustainable food practices. Whether it is the bamboo laden kitchens of Arunachal Pradesh or the earthen pots of Manipur or the sunlit courtyards of Tripura all the fermented delicacies have the history of generation of people who trusted the natural way of attaining

the food security, benefiting the flavor of the food and preserving the health of the community in whole. Although they provide valuable amounts of protein, probiotics, and other valuable micronutrients, their lack of standardization in hygiene raises a barrier towards their increased uptake and commercialization. Finding a balance between cultural authenticity and contemporary food safety will be key towards maintaining these practices within a dynamic food system. As the world grows to view ancestral food systems as new sources of knowledge pertaining to food health, sustainability, and resiliency, the fermented fish nomenclature in Northeast India will be one of the valuable cases. It is not only an academic duty to document, safeguard, and market these foods without dulling their cultural flavors, but it is also in honor of the associating communities that have carried them on throughout the years. The present input does not only help in recording an important segment of the food culture of Northeast India; it also aims at indicating the significance of such knowledge preservation at a time of globalization and cultural homogenisation.

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NANOPARTICLES AND THEIR ANTIVIRAL MECHANISMS

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Abstract:

Viral infections have been reported to cause serious outbreaks worldwide according to recent studies.Nanotechnology can play a vital role in combating these viral infections through diagnosis,treatment,targeted medication delivery,biosensing for the benefit of mankind.Nanoparticles have been recently reported to be active against various viruses like Human Immunodeficiency Virus(HIV), Human Norovirus(HNV), severe acute respiratory syndrome coronavirus(SARS-CoV-2), Hepatitis B virus(HBV), Hepatitis C virus(HCV), Respiratory Syncytial Virus (RSV) and others.In this chapter the different types of nanoparticles are discussed.Further, the application of various nanoparticles in antiviral activities and the different mechanisms associated are discussed.The merits and demerits associated with the nanoparticles are also highlighted along with their scope and future perspectives.

Keywords: Nanoparticles, Antiviral Mechanisms

Introduction:

According to a recent study conducted on worldwide events of infectious disease outbreaks occurring between 1996-2023, out of the 30 most priority pathogens identified, the most frequently reported outbreaks were from the influenza virus, Ebola virus, and Middle East respiratory syndrome-related coronavirus (MERS-CoV). Among the above, high case fatality rates were highest for viruses like Marburg and Ebola (Liu *et al.,* 2025). One of the main causes of death in the world is viral infections. The risk of virus transmission is greatly increased by the expansion of globalization, making it a worldwide hazard to public health in the future. Nanotechnology is a significant advancement with numerous uses in fields such as electronics, textiles, and—most importantly—medicine, including targeted medication delivery, diagnosis, treatment, and biosensing for the benefit of humankind. There is now more growing interest in nanoparticles as a possible new class of antiviral due to the SARS-CoV pandemic and humanity's susceptibility to new viruses and other infectious diseases.

An increasing amount of data in the literature indicates that nanoparticles may be active against various viruses, such as Human Immunodeficiency Virus (HIV), Human Norovirus (HNV), severe acute respiratory syndrome coronavirus (SARS-CoV-2), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Respiratory Syncytial Virus (RSV), and others. The most prevalent transmissible infectious diseases, which have caused significant epidemics and pandemics, are caused by viruses.

Antiviral drugs can be transported by nanoparticles, improving their stability, bioavailability, and targeted delivery to diseased cells or tissues (Kumaraswamy *et al.,* 2024). By delivering therapeutic payloads straight to viral reservoirs, functionalized nanoparticles can reduce viral replication and propagation by avoiding biological barriers like the mucosal epithelium or blood-brain barrier (Delshadi *et al.,* 2021).

Types of Nanoparticles

Nanoparticles are of the following types according to their size, shape, composition, and characteristics, which are as follows:

i. Metallic nanoparticles like silver, gold, iron, or platinum used in catalysis, sensing, imaging, and drug delivery applications (Sharma *et al.,* 2021).
ii. Polymeric nanoparticles are applied in drug delivery systems because of their adaptability, capacity to encapsulate a variety of medicinal compounds, and compatibility with biological systems (Pulingam *et al.,* 2022).
iii. Quantum dots, which are semiconductor nanoparticles used in light-emitting devices, photovoltaics, biosensing, and biological imaging (Sobhanan *et al.,* 2023).
iv. Lipid-based nanoparticles composed of liposomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), and lipid nanoparticles utilized for drug delivery, gene therapy, and vaccine delivery purposes (Sheoran *et al.,* 2022).
v. Carbon-based nanoparticles like graphene, carbon nanotubes (CNTs), and carbon dots applied in electronics, sensors, drug delivery, and biomedical imaging (Sharma *et al.,* 2020).
vi. Ceramic-based nanoparticles like alumina, silica, zirconia, titania useful in a variety of applications, such as drug administration, tissue engineering, biomedical imaging, and catalysis, due to their excellent mechanical strength, thermal stability, and chemical inertness (Treccani, 2023).

vii. Magnetic nanoparticles like iron oxide nanoparticles applied in biomedicine, including targeted drug delivery, hyperthermia therapy, and magnetic resonance imaging (MRI) (Montiel Schneider *et al.,* 2022).

viii. Composite nanoparticles like carbon-metal hybrids, lipid-polymer hybrids, or metal-polymer composites are used in medication administration, imaging, sensing, and catalysis since they integrate the characteristics of their component materials (Liu *et al.,* 2020).

Nanomaterials possess a range of fascinating properties, including shape, optimal size, superparamagnetism, tunable surface charge, luminescence, high surface plasmon resonance, photon upconversion, bioavailability, biocompatibility, immunocompatibility or tolerability, and biodegradability, making them a promising alternative approach (Gurunathan *et al.,* 2020). Additionally, nanoparticles' adaptability allows them to be readily decorated, attached, or conjugated with a variety of functional groups, linkers, and bioactive compounds. Additionally, some nanomaterials can be used for both diagnostic and therapeutic purposes at the same time (Singh *et al.,* 2017; Jiao *et al.,* 2018; Gurunathan *et al.,* 2018; Szunerits *et al.,* 2015).

Application of various nanoparticles in Antiviral activities

Copper, alloys, and oxides such as zinc oxide (ZnO), titanium oxide, iron oxide, and their composites, nitrides, and other ceramic nanoparticles, as well as gold and silver nanoparticles, are the most well-characterized antiviral nanoparticles (Ramrakhiani and Ghosh,2018; Rodrigues *et al.,* 2021; Lin *et al.,* 2021). Antiviral nanoparticles also include Galactan, cellulose, polyethylenimine, chitosan/chitin, and other sulfated polymers, as well as sulfated and nonsulfated polysaccharides.

Potential antiviral action was demonstrated by metal-grafted Graphene Oxide (GO) sheets ornamented with metals as Ag, Fe, Cu, Zn, TiO2, CdS, and MnS2 (Chen *et al.,* 2016; Hang *et al.,* 2015). For instance, GOs coated with copper and silver have the potential to act as antiviral agents against both enveloped, like feline coronavirus (FCoV), and non-enveloped viruses like infectious bursal disease virus (IBDV)(Chen *et al.,* 2016).

When the protective capsid of viruses is removed for replication, nanoparticles like CeO_2 (cerium oxide) bind to the nucleic materials of adeno-associated viruses, adenoviruses, human immunodeficiency viruses, and murine leukemia viruses inside the cell (Nefedova *et al.,* 2022). Besides, gold nanoparticles (AuNPs) have also been used in the recognition of

viruses due to their special photonic, electric, and catalytic qualities as well as the multivalent functionalization of specific biomolecules (Draz and Shafie, 2018).

Mechanisms of Antiviral Activity:

There are four main antiviral action mechanisms at the nanoscale: (1) Nanoparticles directly interact with the virus, as in virucidal; (2) nanoparticles interact with receptors, preventing the virus from entering cells; (3) nanoparticles enter cells and disrupt the assembly and processing of viruses; or (4) nanoparticles stop the virus from replicating.

Some extracellular prevention strategies for nanoscale interaction with viruses include (a) virucidal, in which nanoparticles interact with the viral surface protein (gp120) in both enveloped and unenveloped viruses to cleave inside the viruses, preventing fusion, entry, and infectivity, for example in HIV, silver nanopaticles (Ag Np) act as virucidal agents by preventing binding of virus to host cells though interaction with HIV gp 120 envelope protein (Jain *et al.,* 2021, Elechiguerra *et al.,* 2005).

Recent studies have also reported AgNPs targeting the gp120 protein and preventing binding to the host cell membrane, which is responsible for obstructing entry, fusion, and infectivity (Lara *et al.,* 2010). The nanoparticles have also been shown to possess antiviral effects against Dengue Virus (Sujitha *et al.,* 2015; Gaal *et al.,* 2017), Herpes simplex virus (Hu *et al.,* 2014), Chikungunya virus (Sharma *et al.,* 2019), human norovirus (Bekele *et al.,* 2016), bovine herpesvirus-1(El Mohammady *et al.,* 2018), herpes simplex virus, and human parainfluenza virus type-3 (Gaikwad *et al.,* 2013).

Additionally, polycations exhibit antiviral properties by causing damage to the lipidic envelope, releasing the RNA, and inactivating the influenza virus by adhering to its surface (Rodrigues *et al.,* 2021). In some viruses like murine norovirus (MNV), and feline calicivirus (FCV) (Park *et al.,* 2014), human influenza A (A/PR8/H1N1) (Nakano *et al.,* 2012), and herpes simplex virus 1(HSV-1) (Hajkova *et al.,* 2007), viral biomolecules are harmed by UV photocatalysis exposed through Titanium dioxide (TiO2) films which releases reactive oxygen species, or viral glycoproteins' disulfide bonds are broken, denaturing the virus(Lin *et al.,* 2021).

Copper oxide nanoparticles (CuO NPs) and other copper-based nanoparticles produce reactive oxygen species (ROS) (Murugesan *et al.,* 2024). The hemagglutinin and neuraminidase glycoproteins, which influenza utilizes to attach to its host cells, are broken down by these free radicals. The SARS-CoV-2 spike protein's S1-RBD is expected to directly

interact with iron oxides, causing irreversible conformational changes that would prevent the virus from attaching to host cells (Bhatti and Dang, 2023; Lin *et al.,* 2021).

Viral entry and subsequent infection can be avoided by competitively blocking viral binding to host cell receptors using multivalent nanoparticles coated with viral receptor ligands or glycan moieties (Sarkar *et al.,* 2022). The interactions between particular molecules on viruses and host cells determine whether the virus attaches itself to the cell. Viral infection can be reduced when antibodies bind to the virus in the extracellular space and stop it from attaching to the cell (Milovanovic *et al.,* 2017). According to a study on cadmium telluride (Cd)Te quantum dots, they can alter the structure of the viral surface proteins, preventing them from infecting the host cells. Additionally, virus numbers were reduced when CdTe QDs were bound to the cell membrane receptors (Gurunathan *et al.,* 2020).

A significant Ex vivo study demonstrating the antiviral activity of nanoparticles is the research carried out with EpiVaginal tissues composed of ectocervical epithelial cells isolated from humans that are cultured on a collagen-coated membrane to create a highly differentiated, multilayered tissue which mimicked the human vaginal mucosa (Cagno *et al.,* 2018). The epivaginal tissues were infected with HSV-1, and infectivity was observed. Since NPs coated with a 2:1 mixture of monomeric sulfonated ligand MUS (undecanesulfonic acid) and 1-octanethiol (OT) are the most biocompatible, soluble, and protein-resistant, they were chosen for the current in vivo investigation. The epivaginal tissues showed inhibition of infectivity when incubated with MUS: OT-NPs. This study also showed the inhibitory effect in Invivo models using BALB mice infected with RSV (Cagno *et al.,* 2018).

Merits and Demerits of Nanoparticles

It has occasionally been demonstrated that nanoparticles can interact with undesirable biological molecules, which can also trigger the inflammatory system, opsonize the molecules, cause macrophage absorption, and shorten their plasma half-life (Sanvicens and Marco, 2008).

Nanoparticles have also been reported earlier to have toxic effects. For instance, Titanium oxide (TiO2) Nanoparticles (NPs) have been reported to have their own greater cytotoxicity in comparison to their macro counterparts (Gamedze *et al.,* 2022). One method to capitalize on nanoparticles' antiviral properties and reduce these worries is to coat them onto high-exposure surfaces, like PPE. This method avoids administering the nanoparticles in vivo using a kill-on-contact strategy (Bhatti and Dang, 2023).

Conclusion:

This review highlights the applicability of nanoparticles in preventing viral infections, which have highly impacted global health. In the beginning, we gave an overview of the types of nanoparticles with their properties. We further reviewed the various applications of nanoparticles in antiviral activities. Further, we discussed the mechanisms of antiviral activities, analyzing in vitro and in vivo studies, elaborating significant kinds of nanomaterials that may be employed as antiviral drugs. We analyzed a few merits and demerits associated with nanoparticles. Lastly, we provide our perspective on the potential of nanoparticles in the battle against viral diseases in the future.

Scope and Future Perspectives:

Nanocarriers can be used with antibodies to achieve specific therapeutic effects. Nanoparticles can be administered through empty virus capsids to target particular parts of the human body. Nanoscale drug delivery devices have the potential to transform drug therapy strategies and aid in the development of treatments for viral illnesses.

Numerous nanoparticles, including metal nanoparticles (gold, iron, silicon, and silver), carbon-based nanomaterials (CNTs and graphene), and quantum dots (QDs), have been extensively synthesized and functionalized for a variety of biomedical applications, such as drug delivery, imaging, sensing, and target of interest detection.

The minuscule size of nanoparticles enables them to be used as drug delivery because of their small size; they might exhibit a surface area that is disproportionately enormous compared to their volume, providing numerous opportunities to interact with biological molecules and cellular structures (Manimaran *et al.,* 2022). A recent study supported the continued development of a dengue vaccine based on nanoparticles, which demonstrated the safety and potential effectiveness of PepGNP Dengue in activating virus-specific CD8+ T cells (Miauton *et al.,* 2024).

Nanotechnology is essential in the detection and treatment of viral diseases. Particularly in individuals who relapse after finishing traditional antiviral treatment, nanoparticles exhibit significant promise for medicinal uses. Due to their huge surface-to-volume ratio, surface charge, size, shape, and optical, electrical, biological, and functional features, nanoparticles may address antiviral resistance, a slowly becoming issue with current therapeutic techniques. Additionally, nano-based methods are practical, affordable, non-toxic, biocompatible, and a feasible way to treat several kinds of viral infections, like SARS-CoV-2/COVID-19.

To improve patient care and treatment results, researchers, clinicians, regulatory agencies, and industry partners must work together to translate nanoparticle research into clinical applications. To expand the potential paths of nanoparticle-based treatments, it is necessary to investigate new trends, cutting-edge technology, and possible obstacles.

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PRODUCTION OF VEGAN LEATHER USING KOMBUCHA DRINK

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Abstract:

Most leather produced across the globe is made from the skins of a variety of animals like cattle, sheep, tiger, goats, snakes, fish, leopard and many others. These animals are hunted and killed specifically for their skins. Another biomaterial manufactured without fleshing any animal is environmentally friendly and animal free leather, often known as "vegan leather" or "artificial leather" as an alternative to animal leather. One promising solution is vegan leather derived from bacterial cellulose produced during kombucha fermentation. Kombucha is a fermented tea drink that generates a symbiotic culture of bacteria and yeast (SCOBY), forming a thick cellulose mat on the surface. This bacterial cellulose, when harvested and processed, offers unique properties that make it a viable substitute for conventional leather. The study further highlights the material's potential applications in industries such as fashion, accessories, and upholstery, offering a sustainable and ethical alternative to conventional leather. While the kombucha-derived leather shows promise, challenges related to enhancing its water resistance, scalability, and long-term durability remain. Future research could focus on refining the production process and exploring commercial viability, paving the way for a more sustainable future in materials science.

Introduction:

Leather has been prized for centuries for its durability, flexibility, and aesthetic appeal, making it a preferred material for fashion, accessories, furniture, and automotive industries. However, the production of traditional animal leather poses significant ethical and environmental concerns. The leather industry is a major contributor to deforestation, water pollution, and greenhouse gas emissions, largely due to the raising of livestock and the chemical-intensive tanning process. Additionally, the slaughter of animals for leather production raises ethical questions that have fueled the search for cruelty-free alternatives. Bacterial cellulose (BC) is an environmentally benign natural polymer made from microbial organisms that has been hailed as a material of the future. Due to its distinct physicochemical and mechanical characteristics BC has been used in medicine for a long

time. Wound dressings that are antibacterial (Portela *et al.,* 2019). In the presence of possible nitrogen and carbon sources such as yeast extract, peptone, glycine, glucose, sucrose, mannitol, fructose, and other dietary derivatives, microorganisms metabolize, resulting in increased growth, development, and formation of gel-like cellulose membranes (Hussain *et al.,* 2019; Thorat and Dastager. 2018). The beverage is made by fermenting tea leaf infusions or decoctions with the help of a symbiotic association of bacteria and yeasts known as SCOBY (symbiotic association of bacteria and yeasts) (Chen *et al.,* 2000). Payne, 2016 stated that they had created a cellulose form of kombucha for use in the production of footwear. They used wax to improve the hydrophobic qualities of the created cellulosic fabric without altering its tensile properties or comfortability. They've created footwear employing bacterial cellulose as an alternative to leather as a result of their product. According to their findings, bacterial cellulose may be created in custom shapes and used as a zero-waste manufacturing material (Payne, 2016). Glucose is converted to cellulose through a series of intermediary molecules, including glucose-6-phosphate, glucose-lphosphate, and uridine-5-diphosphate glucose. (Rathinamoorthy and Kiruba 2020). Scientists are currently obtaining valuable products by combining natural/waste resources with better biological synthesis technologies in order to build a "zero waste" society andeconomy (Mateo and Maicas 2015)

Kombucha SCOBY leather is made from the cellulose layers produced by bacteria during the kombucha fermentation process. As the fermentation progresses, the bacteria secrete cellulose fibers that form a thick mat on the surface of the liquid. This mat, often referred to as the “SCOBY pellicle,” grows denser over time and can be harvested, dried, and treated to create a flexible, leather-like material.

The beauty of this process lies in its simplicity and sustainability. Unlike conventional leather, which involves raising livestock and using harsh chemicals for tanning, or synthetic leather, which relies on plastic-based materials, kombucha leather is entirely plant-based and biodegradable. The production process has a minimal carbon footprint and can even be done at home or on a small scale, making it accessible to DIY enthusiasts and eco-conscious designers

Production Process:

Step 1: Brewing Kombucha and Growing Bacterial Cellulose

1. Prepare sweet tea by dissolving sugar in hot water and steeping tea leaves.

Measurement:

Water - 1.5l

Sugar – 100g

Green tea bags - 4

2. Allow the tea to cool to room temperature and pour it into a clean glass jar.
3. Add the kombucha starter culture and cover the jar with a breathable cloth.
4. Ferment the mixture at room temperature for one month. During fermentation, a thick cellulose mat forms on the surface. (Prerak Gala and Bhavana Pandya,2,2022)

Step 2: Harvesting and Processing

1. Remove the cellulose mat (SCOBY) and rinse it thoroughly with water to remove excess acids.
2. Lay the cellulose flat on a drying surface and allow it to air-dry for several days. A dehydrator or oven set at low temperature can speed up the process.
3. To improve flexibility, soak the dried material in a glycerin solution, then airdry again.
4. Natural dyes can be applied at this stage for color customization (optional) (Prerak Gala and Bhavana Pandya,2,2022)

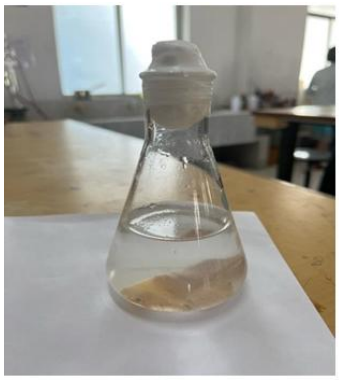
Step 3: Final Treatment

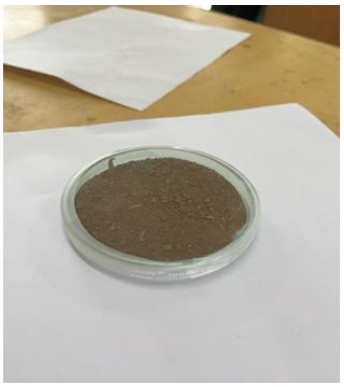
1. Wait until one of your kombucha biofilms becomes thick. The biofilm sheet will shrink significantly during the drying process. Thinner biofilms might not have a leather-like consistency, they may be more similar to tissue paper or printer paper.
2. Once one of your biofilms reaches a thickness, harvest biofilms. Remove the cloth from the container and with disinfect hands, carefully take the biofilm sheet from the liquid.
3. Rinse and wash the biofilm sheet with lukewarm water and a little bit of dish soap.
4. Place your biofilm sheet on a wooden board and measure the final thickness of the wet biofilm.
5. Let the biofilm on the wooden board dry. It might take several days to dry at room temperature.
6. The next step is to condition your kombucha leather. Apply coconut oil to both sides of your leather and it in with your hands. The oil prevents the biofilm from drying out and becoming brittle.

Result and Discussion:

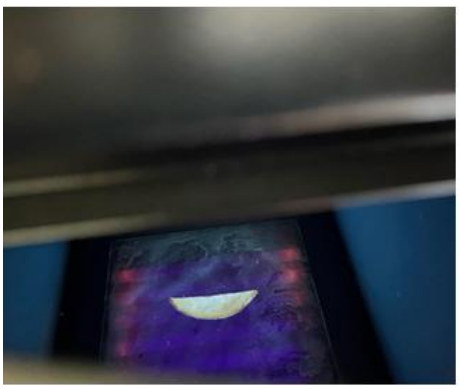
First day (Inoculation) After one month (Harvesting)

Qualitative Tests:





1.Water Solubility Test 2. Biodegradability (soil embedding test)



3. UV Exposure After UV Exposure

Conclusion:

As sustainability becomes a central focus in design and manufacturing, kombucha SCOBY leather holds exciting potential. Researchers are working to improve its durability, exploring treatments to make it more water-resistant and long-lasting. Additionally, advancements in biofabrication could pave the way for large-scale production, making kombucha leather a viable alternative to animal and synthetic leathers in mainstream markets.

In conclusion, kombucha SCOBY leather represents more than just an innovative material — it symbolizes a shift toward more conscious, sustainable practices in fashion and design. With continued research and development, this eco-friendly leather could play a significant role in reducing our reliance on traditional leather and synthetic materials, paving the way for a greener, more ethical future.

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ADVANCES IN PROTOPLAST ISOLATION AND REGENERATION TECHNIQUES IN SOYBEAN

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Abstract:

This chapter explains how protoplast technology can help to genetically improve soybean crops and make genetic research easier. Protoplasts are plant cells without cell wall, which makes them flexible and able to absorb DNA and other molecules more easily. The chapter describes simple, step-by-step methods for isolating healthy soybean protoplasts using enzymes, checking their viability, and growing them into small calli that can later form shoots. Although soybean is a difficult plant to regenerate, the optimized culture conditions used in this study supported cell division, microcallus formation, and the early stages of shoot development. protoplasts are useful for studying how plant cells respond to stress, testing gene functions quickly, and applying modern gene-editing tools like CRISPR. Looking ahead, future improvements in regeneration efficiency, high-throughput screening, and AI-based monitoring can make protoplast technology even more powerful for developing strong and high-yielding soybean varieties.

1. Introduction:

Soybean (*Glycine max*) is one of the world's most important crops, valued for its high protein, oil content, and wide use in food, feed, and industry (2011)**.** As the demand for soybean continues to grow, developing improved and stress-tolerant varieties has become essential Shanmugasundaram & Hymowitz (2000)[13]. Traditional breeding methods are useful, but they often take a long time and are limited by natural crossing barriers Bhatnagar & Basra (2003)[1]. Modern biotechnology offers new ways to improve soybean more efficiently. One of the promising approaches is protoplast technology, where plant cells are isolated without their cell walls Cocking (1960). Protoplasts can be enzymatically isolated from various tissues and have the unique ability to regenerate a new wall, divide, and ultimately develop into whole plants Burgess & Linstead, (1977)[3]; Fowke & Gamborg (1980). These protoplasts can take up DNA easily, divide, form callus tissue, and under the

right conditions, regenerate into whole plants, making them valuable for genetic transformation and genome editing Subburaj & Agapito-Tenfen, (2023)[14] as well as for studying how plant cells grow and respond to stress Davey *et al.,* 2005.

However, soybean is a challenging crop for tissue culture, and success often depends on choosing the right explant, enzyme mixture, culture medium, and growth environment Komatsuda & Ohyama, (1988)[10]. Establishing a reliable protocol for protoplast isolation and callus induction is therefore an important step toward improving soybean through biotechnology Kao & Michayluk, (1975)[9]. Protoplasts also provide an experimental system for a wide range of biochemical and molecular studies ranging from investigations into the growth properties of individual cells to membrane transport Gamborg *et al.,* (1968)[7]; Fowke & Gamborg, (1980)[6]. Protoplasts are highly suitable for transformation and genome editing, including the delivery of CRISPR/Cas9 ribonucleoproteins for DNA-free gene editing Subburaj & Agapito-Tenfen, (2023)[14]. In addition to their use in breeding, they provide a valuable system for studying various physiological and biochemical processes, such as membrane transport, ion uptake, cell wall biosynthesis, and virus–host interactions (Burgess & Linstead, (1977)[3]; Fowke & Gamborg, (1980)[6].

Despite these advantages, efficient and reproducible protocols for protoplast isolation and regeneration in soybean remain limited, particularly for Indian cultivars like MAUS 158. Therefore, developing reliable methods for protoplast isolation, microcallus induction, and callus regeneration is essential for advancing soybean biotechnology. Such protocols would not only accelerate functional genomics and transformation studies but also support the development of improved soybean varieties with higher yields and enhanced resistance to environmental stresses.

2. Historical Background of Protoplast Isolation Technology

The idea of studying protoplasts began many years ago, when early scientists examined plant cells under a microscope and recognized that the soft living material inside the cell was called “protoplasm.” At that time, it was very difficult to isolate a whole plant cell without its wall because removing the strong wall by cutting or crushing usually damaged the cell. A major turning point came in 1960, when E.C. Cocking discovered that plant cell walls could be removed gently using natural enzymes like cellulase and pectinase Cocking, (1960)[4]. This simple but powerful discovery allowed researchers to isolate healthy protoplasts without harming them and marked the true beginning of modern protoplast technology.

In the 1970s, scientists learned how to keep these fragile cells alive, help them rebuild their cell wall, and encourage them to divide. With better culture media, osmotic stabilizers such as mannitol, and improved laboratory conditions, isolated protoplasts were able to grow into tiny cell clusters called microcalli Burgess & Linstead, (1977)[3]. Around the same time, researchers developed protoplast fusion, using chemicals like PEG or electric pulses to join two protoplasts together, enabling hybrid formation between plants that could not cross naturally Power *et al.,* **(**1970)[12]. Many early successes in transferring traits such as disease resistance emerged from this method. By the 1980s and 1990s, scientists succeeded in regenerating whole plants from protoplast-derived callus in several species, demonstrating the totipotency of plant cells Fowke & Gamborg, (1980)[6]. During this period, protoplasts also became valuable tools for studying cell wall formation, nutrient uptake, hormone responses, and interactions with pathogens or environmental stress Davey *et al.* (2005)[5].

In recent years, with the rise of genetic engineering and CRISPR-based genome editing, protoplasts have become even more important. They allow rapid functional testing of genes and efficient delivery of DNA-free editing tools such as CRISPR-Cas9 ribonucleoproteins Subburaj & Agapito-Tenfen, (2023)[14]. Today, protoplast isolation and regeneration play a key role in developing improved, stress-tolerant, and high-yielding crops, including soybean.

3. Overview of Protoplast Isolation Technology and Its Significance in Agriculture

Protoplast isolation technology involves removing the plant cell wall using enzyme mixtures such as cellulase and pectinase to obtain a single, living plant cell called a protoplast. These isolated protoplasts are extremely valuable because, without a cell wall, they can easily take up DNA, RNA, proteins, and other molecules E.C. Cocking, (1960)[4]. Unlike animals, in plants the plasma membrane is bound by a rigid cellulosic wall, and the adjacent cells are cemented together by a pectin-rich matrix. It is mainly for this reason that somatic cell genetics is more advanced with animal than with plant systems Fowke & Gamborg, (1980)[6]. Once isolated, protoplasts can reform their cell wall, divide, form callus, and under suitable culture conditions, regenerate into whole plants Burgess & Linstead, (1977)[3].

This technology is important in agriculture for several reasons:

a. Genetic Transformation and Genome Editing

Protoplasts absorb foreign genetic material more readily than intact cells.

This makes them ideal for:

- CRISPR/Cas-mediated genome editing
- DNA-free genome editing using RNP complexes
- Introducing new genes for disease resistance, stress tolerance, and improved yield

b. Somatic Hybridization

Protoplasts from different species or genera can be fused to create **somatic hybrids**. This allows breeders to combine desirable traits even between plants that cannot be crossed sexually.

c. Selection of Stress-Tolerant Lines

Protoplasts can be exposed to salinity, drought, temperature, or toxic ions in culture to screen for tolerant cells. Selected cells can then be regenerated into improved plants.

d. Study of Plant Cell Biology

Because protoplasts are single, uniform cells, they are used to study:

- Cell wall synthesis
- Nutrient and ion transport
- Plant–pathogen interactions
- Hormone responses and metabolic pathways

e. Rapid Testing of Gene Function

Transient expression in protoplasts allows researchers to test gene activity, promoter function, or protein localization within hours, much faster than whole-plant studies Subburaj & Agapito-Tenfen, (2023)[14].

f. Foundation for Efficient Regeneration Systems

The ability of protoplasts to form callus and regenerate into plants supports large-scale crop improvement through biotechnology (Kao & Michayluk, (1975); Fowke & Gamborg, 1980)[6]. In summary, protoplast isolation technology is a powerful tool in modern agriculture. It enables precise genetic modification, helps overcome barriers in plant breeding, and supports the development of crops that can withstand environmental and biological stresses Cocking, (1960)[4]; Burgess & Linstead, (1977)[3]. As global food demands rise, protoplast-based techniques continue to play a crucial role in advancing sustainable and resilient agriculture Subburaj & Agapito-Tenfen, (2023)[14].

4. Principle of Protoplast Isolation

Protoplast isolation is based on enzymatic digestion of the plant cell wall. The main components of plant cell walls are Cellulose, Hemicellulose and Pectin. These are removed using:

- **Cellulase** (breaks cellulose)
- **Macerozyme** (breaks pectin)

Osmotic stabilizers like mannitol protect the delicate protoplast from bursting

Table 1: Some commonly used commercially available enzymes for protoplast

Sr. No.	Enzyme	Source	Supplier
1	*Cellulase*	*Aspergillus niger*	Duchefa, A. Hofmanweg 71, 2031 BH Harlem, Netherland
2	*Macerozyme*	*Aspergillus niger*	Duchefa, A. Hofmanweg 71, 2031 BH Harlem, Netherland
3	Hemicellulase	*Aspergillus niger*	Duchefa, A. Hofmanweg 71, 2031 BH Harlem, Netherland

Isolation

5. Steps in Protoplast Isolation and Culture of Soybean explants

Step 1: Preparation of Plant Tissue

- Select healthy young leaves, cotyledons, hypocotyls etc.
- Wash and surface-sterilize them.
- Cut into thin strips (0.5–1 mm wide) to expose more surface.

Step 2: Pre-plasmolysis

- Plasmolysis in plant cells happens when they are exposed to a hypertonic solution with high concentrations of sugar or salt.
- This causes water to move out of the cell sap through the plasma membrane, leading to the shrinking of the protoplasm.
- The protoplasm detaches from the cell wall and forms a spherical shape, which is referred to as plasmolysis. Experiments were carried out to induce pre-plasmolysis using CPW salts solution for durations of 30 and 120 minutes, in addition to a control group.

Step 3: Enzyme Digestion

- Prepare enzyme mixture with mannitol for osmotic balance.
- Add soybean leaf strips to the enzyme solution.
- Apply gentle vacuum infiltration for better enzyme penetration.
- Incubate for 4–16 hours (depending on tissue type).

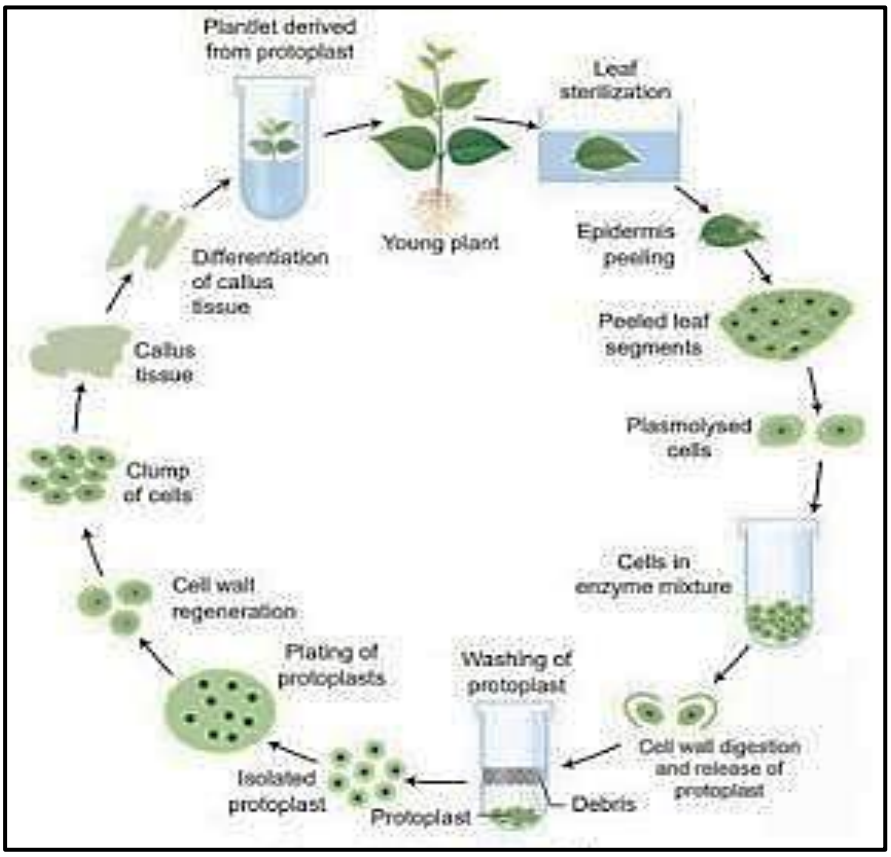
During digestion, the cell wall dissolves and protoplasts are released.

Step 4: Filtration and Collection

- Filter the digest through a mesh (40–75 µm).
- Collect the protoplast-rich filtrate.

Step 5: Washing and Purification

- Centrifuge gently (80–100 × g).
- Wash with W5 solution 2–3 times.
- Optionally use 21% (w/v) sucrose solution for gradient to purify healthy, round protoplasts.



Bhojwani, S. S., & Razdan, M. K. (1996)

Step 6: Viability Test

- Stain with Trypan blue (0.04%).
- Live protoplasts colourless and dead protoplast indicates blue in colour.

Step 7: Protoplast Culture and Callus Induction

a. Culture Medium

Various hormone concentrations were used in the basal tissue culture medium formulations. The media developed by, Kao & Michayluk (1975), Murashige & Skoog (1962)[11], and Gamborg *et al.,* (1968) were the foundational basis for these formulations.

b. Culture of Protoplasts

Freshly isolated protoplasts were cultured in the dark at 25 ± 1°C in KP8 liquid medium with BAP, NAA, and ZT. Their plating efficiency was checked after 7 days. After 10 days, the cultures were shifted to light and periodically diluted with fresh K8 medium. Once small calli formed, they were transferred to gelrite-solidified K8 medium for further growth. When calli reached 2–5 mm, they were shifted to MS medium supplemented with auxins and cytokinins to promote callus development. Finally, compact calli showing morphogenic potential were placed on MSB differentiation medium containing NAA, BA, KT, ZT, and CH to induce shoot formation

c. Shoot Regeneration

Callus is moved to cytokinin-rich medium (e.g., BAP) to initiate shoots.

d. Rooting and Plant Development

Shoots are transferred to rooting medium (NAA/IBA). Rooted plantlets are hardened and grown in soil.

Table 2: Optimal conditions for the isolation of protoplasts from Soybean.

Parameter	**Optimum condition**
Plant material	6- 7 days-old
Cellulase	1.2-2%
Macerozyme	0.5-0.9%
pH of enzyme solution	5.2-5.8
Volume of enzyme solution/ fresh weight of tissue	10 ml g-1
Incubation period	4-6 hours
Incubation temperature	25-27^{o} C
Osmoticum	0.5 – 0.8 Molar

Results:

Fresh and healthy protoplasts were successfully isolated from soybean explants using the optimized enzymatic digestion mixture, consistent with earlier observations on protoplast isolation efficiency Kao & Michayluk, (1975)[9]**.** Under the microscope, most protoplasts appeared round, healthy, and intact, with minimal debris, indicating that the enzyme treatment was efficient. The yield of protoplasts was sufficient for further culture experiments, and viability tests showed that a high percentage of cells remained alive immediately after isolation, similar to previously reported soybean suspension culture studies Gamborg *et al.,* (1968)**.** When cultured in KP8 liquid medium in the dark at 25 ± 1°C, the protoplasts remained stable during the initial days. By the 2nd to 5th day, early cell

wall regeneration was observed, and a portion of the protoplasts began their first divisions. Such progressive cell wall formation and division have also been described in classic tissue culture systems Murashige & Skoog, (1962**)[11].**

The plating efficiency assessed on the 7th day confirmed that a measurable fraction of the isolated protoplasts was actively dividing, showing that the medium composition and culture conditions were suitable for soybean protoplast growth Kao & Michayluk, (1975)[9]. After 10 days, cultures were shifted to low light conditions, which further encouraged cell division and colony formation, a response consistent with earlier studies describing the effect of controlled light on protoplast recovery and growth Kao & Michayluk, (1975[9]; Fowke & Gamborg, (1980)[6]. Small cell colonies became visible, and repeated dilution with fresh K8 medium supported their continuous growth, similar to nutrient-refresh strategies previously used to maintain high division rates in protoplast-derived cells Gamborg *et al.,* (1968).

Over the next 10–15 days, these colonies gradually developed into small, compact calli that could be seen with the naked eye, reflecting early observations on callus formation during protoplast regeneration Burgess & Linstead, (1977)[3]. Once the calli reached 2–5 mm in size, they were transferred onto gelrite-solidified K8 medium, where they continued to grow steadily, supported by the gel matrix and balanced nutrient environment (Kao & Michayluk (1975); Murashige & Skoog (1962)[11]. On MS medium containing MS minerals and B5 organic supplements with auxins and cytokinins, the calli further enlarged and became more compact and nodular. This confirmed that hormonal balance plays a key role in cell proliferation and morphogenesis, as described in classical tissue culture research Murashige & Skoog, (1962)[11]; Gamborg *et al.* (1968)[7]. Morphogenic calli were then transferred to MSB differentiation medium enriched with NAA, BA, KT, ZT, and casein hydrolysate. Under these conditions, several calli produced shoot initials, demonstrating successful initiation of regeneration from protoplast-derived callus, in agreement with established regeneration pathways reported in soybean and other crops Kao & Michayluk, (1975)[9]; Subburaj & Agapito-Tenfen, 2023)[14].

Although regeneration frequency varied among cultures, the development of shoots confirmed that soybean protoplasts in this study were capable of progressing through the complete pathway from protoplast isolation, cell division, and callus formation to early regeneration Kao & Michayluk, (1975**)**[9]; Fowke & Gamborg,(1980); Subburaj & Agapito-Tenfen, (2023)[14]**.**

Future Perspectives:

Protoplast isolation and regeneration in soybean have made significant progress in recent times, yet several opportunities remain to fully exploit this technology for crop improvement. Future research is expected to move toward refining regeneration efficiency, integrating advanced genetic tools, and translating laboratory advancements into practical agricultural applications. (Fowke & Gamborg, 1980)[6].

a. Improving Regeneration Efficiency and Reproducibility

One of the major challenges in soybean protoplast technology is the low and genotype-dependent regeneration rate. Future work will focus on:

- Identifying molecular markers associated with totipotency and regeneration ability.
- Developing universal culture media formulations that work across elite soybean cultivars.
- Using growth regulators, antioxidants, and stress-response modulators to enhance embryo formation and shoot regeneration.

b. High-Throughput Protoplast Platforms

Advances in automation will enable High-throughput protoplast screening systems for rapid testing of gene function, promoter activity, or metabolic pathways.

c. Integration with CRISPR and Next-Generation Gene Editing

Protoplasts offer a suitable system for DNA-free gene editing. Future directions include:

- RNP-based CRISPR delivery for precise, transgene-free modifications.
- Base editing and prime editing performed directly in isolated protoplasts.
- Regeneration pipelines optimized for gene-edited cells to reduce off-target effects.

d. Development of Protoplast-Derived Synthetic Biology Tools

Protoplasts can become powerful platforms for:

- Metabolic pathway reconstruction and engineering.
- Rapid protein expression testing and transient assays.
- Synthetic promoter design and evaluation in soybean cellular environments

e. Enhancing Stress Tolerance Studies

Protoplasts provide a controlled environment to understand soybean's response to:

- Drought, salinity, heat, and cold stress.
- Pathogen and pest interactions at the cellular and molecular levels.

Future research will likely use protoplasts to dissect stress signaling networks and identify new targets for breeding resilient soybean varieties.

f. Genotype-Independent Protoplast Systems

Developing protocols that work across diverse soybean backgrounds is a crucial goal. Future studies should aim to:

- Identify universal enzyme combinations for efficient cell wall removal.
- Create regeneration protocols adaptable to wild relatives and landraces.

This will broaden the germplasm available for biotechnological improvement.

g. Commercial and Industrial Applications

Future perspectives also involve scaling protoplast technology for:

- Production of high-value compounds through cell-based bioreactors.
- Rapid testing of herbicide tolerance and biotic stress responses.
- Seed industry applications, such as quick validation of novel genetic traits.

h. Integration with AI and Digital Phenotyping

AI-assisted image analysis and machine learning will support:

- Automated monitoring of protoplast viability, division, and microcallus formation.
- Predictive modelling to identify ideal culture conditions for regeneration.

This integration will enhance precision in single-cell technologies.

Conclusion:

Protoplast technology has become an important tool in modern plant biotechnology, offering new possibilities for crop improvement and genetic research. By removing the cell wall and working directly with isolated plant cells, scientists can better understand how plants grow, respond to stress, and express important traits. The studies reviewed in this chapter clearly show that successful protoplast isolation depends on several factors such as the type of enzyme used, its concentration, incubation time, osmotic balance, and handling conditions. When these factors are optimized, protoplasts can be efficiently isolated, cultured, and encouraged to divide and form callus. This technology is especially valuable for crops like soybean, which are often difficult to regenerate through conventional tissue culture methods. Protoplast-based approaches open the door to advanced breeding tools such as somatic hybridization, genetic transformation, and genome editing. These methods allow researchers to introduce useful traits like disease resistance, stress tolerance, and improved yield traits that are urgently needed in today's changing climate and agricultural systems.

Overall, protoplast isolation and culture provide a strong foundation for future innovations in crop improvement. As techniques continue to advance, protoplast-based research will

play an even more meaningful role in developing resilient and high-performing crop varieties for sustainable agriculture.

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SYNTHESIS OF CRYSTALS USING GEL TECHNIQUES: A COMPREHENSIVE OVERVIEW

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Abstract:

The crystal grown by gel growth technique offers a simple and economy method for producing crystals that requires very less equipments. Silica hydrogel controls diffusion, prevents convection, and enables crystal formation at room temperature. Crystal forms when inner and outer reactants diffuse and react within the gel to produce sparingly soluble crystals. The polymeric structure of silica gel plays a key role in regulating nucleation and growth. This technique is used for low-solubility materials and those unstable at high temperatures. Although growth is slow and crystal size is limited, the technique provides good purity and morphology control. The present chapter outlines the principles, procedure, advantages, and limitations of gel-grown crystals.

Keywords: Silica Gel, Tartrate Crystals, Nucleation, Gel Growth.

Introduction:

A crystal is a solid composed of a periodic array of atoms, i.e. a representative unit is repeated at regular intervals along all directions in a crystal. In a good or ideal crystal, there is an infinite lattice of periodically arranged atoms in three dimensions. But, in real, it is more difficult to find an ideal crystal. Initial resources of crystals were found in nature alone. With the development of technology and due to the minimum availability of natural resources, a man starts trying to make the crystal and with time he develops the knowledge of making artificial crystals from the materials which are easily available around him.

An orderly investigation of crystallization in gel starts with Leisgang's (1896) well-known discovery of periodic crystallization in gel [1]. This method attracted to many researchers and scientist because of its simplicity and it requires very minimum apparatus. Also, growth can be easily seen during the growth period. This technique can be utilized at the surrounding temperature for those substances, which are slightly dissolvable in water [2-4]. Initially, Henisch (1970) explained the use of the gel method for the growth of the crystal [6]. The crystal product has the extraordinary advantage of a simple process of growth and appropriate for the growth of crystals having low solubility [7-12]. Many

researchers have grown the crystal of tartrates by using gel technique [13-15]. Also, the rare earth tartrate crystals developed by this technique [16-17].

Principle of Gel Growth

Crystal growth by silica gel technique is a simple technique since it requires very less apparatus. Crystals grow under controlled conditions at room temperature in this technique. A solution of two suitable compounds which give rise to the required insoluble crystalline substance by a mere chemical reaction between them is allowed to diffuse into the gel medium and chemically reacts as follows

$$A\,X + B\,Y \rightarrow B\,X + AY \downarrow$$

$$\text{X (solution) + Gel medium} \xrightarrow[\text{Atm. Pressure}]{\text{Room Temperature}} \text{Y (crystal)}$$

$$\text{A (solution) + B (solution) + Gel medium} \xrightarrow[\text{Atm. Pressure}]{\text{Room Temperature}} \text{Y (crystal)}$$

In the above equation AX and BY are the two solutions. After the reaction, it gives the insoluble or sparingly soluble substance AY and BX is the waste product which is highly soluble in water [49]. This method is useful to grow the crystals from the materials which have high solubility. The above reaction takes place in the glass test tube. In reaction, one reactant is incorporated in the gel and another reactant is diffused through small capillary cells.

There are two different methods of diffusion of a reactant which are given below,

a) Single diffusion method

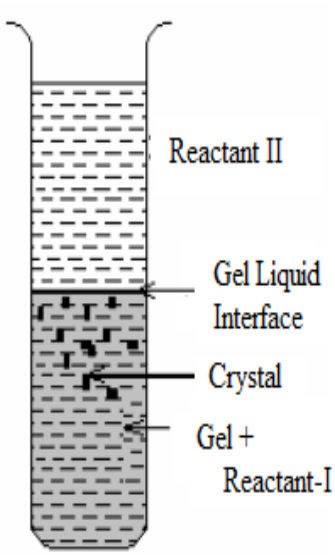
b) Double diffusion method

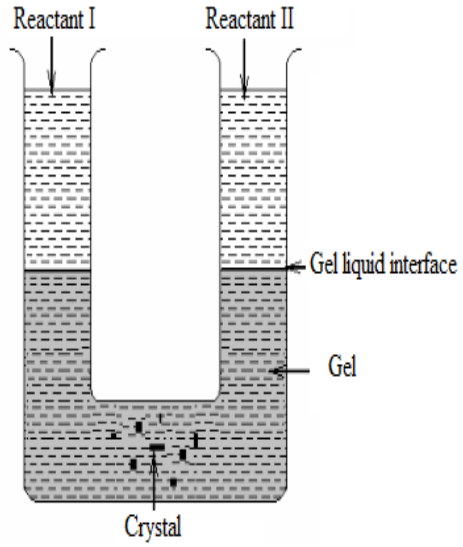
a) Single diffusion method

In this method, one reactant is incorporated in the gel and another reactant is diffused through small capillary cells. These solutions after diffusion react with inner reactant and crystals and other products are formed. The other products are highly soluble in water.

b) Double diffusion method:

In this method, the U-shape tube is used. The reactants in two arms of U-tube are allowed to diffuse by choice of suitable reactant and their concentrations.





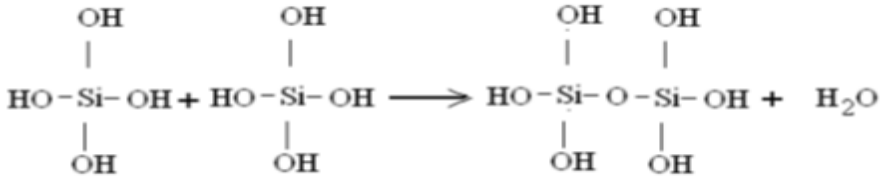
Single diffusion method **Double diffusion method**

Structure of Silica Hydrogel

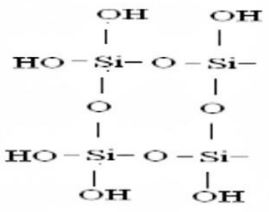
Many researchers have been used the silica hydrogel to grow the different crystals. The studies of the gelling mechanism and structure of gel have great importance. When sodium metasilicate dissolve in water to make an aqueous solution, monosilicic acid is formed according to dynamic equilibrium,

$$Na_2SiO_3 + 3H_2O \rightarrow H_4SiO_4 + 2NaOH$$

This monosilicic acid can polymerize with the liberation of water,



This can occur again and again and a three-dimensional structure formed as silica hydrogel by a network of Si-O links.

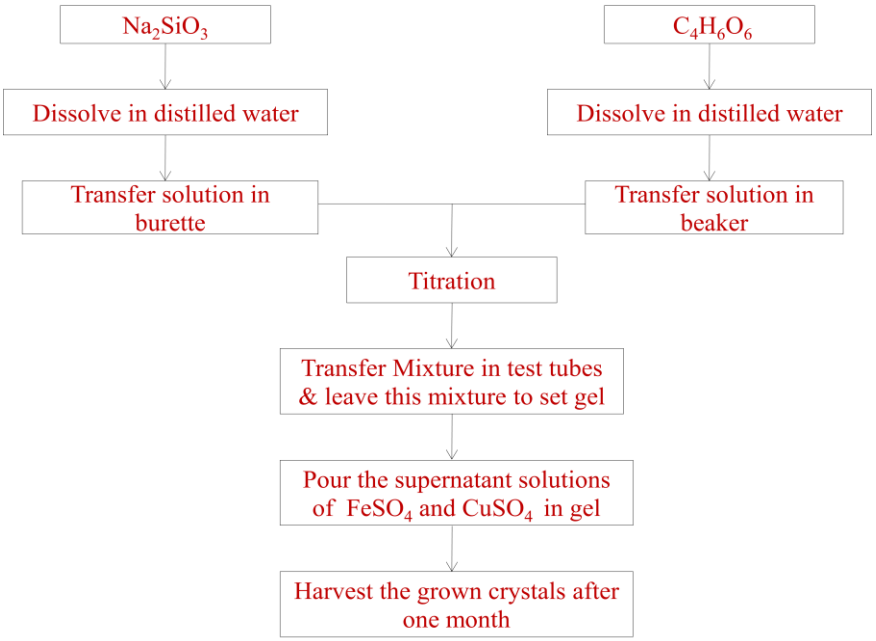


As the formation of the structure of silica hydrogel goes on, water accumulates on the free surface of the gel. This phenomenon of silica hydrogel is known as “Syneresis”. Some of the water has come from the condensation process, and some may arise from purely mechanical factors connected with a small amount of gel shrinkage. The well-known

stability of the silicon-oxygen bonds is responsible for the fact that polymerization is largely irreversible.

Experimental Procedure

Sodium metasilicate was taken in the burette and tartaric acid taken in a beaker. Sodium metasilicate titrated with tartaric acid to attain a specific pH. Then this mixture transferred to a test tube which is used as crystallization vessels and the silica gel was used as a growth media. This mixture allows to set the gel [17,18]. After setting the gel solution of desired compound poured along the wall of the test tube in order to avoid the breakage of the gel surface. After one month crystals were harvested.



Flow chart of the crystal growth process

Specific Gravity of Sodium Metasilicate Solution

The specific gravity is the ratio of the weight of any volume of that substance to the weight of an equal volume of a standard substance. Generally, water is used as a standard liquid with its specific gravity assumed to be unity [13-16]. There are three methods to determine specific gravity which are given below.

- Determinations of specific gravity of liquid by using a specific gravity bottle.
- Determinations of specific gravity by using the principle of Archimedes.
- Determinations of specific gravity of liquid by using the U tube method

Out of these methods, specific gravity by a specific gravity bottle method very useful .

$$\text{Density} = \frac{W_3 - W_1}{W_2 - W_1}$$

Where, W_1 = Mass of empty sp. Gravity bottle

W_2 = Mass of sp gravity bottle + water

W_3 = Mass of sp gravity bottle + gel solution

Parameters controlling growth

In the formation of crystals, the crystal growth depends on various parameters such as,

- Effect of pH
- Effect of concentration of the solution
- Effect of temperature
- Effect of gel aging
- Effect of gel density

Effect of pH

Hydrogen ion concentration in a given solution is the pH of the given solution. Mathematically it is written as the negative logarithm of hydrogen ion concentration. i.e. – log [H] = pH. If the pH value of the solution increases then the thickness of gel increases. i.e. higher the pH denser the gel [50]. It is also observed, that if the pH value reduces, the gel setting period increases and if the pH value increased, the gel setting period is decreases [51]. As pH value goes on increasing then the number of crystals decreases [52].

Effect of Concentration

To study the effect of concentration of reactants, it is good to start with lower concentration. If the concentration of reactants goes on increasing, the growth rate also increases. The high concentration of solvents has a faster growth of crystals as compared to lower concentrations of solvents. But the defective crystals may be formed due to faster growth of crystals while slowly grown crystals have fewer defects [53].

Effect of Temperature

The effect of temperature plays an important role in crystal growth [54]. At a higher temperature growth rate of crystals is faster as compared to crystal growth at the lower temperature. At a higher temperature nucleation period is reduced. Variations in the temperature do not permit desired growth so to have better quality crystals; one should maintain the constant temperature to reduce the density of defects [53]. Hence in summer gel setting time is less than in winter.

Effect of Gel Aging

Gel aging is very important in the growth of crystals to prevent from breaking of gel; otherwise, there may be a production of defective crystals. Once the gel sets then leaving it for aging from some hours to some days. Gel aging reduces pore size and which affects the quality of crystals [55]. The evaporation of water from the gel before the gel setting

increases the gel density. Therefore it decreases the porosity of gel and in turn decreases the number of nucleation sites. After the gel is set, evaporation of water causes a lack of ionic carriers in the channel due to shrinkage of gel.

Effect of Gel Density

Gel density affects the porosity of the gel column and in turn affects the crystal size, nucleation density, and quality of crystals. If gel density increases then gel setting time reduces. i.e. higher the gel density, quick is the gel setting. If the density of sodium meta silicate increases the transparency of the gel decreases. Pore size depends on gel density [56].

Advantages and Limitations of Gel Method

The crystal growth by gel method has some advantages and some limitations as follows,

Advantages of Gel Method

- Gel method is a very simple method that requires very fewer materials.
- The progress of crystals can be observed at all stages during the growth of crystals.
- The gel medium prevents convection current and turbulence.
- Crystals can be grown at room temperature and due to which grown crystals are having relatively fewer defects.
- The gel reduces the effect of speed of chemical reaction.
- The diffusion rate and nucleation probability can be controlled. One can design crystallization equipment for obtaining the required size and morphology of different crystals.
- One can obtain the mixed crystals by the gel method.
- The growth of crystals can be possible for the materials which have no suitable solvents for re-crystallizations.
- The growth of crystals can be possible for the material which decomposes at a temperature below their melting point.
- All nuclei are spatially separated, minimizing many effects due to precipitate interaction.

Limitations of Gel Method

- Crystal growth period is generally very large, so it requires patience.
- Crystal size is relatively small.
- Grown crystals may stick to the walls of a test tube.
- Chances of lattice contamination by impurities from the gel itself have profusely increased.

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NENOFERTILIZERS – A TOOL FOR SMART NUTRIENT DELIVERY TO CROP

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Abstract:

Nanofertilizers are a new generation of fertilizers that employ advanced nanotechnology for fertilizing crops in an efficient and sustainable manner and at the same time, reducing the environmental impact of fertilizer application. Nanoparticles are used in nanofertilizers to control the release of nutrients with an aim to make them more efficient and cost-effective in comparison to traditional fertilizers. Nanofertilizers comprise one or more plant nutrients within nanoparticles where at least 50% of the particles are smaller than 100 nanometers. Carbon nanotubes, graphene, and quantum dots are some examples of the types of nanomaterials used in the production of nanofertilizers. They are designed to deliver plant nutrients in a controlled manner, ensuring that the nutrients are gradually released over an extended period, thus providing a steady supply of essential elements to the plants. These nanomaterials have a high surface area-to-volume ratio, making them ideal for holding and releasing nutrients. Naturally occurring nanoparticles are found in various sources, including volcanic ash, ocean, and biological matter such as viruses and dust. However, relying solely on naturally occurring nanoparticles may not be sufficient in case of large-scale production. Nanofertilizers are especially beneficial in areas where traditional fertilizers are inefficient or ineffective. At present, nanofertilizers face restrictions, including higher costs of production and potential environmental and safety concerns due to the use of nanomaterials, therefore further research is required to fully understand their long-term effects on soil health, crop growth, and the environment.

Introduction:

Fertilizer is mostly used in agriculture to provide macro and micronutrients that the soil generally lacks. Fertilizer accounts for 35–40% of crop productivity, however some fertilizer directly influences plant growth and development. The widespread presence of nutrient deficiencies in soils result in large financial loss for farmers as well as notable reduction in the quantity and nutritional quality of grain consumed by human and animals. Crop productivity can be increased through fertilizer application. Nevertheless, plants do

not have complete access to the available nutrients found in the bulk chemical forms. Additionally, because most macronutrients are converted to insoluble forms in soil, their consumption is extremely low. Usually, fewer than half of the chemical fertilizers are used by crop plants.

Furthermore, the absorption of the majority of macronutrients is significantly diminished d ue to their conversion into insoluble forms in the soil. Crop plants generally utilize less than half of the chemical fertilizers that are applied (Loomis and Connor 1992). The leftover minerals can leach into the soil, where they either become trapped or contribute to air pollution. Therefore, relying on extensive chemical fertilizer usage to boost crop productivity is not a sustainable strategy in the long term. Overusing these fertilizers can lead to significant and lasting damage, impacting soil structure, mineral cycles, and the microbial life within the soil. This disruption can affect plants and, ultimately, the entire food chain, potentially causing heritable mutations in future generations of consumers. Considering the above points, there is an urgent need to develop smart materials that can systematically release chemicals to specific targeted sites in plants which could be beneficial in controlling nutrition deficiency in agriculture. To overcome all these drawbacks a smarter way i.e., nanotechnology can be one of the sources. Since fertilizers are the main concern, developing nano-based fertilizer would be a new technology in this field.

Nanomaterials are at the leading edge of rapidly developing field of nanotechnology. According to the National Nanotechnology Initiative (NNI), “Nanotechnology research and development is directed towards understanding and creating improved materials, devices and systems that exploit nanoscale properties” (Nanoscale Science Energy and Technology Subcommittee 2007).

Nanotechnology is defined by the US Environmental Protection Agency19 as the science of understanding and control of matter at dimensions of roughly1–100 nm, where unique physical properties make novel applications possible. This definition is slightly rigid with regard to size dimensions. Greater emphasis could have been placed on the problem-solving capability of the materials. Other attempts to define nanoparticles from the point of view of agriculture include “particulate between10 and1,000 nm in size dimensions that are simultaneously colloidal particulate”.

The term “nano” is adapted from the Greek word meaning “dwarf.” The word “nano” means 10^{-9}or one billionth part of a meter. Particles with at least one dimension less than 100 nm

are considered as "nanoparticles" (Thakkar *et al.,* 2010). Nanoparticles have high surface area to volume ratio, nanometer regime, and unique properties, which makes them highly applicable. Nanoparticles have potential applications in agriculture system, viz., detection of pollutants, plant diseases, pests, and pathogens; controlled delivery of pesticide, fertilizers, nutrients, and genetic material; and can act as nano architects in formation and binding of soil structure (Ghormade *et al.,* 2011). Nanoparticles can result in modification of plant gene expression and associated biological pathways which ultimately affect plant growth and development (Nair *et al.,* 2010).

One particular area in this nanotechlogy is that of production of nano fertilizers. Nano fertilizers may be defined as the nano particles, which can supply essential nutrients precisely for maximum plant growth, have higher use efficiency and can be delivered in a timely manner to a rhizospheric target or by foliar spray. Nano fertilizers are molecular modified or synthesized materials, with the help of nano technology used to improve the fertility of soil for a better yield and increased crop quality. Nano fertilizers are nutrient carriers of nano dimensions ranging30 to 40 nm.

Advantages of Nano fertilizers:

Nano fertilizers play an important role in increasing the quality of agricultural products and in removing the soil and environmental hazards. The purity of elements is very high in such fertilizers. One of the advantages of such fertilizers is that they can be used in tiny amounts, as a result it saves cost by less expenditure on fertilizers which is economical from farmer's point of view. In general advantages of application of nano fertilizers are

(i) increased yield of crop by an average of 20%,

(ii) enhanced nutritional value of the produce and

(iii) overall health of the crop is enhanced, making it more resistant to severe weather and extreme atmospheric conditions. Immunological response is heightened allowing the plant to fight disease and prevent infections.

(iv) According to Subramaniun and sharmila rahale (2013) the nano-clay based fertilizer formulations (zeolite and montmorillonite with a dimension of 30-40nm) are capable of releasing the nutrients particularly N for a longer period of time (> 1,000 h) than conventional fertilizers (<500 h).

(v) Fertilizer particles can be coated with nano-membranes that felicitate in slow and steady release of nutrients. This process helps to reduce loss of nutrients while improving fertilizer use efficiency of crops.

(vi) Major portion of nutrient fixation occurs in the broken edges of the clay particles. Zero valence nanoparticles adsorb onto the clay lattice, thereby preventing fixation of nutrient ions. Further, nano-particles prevent the freely mobile nutrient ions to get precipitated. These two processes assist in promoting the labile pool of nutrients that can be readily utilized by plants.

Conventional Fertilizers versus Nano-fertilizers

Comparison of nanotechnology-based formulations and conventional fertilizers applications: (Cui *et al.,* 2010)

Properties	**Nano-fertilizers-enabled technologies**	**Conventional technology**
Solubility and dispersion of mineral micronutrients	- May improve solubility and dispersion of insoluble nutrients in soil, - Reduce soil absorption and fixation, and - increase the bioavailability	Less bioavailability & less solubility due to large particle size
Controlled release mode	Both release rate and release pattern of nutrients for water-soluble fertilizers might be precisely controlled through encapsulation in envelope forms of semi permeable membranes coated by resin-polymer, waxes, and sulfur	Excess release of fertilizers may produce toxicity and destroy ecological balance of soil
Nutrient uptake efficiency	- Might increase fertilizer efficiency - Uptake ratio of the soil nutrients in crop production and - Save fertilizer resource	Bulk composite is not available for roots and decrease efficiency
Effective duration of nutrient release	Can extend effective duration of nutrient supply of fertilizers into soil	Used by the plants at the time of delivery, rest is converted into insoluble salts in the soil
Loss rate of fertilizer nutrients	Reduces loss rate of fertilizer nutrients by leaching and/or leaking	High loss rate by leaching, rain off, and drift

Synthesis of Nano fertilizers

An essential feature for the nanoparticle synthesis is the preparation of the particles of specific size and shape. For agricultural use it is referable to have article having size less than 30-40 nm. The methods for making nanofertilizers can generally involve either a "top down" approach or a "bottom up" approach. In top-down synthesis, nanoparticles are produced by size reduction from a suitable starting material. Size reduction is achieved by various physical and chemical treatments. In bottom up synthesis, the nanoparticles are built from smaller entities, for example by joining atoms, molecules and smaller particles. In bottom up synthesis, the nano structured building blocks of the nanoparticles are formed first and then assembled to produce the final particle (Thakkar *et al.,* 2010). The bottom up synthesis mostly relies on chemical and biological methods of production. Biological methods offer a safe and ecologically sound approach for nanoparticle fabrication as an alternative for physical, chemical and aerosol methods. The main advantages of biological methods are that the particles are usually encapsulated by mother protein, therefore, unless the protein layers break, the particles are stable, so these can be used for agricultural purposes. Fig. 1 describes various processes of nanoparticle production.

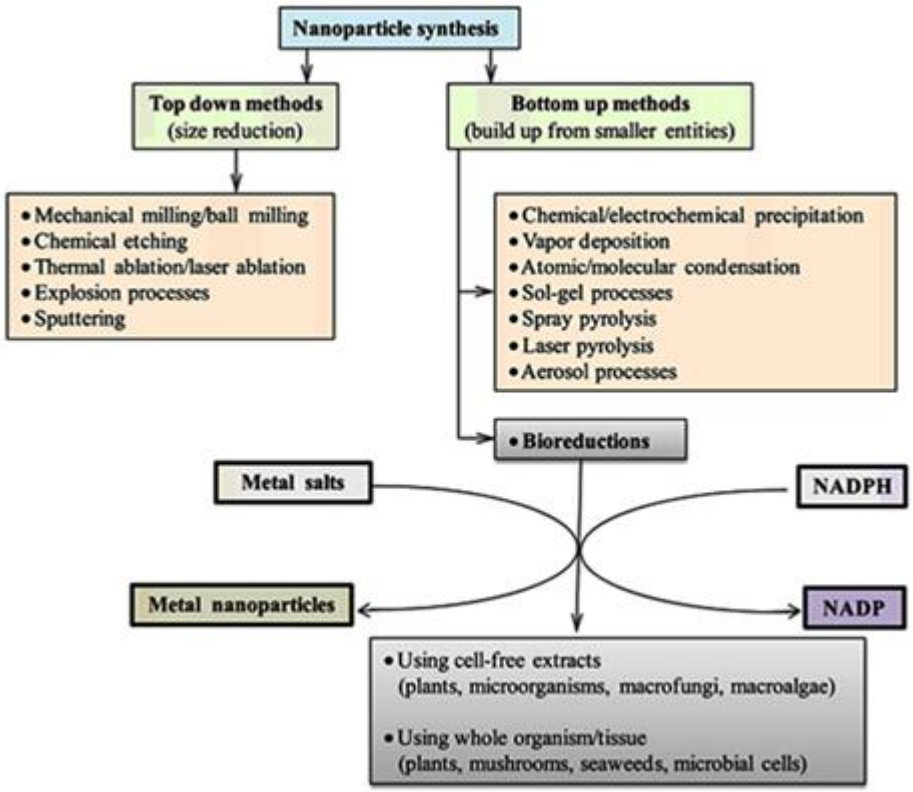

Figure 1: Various approaches for making nanoparticles and co-factor dependent biosynthesis

Nano-Fertilizer Formulations and Their Smart Delivery Systems

The nano fertilizer formulations should have some desired properties such as high solubility, stability, effectiveness, time-controlled release, enhanced targeted activity with effective concentration, and less eco-toxicity with safe, easy mode of delivery and disposal. The loading of nutrients on the nanoparticles is usually done by (a) absorption on nanoparticles, (b) attachment on nanoparticles mediated by ligands, (c) encapsulation in nano particulate polymeric shell, (d) entrapment of polymeric nanoparticles, and (e) synthesis of nanoparticles composed of the nutrient itself (Sulanki et.al., 2015).

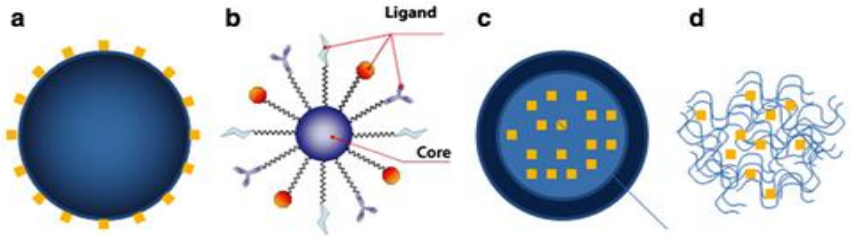

Figure 2: Loading of nutrients on the nanoparticles

Different Types of Nano Fertilizers in India: Nanofertilizers can be classified on the basis of their actions, consistency and nutrient composition as discussed below:

1. Action Based Nanofertilizers

- Controlled release
- Targeted delivery
- Plant growth stimulating
- Water & nutrient loss controlling

2. Nutrient Based Nanofertilizers

- Inorganic
- Organic
- Hybrid
- Nutrient-loaded

3. Consistency Based Nanofertilizers

- Surface coated
- Synthetic polymer coated
- Biological product coated
- Nanocarrier based

The mode of fertilizer application influences their efficiency and impact on plant systems. The following methods can be used for nano-fertilizer delivery to plants:

Soil Application

Soil application is the most common method of nutrient supplement using chemical and organic fertilizers. As nano-fertilizers are required in less quantity, the nano-fertilizers can be mixed well with finely ground soil of farmward manures before localized placement surrounding the root zone of the plants. The reason in support of this practice is that two phenomena will occur after placing of nano-fertilizers viz. 1. More number of nano particles can come in intimate contact with soil particles and thereby will be subjected to greater action of soil solvents and 2. Nano particles directly interact with the plant root surface and penetrate through the cell wall and enter inside the cell.

Foliar Application

In this method, liquid fertilizers are directly sprayed onto leaves. As stomata and leaf epidermal cells are majorly involved in nutrient uptake, foliar application method can have agronomic advantage if used for nano-fertilizers. Foliar application with effective surfactants, seed coating etc. of nano-fertilizers can be adopted to enhance the use efficiency of the fertilizers.

Seed Nano-Priming:

Seed priming is a pre-sowing treatment that induces physiological changes within seeds, allowing for faster germination and promoting plant growth and development by regulating metabolic and signaling cascades. The method involves soaking seeds in nanofertilizers, which has been shown to reduce fertilizer application by half while achieving excellent results. Nanobiofertilizers act as stimulants, enhancing germination and development by penetrating seed pores, dispersing within, and activating plant hormones that promote growth.

Optimization of Concentration of Nanoparticles

Aerosol spray is found much superior than traditional spray for the application of nanoparticles to plants. In general, by normal spray 33% of the nanoparticles were untraceable after spray and were considered as a loss while aerosol spray resulted only 15% loss. Higher the particle size lower is the penetration. Nanocube is the better shape to spray to plants. Two weeks old plants are found to be more suitable for foliar application of nanoparticles.

Mode of Entry

The uptake, translocation, and accumulation of nanoparticles depend on the plant species, age, growth environment, and the physicochemical property, functionalization, stability, and the mode of delivery of nanoparticles.

There is also a chance for enlargement of pores or induction of new cell wall pores upon interaction with engineered nanoparticles which in turn enhance nanoparticle intake. Further internalization occurs during endocytosis with the help of a cavity-like structure that forms around the nanoparticles by plasma membrane. They may also cross the membrane using embedded transport carrier proteins or through ion channels. In the cytoplasm, the nanoparticles are applied on leaf surface, they enter through the stomatal openings of through the base of the trichomes and then tranlocated to various tissues. However, accumulation of nanoparticles on photosynthetic surface causes foliar heating which results in alterations of gas exchange due to stomatal obstructions that produce changes in various physiological and cellular functions of plants.

Nutrients Enter Plant through Root Hairs and Deliver Nutrients:

The macro and microelements, such as Ca, Mg, Fe, S and Zn are encapsulated into microspheres, and these polymer microcapsules are, with time, incorporated and dissolved into the soil. Once close to the root, the chemical bonds of the microcapsule's wall polymer are broken down by the organic acids or phenolic substances from root exudates. These root exudates are typically released to enhance plant feeding during the plant growth process and represent the stimuli activating CPA release.

After entering the cell, nanoparticles can transport apoplastically or symplastically. They may be transported via plasmodesmata from one cell to the other (Rico et al.2011). In the cytoplasm, nanoparticles approach to different cytoplasmic organelles and interfere with different metabolic processes of the cell (Moore 2006).

Conclusion:

Development of nanofertilizers represents a stimulating prospect to transform the agricultural sector and promote sustainable goals. By focusing on ongoing research, addressing potential risks, fostering interdisciplinary collaboration, educating farmers, and ensuring affordability and accessibility, the potential of nanofertilizers to contribute to global food security and agricultural sustainability significantly can be realized.

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ARTIFICIAL INTELLIGENCE IN MICROBIOLOGY

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Abstract:

Artificial intelligence (AI) is fundamentally transforming microbiology by significantly enhancing data analysis, accelerating discoveries, and optimizing experimental design. AI-driven tools enable researchers to analyze complex microbial interactions, predict disease outbreaks, and develop sustainable solutions across various sectors. Machine learning algorithms process vast microbiological datasets to identify patterns that would otherwise go undetected, boosting research efficiency and innovation. AI applications span multiple domains of microbiology: Medical Microbiology: AI assists in pathogen detection and antimicrobial resistance prediction, leading to more precise treatment strategies and improving outbreak preparedness. Environmental Microbiology: Models analyze microbial communities in soil, water, and air, aiding in pollution monitoring and predicting microbial functions related to ecosystem stability and climate resilience. Industrial Microbiology: AI algorithms optimize fermentation processes and microbial enzyme production, increasing efficiency in pharmaceuticals, food, and biofuels. Agricultural Microbiology: AI identifies beneficial microbial antagonists for crop disease management, supporting sustainable pest control and precision agriculture. Despite its transformative impact, the integration of AI faces several limitations. Key challenges include the reliance on large, high-quality datasets, the interpretability of complex AI-driven findings, potential biases in models, and the need for interdisciplinary expertise. Addressing these requires standardized microbial datasets, advances in explainable AI, refining algorithmic models, and promoting collaboration between microbiologists and AI specialists. The future prospects for AI in microbiology are immense, pointing toward advancements like personalized medicine based on microbiome analysis, integration with synthetic biology for microbial engineering, and fully automated laboratories. Continued research and adherence to ethical, regulatory frameworks will ensure AI remains a reliable and accessible tool for groundbreaking microbiological discoveries, fostering sustainability and improvements in global health.

Keywords: Artificial, AMR, Machine Learning, Synthetic Biology Diagnostics

Introduction:

Artificial intelligence (AI) has revolutionized microbiology by enhancing data analysis, accelerating discoveries, and optimizing experimental design. AI-driven tools enable researchers to analyze complex microbial interactions, predict disease outbreaks, and develop sustainable agricultural solutions. Machine learning algorithms process vast microbiological datasets, identifying patterns that might otherwise remain undetected (Zhou *et al.,* 2020). In food microbiology, AI assists in pathogen detection and food safety monitoring, reducing risks associated with contamination (Sharma *et al.,* 2021). Moreover, AI applications extend to antimicrobial resistance research, where predictive models help design novel therapeutic approaches (Smith *et al.,* 2019). As AI continues to evolve, its integration into microbiology is expected to refine our understanding of microbial ecosystems and pave the way for innovative biocontrol strategies. In addition to its applications in food safety and antimicrobial resistance, AI is transforming microbiological research by enhancing microbial strain identification and optimizing biocontrol strategies. Advanced machine learning techniques enable rapid genome sequencing and functional annotation, helping scientists classify microorganisms with greater accuracy (Chen *et al.,* 2021). AI-driven models also facilitate the prediction of microbial antagonists and their effectiveness in agricultural disease management, supporting sustainable crop protection efforts (Patel & Singh, 2022). By integrating AI into microbiology, researchers can streamline data analysis, improve experimental reproducibility, and uncover novel microbial interactions that contribute to ecosystem stability and biotechnological innovation.

Applications in Various Sectors of Microbiology

Artificial intelligence (AI) has been increasingly integrated into various sectors of microbiology, enhancing research efficiency and innovation. In medical microbiology, AI-driven diagnostic tools assist in pathogen detection and antimicrobial resistance prediction, allowing for precise treatment strategies (Zhang *et al.,* 2021). AI-powered genomic sequencing has also contributed to the identification of emerging infectious diseases, improving outbreak preparedness and response (Singh *et al.,* 2020).

In environmental microbiology, AI models analyze microbial communities in soil, water, and air, aiding in pollution monitoring and biodegradation studies (Patel & Kumar, 2019). AI-assisted microbial metagenomics enables researchers to predict microbial functions that influence ecosystem stability and climate resilience (Ghosh *et al.,* 2022).

The field of industrial microbiology benefits from AI algorithms that optimize fermentation processes and microbial enzyme production, boosting efficiency in pharmaceuticals, food, and biofuels (Sharma & Joshi, 2021). AI also plays a crucial role in strain selection, helping scientists engineer microbes for improved biotechnological applications (Wu *et al.,* 2020). In agricultural microbiology, AI aids in identifying beneficial microbial antagonists for disease management in crops, contributing to sustainable pest control strategies (Kumar *et al.,* 2018). Additionally, AIdriven predictive models assess soil microbial diversity and its influence on plant health, guiding precision agriculture techniques (Verma & Singh, 2021).

Across these sectors, AI enhances microbiological research by accelerating discoveries and improving data analysis. As AI evolves, its integration into microbiology will continue to shape innovations in healthcare, environmental conservation, industrial production, and sustainable agriculture.

Limitations

While artificial intelligence (AI) has significantly advanced microbiology, several limitations must be considered. One major challenge is the quality and availability of data; AI models rely heavily on large, high-quality datasets, but microbiological data can be fragmented, inconsistent, or limited in certain fields (Chen *et al.,* 2021). Another limitation is the interpretability of AI-driven findings, as complex machine learning algorithms often produce results that are difficult for researchers to fully understand or validate (Patel & Singh, 2022).

Additionally, AI models can exhibit biases based on training data, potentially leading to inaccurate predictions or misinterpretations in microbial classification and disease diagnostics (Ghosh & Banerjee, 2020). The integration of AI into microbiology also requires interdisciplinary expertise, demanding collaboration between microbiologists and AI specialists, which can slow adoption in traditional laboratory settings (Sharma & Joshi, 2021). Finally, ethical concerns regarding data privacy and algorithm-driven decision-making in medical microbiology pose regulatory challenges that must be carefully addressed (Verma & Rao, 2019).

Despite these limitations, continued research and refinement of AI methodologies hold promise for overcoming these hurdles, ensuring responsible and effective applications in microbiological studies.

Proposed Solution for Limitations

Addressing the limitations of artificial intelligence (AI) in microbiology requires a combination of improved methodologies, interdisciplinary collaboration, and regulatory frameworks. One key solution is enhancing data quality and accessibility by developing standardized microbial datasets and improving data-sharing initiatives across institutions (Chen *et al.,* 2022). Advances in explainable AI can also make AI-driven findings more interpretable, enabling researchers to validate results more effectively (Patel & Singh, 2023).

To mitigate biases in AI predictions, researchers are adopting diverse training datasets and refining algorithmic models to reduce inaccuracies in microbial classification and diagnostics (Ghosh & Banerjee, 2021). Promoting interdisciplinary collaboration between microbiologists and AI specialists is essential to facilitate effective implementation, with universities and research centers increasingly offering joint programs and workshops (Sharma & Joshi, 2022).

Ethical challenges, including data privacy and AI-driven medical decisions, can be addressed through stringent regulatory frameworks and transparent AI governance policies (Verma & Rao, 2020). As AI continues to evolve, fostering responsible innovation and ensuring AI tools remain ethical, unbiased, and accessible will drive sustainable advancements in microbiology.

Future Prospects

The future of artificial intelligence (AI) in microbiology holds immense promise, with emerging advancements expected to revolutionize research and practical applications. AI-driven predictive analytics will enable early detection of microbial pathogens, improving disease prevention strategies in medicine and agriculture (Chen *et al.,* 2024). The integration of AI with synthetic biology will enhance microbial engineering, facilitating the design of custom microbes for bioremediation, biofuel production, and sustainable agriculture (Patel & Singh, 2025).

Additionally, AI-powered automated laboratories will accelerate microbiological experiments, reduce human intervention while enhance precision and reproducibility (Ghosh & Banerjee, 2023). The application of deep learning models in metagenomics will uncover hidden microbial interactions, refining our understanding of microbiomes in human health and environmental ecosystems (Sharma & Joshi, 2025). AI will also play a crucial role in **personalized medicine**, tailoring microbiome-based therapies to

individual patients and optimizing probiotic formulations based on genetic and microbial profiles (Verma & Rao, 2024).

As AI technology evolves, ethical considerations and regulatory frameworks will remain essential to ensure responsible implementation. With continued interdisciplinary collaboration, AI is set to drive groundbreaking discoveries, enhancing microbiology's role in healthcare, environmental sustainability, and biotechnological innovation.

Conclusion:

The integration of artificial intelligence (AI) into microbiology has profoundly enhanced research methodologies, enabling rapid data analysis, predictive modeling, and automation of laboratory processes. AI-driven advancements in medical microbiology have improved disease diagnosis and treatment strategies, helping predict antimicrobial resistance patterns and optimizing therapeutic interventions. Similarly, AI applications in environmental microbiology allow researchers to analyze microbial communities in soil, water, and air, aiding in pollution control and climate resilience. In agricultural microbiology, AI enhances sustainable farming by identifying beneficial microbial antagonists that support disease resistance in food crops, contributing to precision agriculture and biocontrol strategies. The field of industrial microbiology benefits from AI's ability to optimize microbial fermentation and enzyme production, increasing efficiency in food processing, pharmaceuticals, and biofuel production.

Despite its transformative impact, AI in microbiology faces limitations, including data accessibility challenges, biases in AI models, and the complexity of algorithmic interpretations. Addressing these hurdles requires interdisciplinary collaboration, improved data standardization, and regulatory frameworks that ensure responsible AI implementation. The future prospects of AIdriven microbiology point toward further advancements in synthetic biology, metagenomic analyses, personalized medicine, and automated laboratory technologies, ushering in a new era of microbial research.

As AI continues to evolve, microbiologists must balance technological innovation with ethical considerations, ensuring AI remains a reliable, unbiased, and accessible tool for scientific discovery. Through continuous refinement and collaboration, AI will contribute to groundbreaking microbiological research, fostering sustainability, healthcare improvements, and industrial innovations that benefit global ecosystems and human health.

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Converging Technologies: Nanobiotechnology and Biomaterial Innovations

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